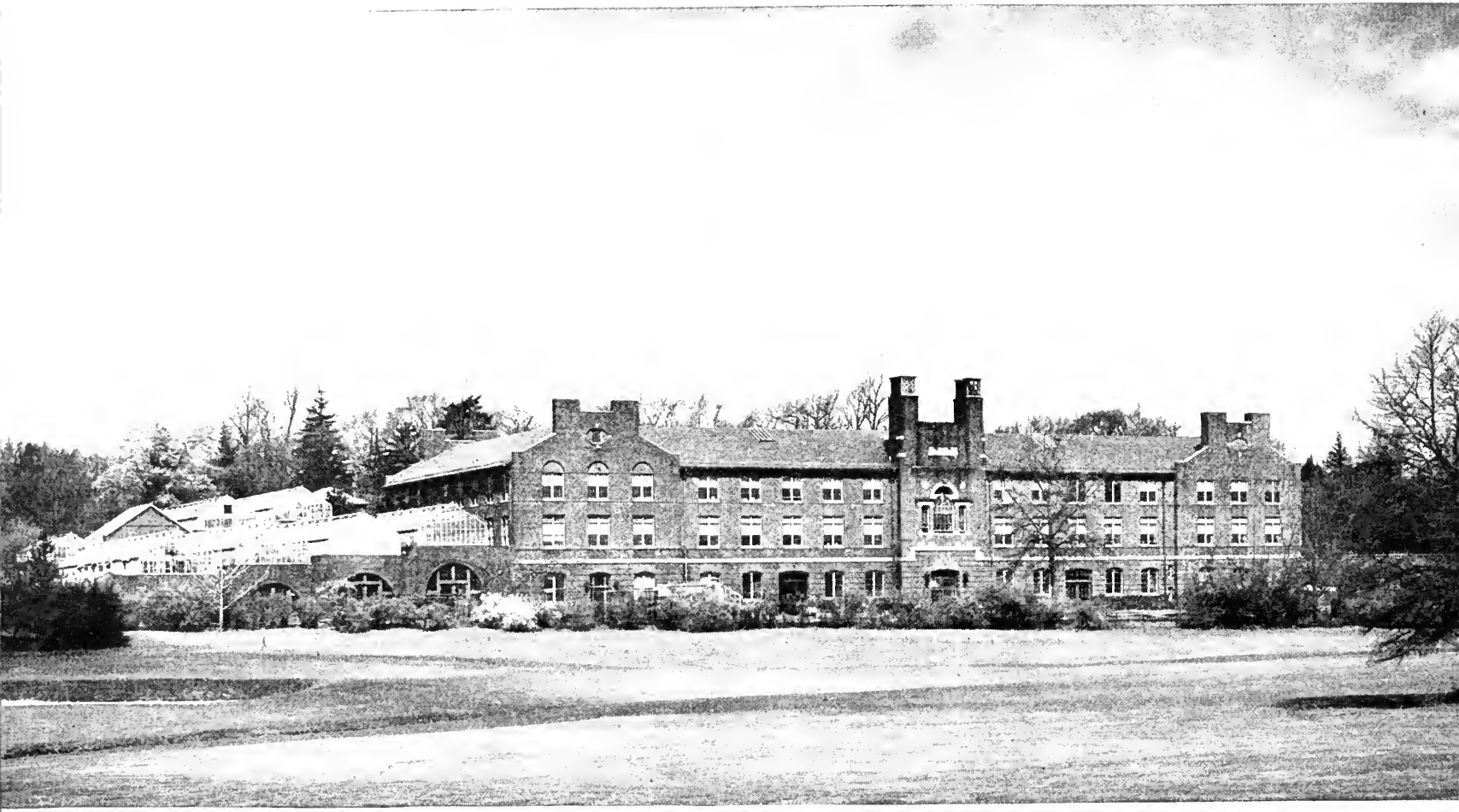

FIGURE 1. Boyce Thompson Institute for Plant Research, Inc., east elevation.

GROWTH
of
PLANTS

Twenty Years' Research at Boyce Thompson Institute

by

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REINHOLD PUBLISHING CORPORATION
330 West 42nd St., New York 18, U. S. A.
1948

Printed in U. S. A. by
NORWOOD PRESS, NORWOOD, MASS.

Contents

FIGURE 2. Colonel William Boyce Thompson.

Introduction

The Founder of the Boyce Thompson Institute

Boyce Thompson Institute for Plant Research, Inc., was founded and endowed by Colonel William Boyce Thompson of Yonkers, New York. His total gift to the Institute in property and endowment amounted to somewhat more than ten million dollars. The Institute was formally dedicated on September 24, 1924.[2]

The writer is often asked, "Who was Colonel Thompson and why was he so much interested in research on plants?" Colonel Thompson * was born in Alder Gulch, near Virginia City, Montana, in 1869, and spent the first seventeen years of his life in the wild frontier towns of Virginia City, Glendale, and Butte, Montana. It is not strange that his main interest was in mining, for that was the industry of the region; besides, his father formed an investment company that dealt in mining properties. After a period at Phillips Exeter Academy and Columbia School of Mines, Colonel Thompson returned to Montana and engaged in various mining operations there until 1899. At this date he moved to the East; operating in New York and Boston, he began dealing in mining stocks and developing mines. His first success was the development of the Shannon Mine at Clifton, Arizona, 1899 to 1903. This netted enough to pay off all indebtedness of the Thompson Investment Company with an additional net personal asset of $70,000. The stockholders profited similarly. This was only pocket change compared with what was to come later. In the development of this property, efficiency of operation was the keynote of success, as was the case in his future operations: for example, when copper prices were low, the cost of production must be reduced to meet the price.

Later the Colonel developed or aided in developing a series of mines that were highly profitable: Nipissing, Ely, and Mason Valley, Nevada, Bingham Canyon, Utah, and Inspiration and Magma in Arizona. The last two were models in efficiency, and the efficiency grew out of careful and extensive researches preliminary to carrying out the plan of operation and building the concentrators and refineries. The former especially because of its size gave great returns in dividends during World War I. If the Colonel's savings at this point reached the fifty million mark, they were to be greatly increased by his part in the development of Texas Gulf Sulphur and by the organization of the Newmont Mining Corporation, the latter an investment corporation handling mining stocks.

* The data for the brief biography of Colonel Thompson are taken mainly from Hermann Hagedorn's book, "The Magnate: William Boyce Thompson and His Time, 1869–1930." [1] The author includes some statements based on his personal contacts.

While the Colonel's biggest philanthropies were the financing of Boyce Thompson Institute and numerous big gifts to Phillips Exeter Academy, other sizable philanthropies could be named by the score: Belgian relief, Roosevelt Memorial, financing the Red Cross Commission to Russia, 1917, very large relief gifts to Russia during the sojourn of the Red Cross Commission there, and many contributions to worthy charities during and after World War I.

One of the very interesting things about Colonel Thompson was his friendships. His close personal friends ranged from ditch diggers to able scientists, statesmen, businessmen, and rulers. These friendships were generally determined by positive worthy characteristics of the friend, especially insight, integrity, and sincerity. The friendship with Doby Tom, a Slovak ex-sailor, working in Boyce Thompson Southwestern Arboretum, was a matter of mutual entertainment. Tom had definite ideas about his own rights and began answering fanciful criticism of the Colonel. Later their arguments often took on the appearance of bitter personal quarrels, to the great amusement of the Colonel as well as of Tom. The friendship with Johnny Schaller, a stone-mason, was unique. Johnny was for nineteen years, up to his death, superintendent of "Alders," Colonel Thompson's estate at Yonkers, N. Y. The friendship and the employment of Johnny as superintendent was an outgrowth of the Colonel's admiration of Johnny's excellence as an artisan and approval of sincere and sturdy German character in which realism far outweighed imagination.

The Colonel's friendship with Dr. Robert Kennedy Duncan, organizer and first Director of Mellon Institute, started with the reading of Duncan's books, "New Knowledge" (1905) and "The Chemistry of Commerce" (1907), which gave a preview of the coming importance of chemistry in the industries. This friendship was deepened by frequent visits while the Colonel was having research done with Duncan on leaching and flotation of copper ore and on certain sulfur problems. These copper researches aided in making Inspiration and Magma efficient and profitable mines, and the research on sulfur assisted in building up a fine technical organization under Walter Aldridge that made Texas Gulf Sulphur technically so nearly perfect and financially so highly profitable.

Dr. Duncan's books were not only excellent expositions of the subjects discussed, but they were simple, direct, and highly inspirational. Such passages as: "the romance of untrodden ways, the romance of unguessed to-morrow" and "man can really live only when he has the chance to live. There is but one way of lifting man to a higher moral and spiritual plane, and that is by lifting to a higher plane the condition of his material surroundings" evidently impressed the Colonel. The effect of association with Duncan through his books and personally was to convince Colonel Thompson of the great contribution that research could make to human progress. It also convinced him that one of the best investments one could make for society was properly chosen and directed research. Colonel Thompson had

practically decided to finance Dr. Duncan's research in industrial chemistry when A. W. Mellon made his gift of the Mellon Institute. If Mr. Mellon had not anticipated the Colonel in financing Dr. Duncan's researches, it is almost certain that there would not have been a Boyce Thompson Institute nor a Mellon Institute under that name.

After the death of Dr. Duncan, Dr. Raymond Foss Bacon, the next Director of Mellon Institute, assumed the place of Dr. Duncan as a friend and scientific counsellor to Colonel Thompson. It was with Dr. Bacon's advice and assistance that plans for Boyce Thompson Institute developed. It was also through Dr. Bacon's suggestion that Prof. John Merle Coulter was called in to aid in developing plans for the Institute.

One of the Colonel's most interesting friendships was that with Colonel Raymond Robins, a well-known social worker. Neither had met before being appointed to the Russian Red Cross Commission in 1917, and both apparently avoided meeting as long as possible after going on the Commission. Colonel Robins was said to have asked, "Why was that several-sorts of a plutocrat, Thompson, appointed on the Commission?" Thompson was supposed to have asked a like question about that several-sorts of a socialist. When they did meet and exchange views, they found themselves in agreement on most matters, even on social views, and especially on the social needs of Russia at that time. As one reads the operation of this Commission as reported in "The Magnate,"[1] he is inclined to believe that if the advice of Thompson and Robins had been followed by the Allies, including our own country, Russia would have been kept active on the Eastern front and the future history of Russia would have been very different. The Thompson-Robins recommendation that the Allies authorize Kerensky to parcel out the land of Russia immediately to the peasants and let the remuneration for the land rest for later adjustment, brought a loud howl of disapproval from the Allies. Also, the recommendation that Russia be given adequate material help of all kinds fell on deaf ears. Even when Kerensky lost out to the Soviets and Lenin, largely because of his failure to distribute the land to the peasants, Thompson and Robins still recommended full cooperation with Russia. This was still more bitterly opposed by the Allies, and both the magnate and the socialist, now mutually called "Chief" and "Panther," were considered dangerous radicals. A friendship which started and grew under the trying conditions of the Red Cross Commission in Russia was intensified with time and association only to be ended by the death of Colonel Thompson in 1930.

As a boy and throughout life, the Colonel seemed to have two deep-seated longings: a desire to understand the logical order behind life and the universe, and a longing for beauty. Neither of these longings received much satisfaction in the rough, helter-skelter, sulfur dioxide-scorched Butte of that day. The Colonel was very fond of his cultured, sympathetic mother, who also had a keen sense for beauty. His father was stern, with a strict Methodist interpretation of conduct and with little understanding of the

aspirations of a growing boy. To this boy the Methodist religion represented a vengeful rather than a just and sympathetic God. Arthur Cotton Newell, a classical student from Baliol College, Oxford, and a high-school teacher at Butte, seems to have given the boy the first start in proper orientation with the world, both by personal contact and by placing in his hands Olive Schreiner's "African Farm" and Herbert Spencer's "Data of Ethics." The logical orientation with the world was furthered still more during his happy days at Exeter. Here he came under the influence of able teachers and outstanding characters: George Wentworth, teacher of mathematics and author of textbooks on the subject; Professor Tufts, English; George Lyman Kittredge, Latin; and Bradbury Cilley, Greek.

After two very busy decades in developing mining interests, Colonel Thompson at last found some leisure time to indulge his two natural bents: the desire for beauty and the desire to understand and contribute to the logical order of society. Furthermore, he had acquired the wherewithal to gratify these interests. His fine home on North Broadway, Yonkers, with its beautiful landscaping, largely planned and developed by himself, is an expression of his sense of beauty. With the aid of the artistic touch of Dr. Fred J. Pope, a mining and chemical engineer in the Colonel's employ, he collected and arranged in the basement of his home two valuable and beautiful exhibits: one of minerals and another of carved jades.

While Dr. Duncan had impressed the Colonel with the social value of research, the Colonel had to decide the area in which such research was most needed. In planting his estate, he learned from consulting experts that there was much still unknown about factors affecting plant development, especially about controlling insect pests and plant diseases. His experiences in starving and freezing Russia emphasized the significance of plants to man as the ultimate source of all his food, nearly all his clothing, and much of his shelter. Statements of the Colonel quoted from "The Magnate" (pp. 290–291) show how his mind was working. "'When I have enough money,' he said one day, 'I am going to build a laboratory to study some of the fundamental things. I want to do something to get at the bottom of the phenomena of life processes and I think a good place to study them would be in the realm of plants. Any principles concerning the nature of life that you can establish for plants will help you to understand man, in health and in disease. So, by helping men to study plants, I may perhaps be able to contribute something to the future of mankind.'

"The thought made contact in his mind with other thoughts rising out of his Russian experience, his impatience with the ineffectiveness and unreality of the political approach to national problems, the waste and stupidity of politics. The phrase, 'when there are two hundred million of us' came again and again to his lips. He saw hope only in an order based on economics, illuminated and disciplined by science. He sent his imagination playing along the highways of tomorrow. 'What you are doing in politics and social welfare is all right, Panther,' he said to Robins one day as they stood to-

gether in the sunken garden. 'But there will be two hundred million people in this country pretty soon. It's going to be a question of bread, of a primary food supply. That question is going to be beyond politicians and sociologists. I think I'll work out some institution to deal with plant physiology, to help protect the basic needs of the two hundred million. Not an uplift foundation but a scientific institution dealing with definite things, with germination, parasites, plant diseases, plant potentialities. I can understand a thing of that sort. I could do something with it.'"

In 1919 the Farm and Research Corporation was formed, but no plans had been developed as to what it was to do or how it was to be done. Evidently the Colonel assumed that Dr. Pope would plan and build the laboratory. Pope was innocent of any considerable knowledge about plants, and nobody knew better his innocence of such knowledge than Pope himself. The Colonel advised Pope that if he didn't know about such work, he'd better pack his grip and go where he could get sound advice. Pope visited various universities and agricultural colleges, among them Cornell, University of Wisconsin, and University of Illinois, with the question, "What should the proposed institution undertake, mainly research or mainly extension?" Eugene Davenport, Dean of Agriculture of the University of Illinois, gave the deciding answer when he said in effect, "Agricultural extension is well organized and cared for in the United States, but if we do not have more fundamental knowledge, we may soon have nothing more to extend." Basic research on plants became the function of the corporation. Then followed a series of conferences with plant scientists in several institutions as to the best organization for such an institution and the most significant problems that could be undertaken. The early suggestions were not satisfactory to Colonel Thompson, so Dr. Bacon suggested that Prof. John M. Coulter, Professor of Botany at the University of Chicago, be called in as an adviser.

Prof. Coulter visited Colonel Thompson at his home in Yonkers in the fall of 1920 and presented a proposed outline for the organization of the Institute. This was accepted by the Colonel and the selection of a Director to plan and build the Institute was taken up immediately. Prof. Ezra J. Kraus of the University of Wisconsin was offered the position, but after due consideration decided not to accept. Later, the author, then Associate Professor of Plant Physiology at the University of Chicago, was offered the position and accepted. On February 1, 1921, the Director and Mr. John M. Arthur, an assistant and graduate student in plant physiology at the University of Chicago, began planning the Institute. Their duties at Chicago required one half of their time until August last of that year. The other half was used in visiting research laboratories in the United States, in searching for and buying books for the new library, and in considering projects that ought to be investigated, along with the type of equipment and building and personnel needed to develop the projects effectively.

The Director spent the fall of 1921 and a portion of the winter of 1922 in

Europe, visiting biological laboratories and experiment stations and consulting with prominent men in biology. During this trip the nucleus of the present library was purchased. The purchases consisted mainly of complete bound sets of German, English, and French periodicals in botany, biology, and chemistry, including a complete set of Justus Liebig's *Annalen* and several thousand German dissertations. The German publications and some publications in other languages that were for sale in Germany were purchased on very favorable terms because of the highly inflated German currency.

Mr. Arthur and the writer spent the rest of 1922, 1923, and some of 1924 in working with The J. G. White Engineering Corporation, the builders of the laboratories, in designing and equipping the laboratories. Because of the great amount of control equipment that was installed for the first time in biological laboratories this proved to be a rather arduous task. A refrigeration room was built to run at a regulated temperature considerably below freezing in which several thermostatically controlled chambers were placed to give the constant temperatures needed for plant studies. A scrubber system was installed to scrub the flue gas from the boilers as a source of carbon dioxide for the greenhouses. The greatest amount of control apparatus was developed for the proposed studies on light. Much of this is described and illustrated in Chapter IX, "Plants Grown under Controlled Environmental Conditions."

During the period of building, much attention was given to selecting the scientific staff for the Institute. We were anxious to get research started at an early date. Dr. L. O. Kunkel came to Yonkers in the spring of 1923 and started his work on the yellows disease of plants. This work is described later in the first chapter. Mr. Arthur was working on the *Botrytis* disease of tulips under the direction of Prof. H. H. Whetzel of Cornell, and on the fireholding qualities of tobaccos treated with various salts, and the writer was studying germination problems. Research did not start in full force, however, until the building was finished and a bigger staff was assembled in the fall of 1924.

Aims and Scope of Work of the Institute

From our studies of biological laboratories in the United States and Europe, we came to six conclusions concerning the aims, organization, and scope of the work to be undertaken at the Institute that would make it most serviceable in plant science at the time.

(1) It should do basic research, as had been determined by the founder, but basic research should not be contrasted with applied research as pure and applied research had been contrasted in some European laboratories, with harm, as we believed, to best progress. We felt that any project or problem tackled should be studied in all its relations, including its meaning in nature, in agriculture, and in the industries. Our later experience has

shown that much is added to knowledge in applying findings in the laboratory to behavior in nature and to practice.

(2) We had found too many botantists working on problems as individuals, hence with inadequate techniques for proper solution of the problems, likewise with inadequate equipment. We decided that we would not organize as departments based on techniques, but would organize to attack projects focusing enough different techniques on the projects to bring evidence on them from many, if not from all, angles. We believe this has minimized department jealousies such as sometimes appear in universities where, because of the necessity of teaching, departments must be organized on the basis of technique. It has also led to excellent cooperation between workers. This, of course, called for a staff with a great range of techniques and knowledge of the subject; not only must we have personnel covering every phase of botany needed for the projects, but we must have several kinds of chemists, physicists, entomologists, *et al.*

(3) We decided that botanical laboratories were generally inadequately equipped for effective work and that botanists should be supplied with many accurate instruments and controls to make their researches thoroughly reliable. The Institute was, as originally organized, almost a model of equipment for the projects in line for solution, and it has always given great attention to equipment since, so far as finances made this possible. Sometimes this ideal has brought embarrassment. One worker on light effects asked for a quartz prism that cost $1800.00 and two quartz lenses costing $300.00 each. He got the three desired gems.

(4) We found many botanists spending their time in doing work that they were not trained to do and doing it poorly or only moderately well. So we early installed an excellent photographic and illustration division, a library force that furnished citations of all literature bearing on the several projects, an engineering and mechanical division that kept all controls in operation and built any apparatus that was not available on the market, and finally a trained greenhouse and garden force that could carry through many of the larger scale experiments under the guidance of the investigators. The investigators were also provided with all needed laboratory helpers. These were generally high-school graduates who were encouraged to take college work while working for the Institute. A number of the laboratory helpers have since received college degrees, some Master's degrees, and one a Doctor's degree.

(5) Prompt publication of the researches was also considered desirable. Consequently at first extra space was purchased in the *Botanical Gazette* and the *American Journal of Botany* and finished researches published promptly. It is interesting to note that at least one botanist protested to these journals against this practice as unfair in that it gave the scientists of the Institute an unfair advantage over other scientists, namely, opportunity for prompt publication of their results. The Institute merely held that prompt publication is a desirable part of research and that all research institutions should

consider it as such and provide for it as an integral part of research. Later, the quarterly journal, *Contributions from Boyce Thompson Institute,* was started, as well as a series of *Professional Papers* dealing with practical applications of researches at the Institute. The latter consist mainly of separates published in trade journals or other professional periodicals. Several members of the staff have published one-chapter monographs in treatises on various phases of biological science or in review periodicals.

(6) Finally it was decided not to duplicate botanical research that was already being adequately covered in the northeastern United States. This was especially true of systematic botany and genetics. This left fundamental researches in plant physiology, pathology, and biochemistry as the main fields of activity for the Institute.

Organization and Purpose of the Book

The first eleven chapters of this book describe the researches on twelve larger projects with their many scientific and practical ramifications. Most of these projects have been in operation throughout the life of the Institute and some phases of all of these are still under study except for the Duck Food and Plant Cell Membranes projects, the first of which was finished and the second discontinued. Chapter XII presents fourteen shorter projects which, in the main, have been finished or discontinued.

This book is a critical summary of the researches carried on at Boyce Thompson Institute. Only such outside researches are discussed as are necessary to orient the work at the Institute generally in the whole field of plant science. A critical discussion of all the literature on every subject treated would expand the work far beyond a moderate sized volume.

Acknowledgments

The author expresses his gratitude to each member of the scientific staff of Boyce Thompson Institute for critically editing the portions of the manuscript covering his researches. Gratitude is also due the Publication Committee (F. E. Denny, A. E. Hitchcock, N. E. Pfeiffer, L. P. Miller, S. E. A. McCallan, Z. Troy, B. M. Brooks) of the Institute for editing all the manuscript for the book. The author gives special recognition to the several members of the Institute who wrote chapters or sections of the book. The following is a list of co-authors with the portions written by each: Dr. P. W. Zimmerman, Chapter VI — "Plant Hormones"; Mrs. Wanda K. Farr, Chapter VIII — "Plant Cell Membranes"; Dr. Albert Hartzell, Chapter X — "Research on Insecticides"; Dr. S. E. A. McCallan, Chapter XI — "Fungicide Investigations"; and Dr. W. J. Youden, last two topics of Section 13 and all of Section 14 in Chapter XII — "Miscellaneous Chapter." Special gratitude is due Mrs. Bettie M. Brooks for directing the typing and looking after the details of arranging chapters, sections, and figures

for the book, to Dr. L. P. Miller for a critical reading of all chapters, to Mrs. Lillian Teller for indexing the volume, and to Mr. L. P. Flory for the curves and most of the photographs used.

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1. Hagedorn, Hermann, "The Magnate: William Boyce Thompson and His Time, 1869–1930," 343 pp., John Day Company, New York, 1935.
2. "Organization — equipment — dedication," *C. B. T. I.** **1**: 1–58 (1925).

* *C. B. T. I.* will be used throughout the citations in this volume to indicate Contributions from Boyce Thompson Institute.

CHAPTER 1

Early Problems

Yellows and Virus Diseases of Plants

At the time Dr. L. O. Kunkel started his researches at the Institute, "yellows" were very obscure plant diseases. Peach yellows was described in 1791 by Judge Richard Peters of Philadelphia. There were violent outbreaks of it, some of them destroying whole orchards, in 1791, 1806–07, 1817–21, 1845–58, 1874–78, 1886–88, and 1920. The symptoms of the disease are as follows: the fruits ripen early with deep red color of both skin and flesh, and the flesh is bitter; the leaves are yellowed, rolled, and drooping; the new shoots are thin and wiry, growing upright and bearing narrow yellow leaves; the buds that remain dormant on healthy trees grow prematurely on diseased trees, producing witches' broom effect; the diseased tree becomes worthless and dies in two to six years. The disease is limited mainly to southeastern Canada and northeastern United States. Some outbreaks have occurred as far south as Texas and as far west as Arkansas and Nebraska. The disease had been fairly extensively studied by able pathologists, including Penhallow (1882–83) and Erwin F. Smith (1888–94), but neither the causative agent nor the method of transmission had been learned.

Dr. Erwin F. Smith described aster yellows in 1902 and suggested that it might go to other composites closely related to the China aster. The causative agent and method of transmission of this disease was likewise unknown when Dr. Kunkel started his work on yellows diseases. He had recently returned from his research on sugar mosaic, the insect vector for which he had discovered. Dr. Kunkel did not seem enthusiastic about the problem, although his later discoveries in the field did much to enhance his already high standing as a plant pathologist. Recently the author once accused him of showing little en husiasm for this new undertaking. His answer was that he never liked to undertake a new problem. Anyone who knows Dr. Kunkel, able and a bit phlegmatic as he is, would know that his lack of enthusiasm did not augur lack of future accomplishment in the problem. Prof. L. R. Jones, a member of the Board of Directors of the Institute, had some doubts of the wisdom of having a scientist undertake such an obscure problem. He might spend many years on it without success.

Dr. Kunkel started on aster yellows searching for possible insect vectors by the methods he used in discovering the insect vector of sugar cane mosaic. About six weeks after he got the insect isolation cages, he announced that of the several insects feeding commonly on asters, one and only one

FIGURE 3. A, Diseased aster plant in blossom. Yellowed foliage, green-colored flowers, and upright secondary shoots are shown. The plant is badly stunted. B, Healthy young aster plant. C, Yellowed plant of the same age, showing chlorosis and upright habit of growth of diseased leaves.

transmitted the disease. This was a leafhopper, *Cicadula sexnotata* Fall.[7, 8] The disease is not transmitted by means of the juice from diseased plants but can be transmitted by budding or grafting portions of a diseased plant on healthy plants. Either nymphs or adult leafhoppers can transmit the disease, but the causative agent has to be incubated in the body of the insect about ten days before the disease can be transmitted to a healthy plant.

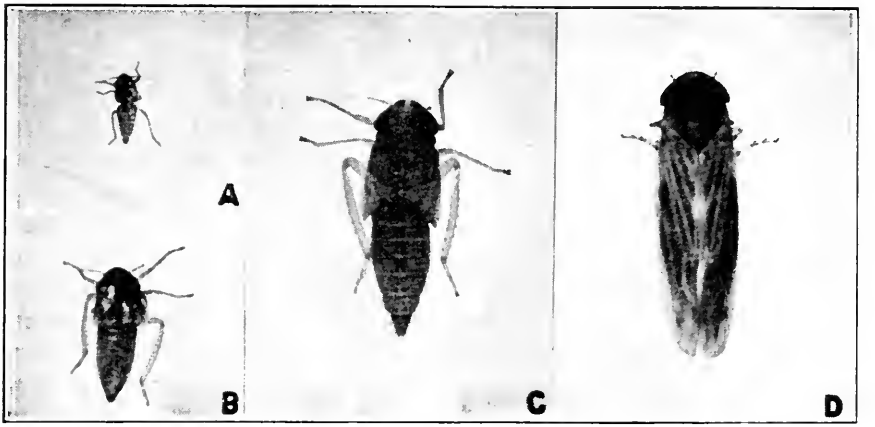

FIGURE 4. *Cicadula sexnotata.* A, Nymph in the first instar. B, Nymph in the second instar. C, Nymph in the fifth instar. D, Full-grown adult.

In his first paper, he reports that the insect can transmit the disease to 50 different species of plants belonging to 23 families. In a later paper, he reports 120 additional hosts belonging to 30 different families of flowering plants. Dr. Kunkel showed that perennial hosts such as *Plantago major* L. and *Chrysanthemum leucanthemum* L. carry the disease over winter and are a source of infection for the annual China aster. The insect over-winters in the egg stage, but eggs do not carry the disease. Aster yellows proved to be the same as white heart of lettuce and an undescribed disease of buckwheat and, of course, it is a disease on all the great number of hosts found giving symptoms more or less similar to those on aster yellows. It is not identical with peach yellows, curly top of beets, strawberry yellows, cranberry false blossom, or *Dahlia* stunt.

The symptoms of the disease on aster yellows are shown by the accompanying colored plate (Fig. 3). Fig. 4 shows four stages in the development of the insect vector. The disease, of course, would not appear on China asters in locations where no perennial or biennial carrier grew. Such regions are scarce. The sure control is to grow asters inside of areas caged with cheesecloth or other screenings of 22 × 22 meshes to the inch or finer. Even in such cages yellows plants must be rogued as soon as they appear. The discovery of the method of transmission of aster yellows and the means of controlling the disease led to a great revival of aster production commercially. The cages not only prevent the disease, but the conditions within the cages pro-

duce better flowers and continued production later in the fall by protection against early frosts. The failure of the disease to appear in greenhouse culture is probably due to the shyness of the insect carrier.

With aster yellows out of the way, Dr. Kunkel tackled the century-old mystery of peach yellows. Here he found again only one insect (Fig. 5), a leafhopper (*Macropsis trimaculata* Fitch), out of 15 commonly feeding on the peach, capable of transmitting the disease. Dr. Albert Hartzell later confirmed this conclusion on the specific carrier and found 47 other species of insects and mites feeding on the peach incapable of transmitting the yellows. Manns likewise found [6] that *M. trimaculata* transmits the disease, and by very limited tests concluded that the froghopper or spittle bug (*Philaenus leucophthalmus*) is an even more effective vector. In 1942, Manns [11] seems to throw some doubt on this conclusion. Hartzell states that peach yellows has a necessary incubation period in the insect of 10 to 26 days with an average of 16 days, also that the nymphs rather than the adults are the main, if not the sole, carriers of the disease. He implies that the low percentage of transmission found by Kunkel and himself may be due to the fact that individuals or strains of the species are unable to act as carriers, due perhaps to the impermeability of their intestines to the causative agent and hence to failure of the causative agent to enter the blood and finally the saliva of the insect. The insect decidedly prefers various wild and even domestic plums to the peach as food. Although plums take the disease and are injured by it, the disease symptoms are largely masked in plums. The patchiness of peach yellows on the peach both in time and location may be explained by the feeding preferences of the insect, the proximity of plum trees to the peach orchards, and other ecological factors.

Kunkel [9] later found that the causative agent can be killed in dormant peach trees by heating the trees at various temperatures for the proper length of time without seriously injuring the trees. Heating to 35° C (95° F) * for 19 to 24 hours, to 50° C (122° F) for 10 minutes, or to 54° C (129° F) for 1.5 minutes, the latter two in a water bath, destroys the infective agent without injury to the peach trees. The proposed methods for control of yellows on peaches are: destruction of all diseased trees in the orchard, removal of wild plums within a mile of the orchards, and contact sprays to destroy the insect vector or vectors.

Hildebrand, Berkeley, and Cation [6] mention about a score of other diseases of the peach that resemble peach yellows in that they are transmitted artificially by blending of tissues, budding or grafting, and not by mechanical transfer of juice. In nature the transmission must be by insects. Whether the vectors are or are not specific for these diseases is not known, for, in the main, the vectors have not been determined. It will suffice here to mention only a few of these diseases: rosette, little peach, red suture, phony disease,

* The temperature first given for any reaction is that used by the author of the article being described, and may be either in Fahrenheit or in Centigrade, followed by the temperature equivalent of the other scale in the nearest full unit.

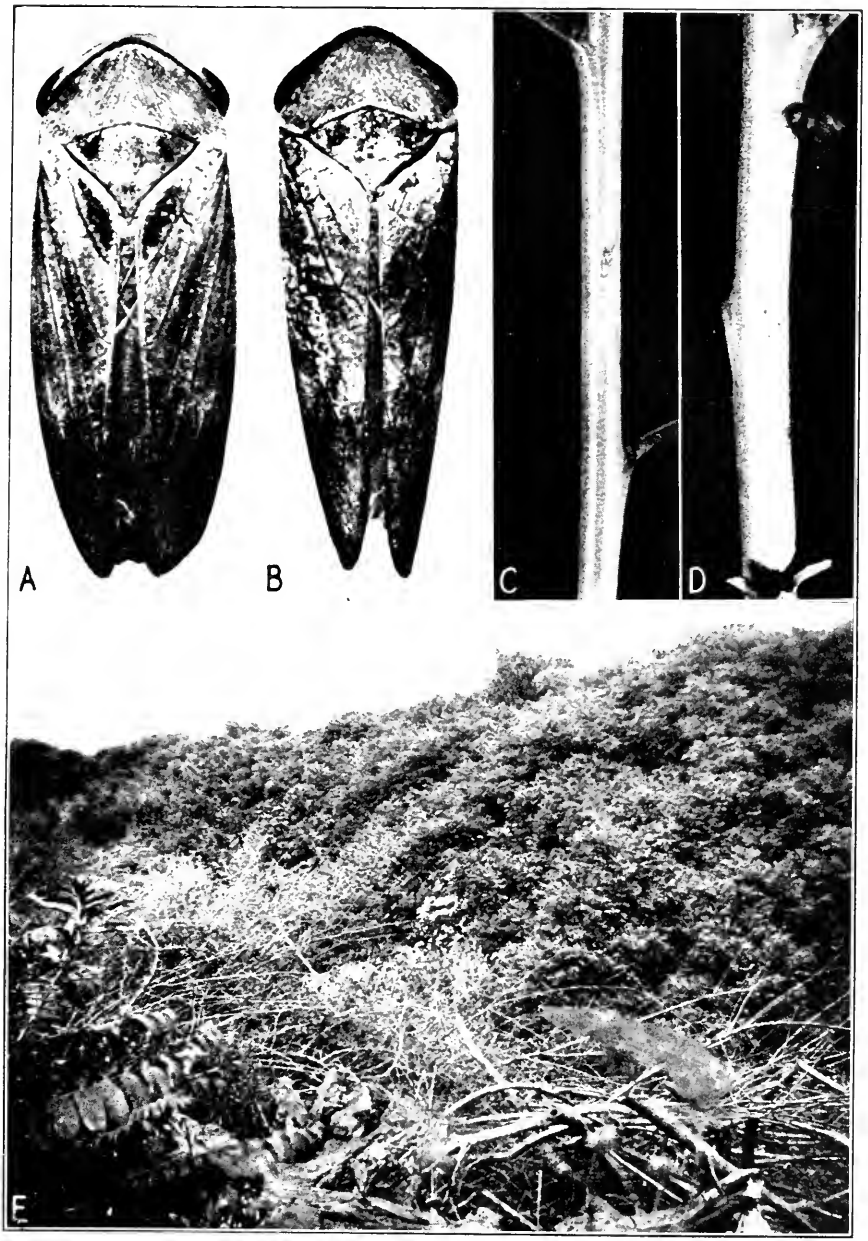

FIGURE 5. The plum and peach leafhopper, *Macropsis trimaculata*. A, Adult female (15 ×). B, Adult male (15 ×). C, Egg punctures. D, Feeding punctures. Note adult resting at top of twig. E, Sink hole in center of peach orchard in which all trees were healthy except for several diseased ones on the periphery of this sink hole.

mosaic, and rosette mosaic. Some of these diseases were first described before Kunkel discovered *M. trimaculata* as the vector of peach yellows but more of them have been described since. Much research will be required to determine the natural vectors of these diseases and determine their interrelations with each other. There is also much to learn about many similar diseases of other stone fruits of the genus *Prunus*.

Peach and aster yellows have been described as virus diseases, but are we sure that this is so? Certain it is that we have not proved that they are caused by filterable viruses, as is tobacco mosaic, for we cannot transmit them mechanically by transfer of unfiltered juice from diseased to healthy plants, let alone by the transfer of filtered juice. Such facts as incubation periods in the insect vector, transmission by blending of diseased and healthy tissue (grafting or budding) and not by mere transfer of juice and the extreme heat lability of peach yellows ought to lead to open-mindedness on the nature of the inoculum.

Dr. Kunkel early associated with himself a number of workers of diverse training to work on several of the phases of the yellows and virus disease problems. There were two entomologists. Some of Dr. Hartzell's work on yellows diseases is described above. He has also shown that peach yellows is not transmitted by pollen as was already known for seeds. Dr. Irene D. Dobroscky made a cytological study of the salivary glands and alimentary tract of aster yellows-infected *Cicadula sexnotata* and found no evidence of the presence of organisms or other visible changes. She also showed that cranberry false blossom was transmitted by *Euscelis striatulus* Fallen but not by some other insects feeding on cranberries or by mechanical means. She found no cytological changes in the glands and alimentary tract of viruliferous insects and suggested control of the disease by spraying to control the vector. Dr. F. O. Holmes, a protozoologist, studied the cytology of intracellular bodies of *Hippeastrum* mosaic, the *Herpetomonas bancrofti* in latex of *Ficus*, movement of tobacco mosaic within the plant, and several other phases of tobacco mosaic. He also attempted to photograph virus by use of ultraviolet light. Dr. Helen Purdy Beale, a plant pathologist, attempted the culture of organisms from tomato mosaic, found that slugs do not transmit tobacco mosaic, and studied the multiplication of tobacco mosaic in isolated leaves. She discovered a crystalline form of virus in the tobacco leaf. She also made an extensive study of serum reactions of viruses which developed a new technique for plant virus research. Dr. W. C. Price showed that tobacco plants acquired immunity to the ring spot disease and made a study of local lesions in the bean leaf inoculated with tobacco mosaic. Dr. C. G. Vinson and Mr. A. W. Petre, biochemists, isolated, purified, and crystallized tobacco mosaic virus. They concluded that it acted like a chemical rather than an organism. When heated, the juice containing the virus gives a precipitate at 85° C (185° F), also another precipitate at 90° C (194° F). Only the first contains an appreciable amount of nitrogen, and the juice shows little virus after removal of the first precipitate.

Doctors Mary Lojkin and Vinson found that trypsin and pancreatin inactivated purified tobacco virus but did not do so in the crude leaf extract. Emulsin, pepsin, and yeast extract showed no effect on the purified virus, except pepsin after many days' incubation. The work of Vinson and his associates laid the foundation for the later epochal work that Dr. W. L. Stanley did at Rockefeller Institute in purifying tobacco mosaic and identifying it as a large protein molecule. C. N. Priode, M. W. Woods, H. H. Thornberry, and others also worked with Dr. Kunkel on virus diseases while he was at the Institute during the ten years from 1923 to 1932.

During the decade Dr. Kunkel and his associates had made the Institute outstanding headquarters for contributions to the knowledge on virus and yellows diseases of plants. Rockefeller Institute for Medical Research, including the branch at Princeton, were, of course, interested in virus diseases as well as other diseases of both humans and animals. They concluded that researches on human, animal, and plant diseases could be carried on profitably in close association, the advance in each contributing to and benefiting by advances in the others. This is especially true of virus diseases. Consequently, Rockefeller Institute employed Dr. Kunkel to head a new division of plant pathology at Princeton, New Jersey, as a part of their now newly named Department of Animal and Plant Pathology. Dr. Kunkel planned and built at Princeton a fine modernly equipped plant pathology laboratory and manned it with scientists well trained for handling the several phases of plant pathology, especially virus and yellows diseases. He took with him three scientists who had formerly worked at the Institute. Following the policy of avoiding duplication of phases of plant science already strong in northeastern United States, the Institute did not attempt to reorganize and re-man the virus disease project. Some of the scientists on the project at the Institute shifted their efforts to other projects, but Dr. Beale and associates [1, 2, 10] have continued to date with their work on the serum reaction of plant viruses and other phases of the problem.

A Duck Food Problem

The work on the duck food problem described below was carried out by Dr. W. S. Bourn with advice and help of the scientific staff of the Institute. Bourn defines the problem, its support, and execution in essentially the following language.[3, p. 425]

The inland waters of North Bay and Back Bay in Virginia and Currituck Sound in North Carolina have long been known to be one of the most important winter feeding grounds for migratory wild fowl in the United States. In these waters there formerly thrived in great abundance such submerged angiosperms as *Potamogeton pectinatus* L. (sago pondweed), *P. perfoliatus* L. (redhead grass), *P. foliosus* Raf., *Naias flexilis* (Willd.) Rostk. & Schmidt, *Vallisneria spiralis* L. (wild celery), *Ruppia maritima* L., and *Ceratophyllum demersum L.*, all being valuable food plants, according to McAtee, for wild

ducks, geese, and swan. In 1918, almost simultaneously with the opening and enlargement of the Albemarle and Chesapeake Canal, however, these plants began to die out, and by the end of 1926 vast areas were practically denuded of their aquatic seed plants. This destruction of the plant life produced an enormous economic loss affecting thousands of the residents, who derived their living from gunning and fishing. Shooting clubs and sportsmen practically abandoned their large investments in the region because wild ducks and geese in any appreciable numbers were no longer attracted there. At the request of Mr. William E. Corey, a prominent sportsman of New York, who had been interested in the region for many years, the Boyce Thompson Institute for Plant Research, Inc., in 1925 undertook a study of the causes for the disappearance of the duck food plants, and of methods of re-seeding depleted areas. Early the fol owing year Dr. Bourn was assigned to the investigation.

Studies were made continuously from 1926 to 1930, inclusive, o the physical, chemical, and biological conditions of the water. During this time ecological experiments were carried on for the purpose of determining varieties of plants resistant to existing conditions and to determine the best methods of re-seeding the barren areas. The ecological investigations led naturally to physiological experiments in the laboratory to determine the factors limiting the growth of submerged angiosperms. These experiments were continued for six years.

Fig. 6 shows the location of North Bay and Back Bay, Virginia, and Currituck Sound, North Carolina, as well as land and other bodies of water in the general region. The three bodies of water involved in this study are protected from the salty waters of the Atlantic Ocean by a continuous sand bar that partially encloses Albemarle Sound to the south as well. On rare occasions very heavy storms drive some ocean water over the lower, narrower places in the sand bar. Currituck Sound is connected with the waters about Norfolk and Portsmouth, Virginia, including the estuary of the James River and Hampton Roads by the North Landing River and Chesapeake and Albemarle Canal. During the first World War this canal was widened, the sea-level locks were abandoned and not replaced after widening. This gives a free water connection between Currituck Sound and the salty waters about Norfolk. Before the locks were restored, as a result of this study, there was considerable movement of water from the Norfolk region toward Currituck Sound when the wind blew from the north and from Currituck Sound toward the Norfolk region when the wind blew from the south.

Early in these studies we began determinations of the salt content of the water of the various regions involved by a titration method developed by Dr. F. E. Denny.[4] Titrations were made monthly for four years at 26 stations shown on the map in Fig. 7. Fig. 8 shows the average of the 48 determinations for each station. By examination of this curve it will be seen that the average salt concentration at the stations gradually increased from Station Number 1, Corey's boathouse, to Station Number 26, Pungo Ferry,

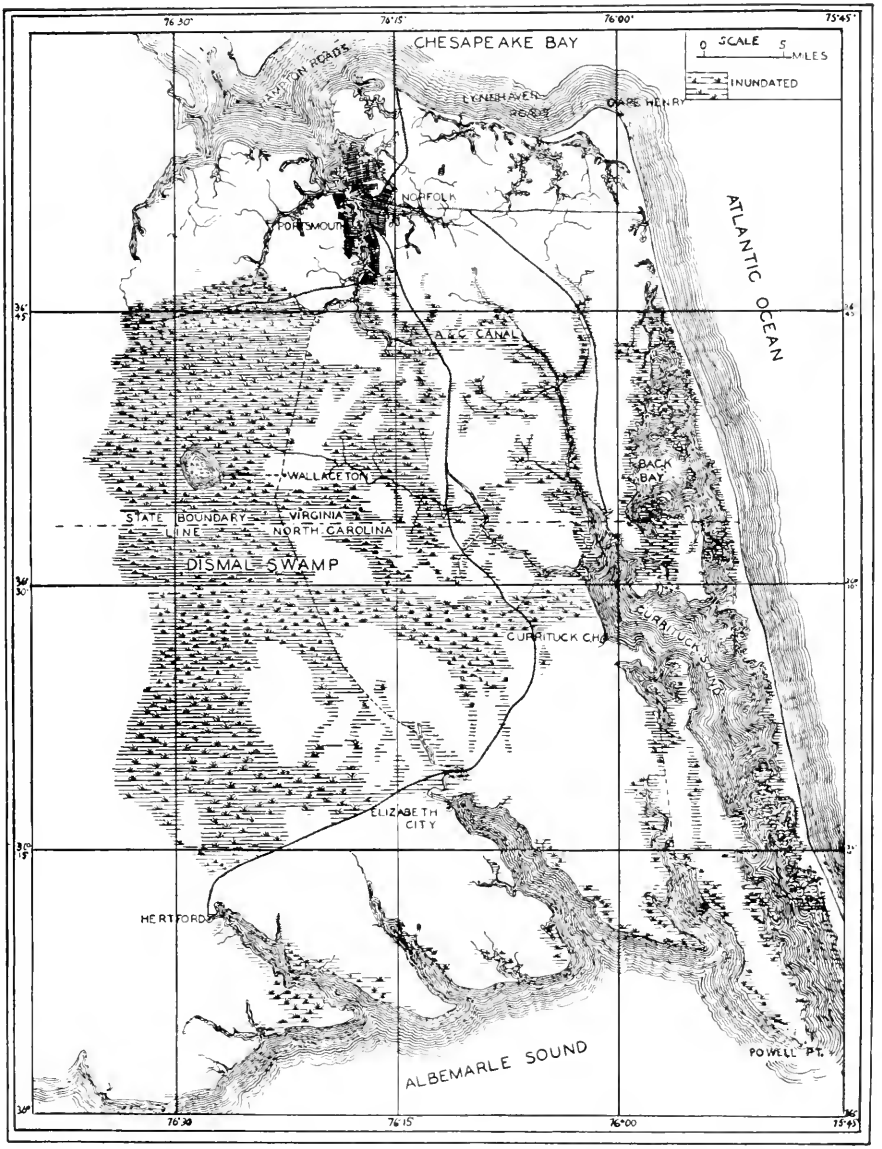

FIGURE 6. The Back Bay, Virginia, and Currituck Sound, North Carolina, region, showing the relation of these waters to the Albemarle and Chesapeake Canal, Norfolk Harbor, and Hampton Roads.

that is, from the most distant point from the North Landing River to the station farthest north in this river. This indicates that the salt in Currituck Sound and Back Bay had its main source from the canal and North Landing River. When a south wind blew for long periods the salt content at Station 26, Pungo Ferry, reached a concentration of 7 to 10 per cent of sea water.

When the north wind blew for a considerable time the concentration of salt at Station 26, Pungo Ferry, reached values as high as 60 per cent of sea water, nearly as high as the salt concentration in the waters about

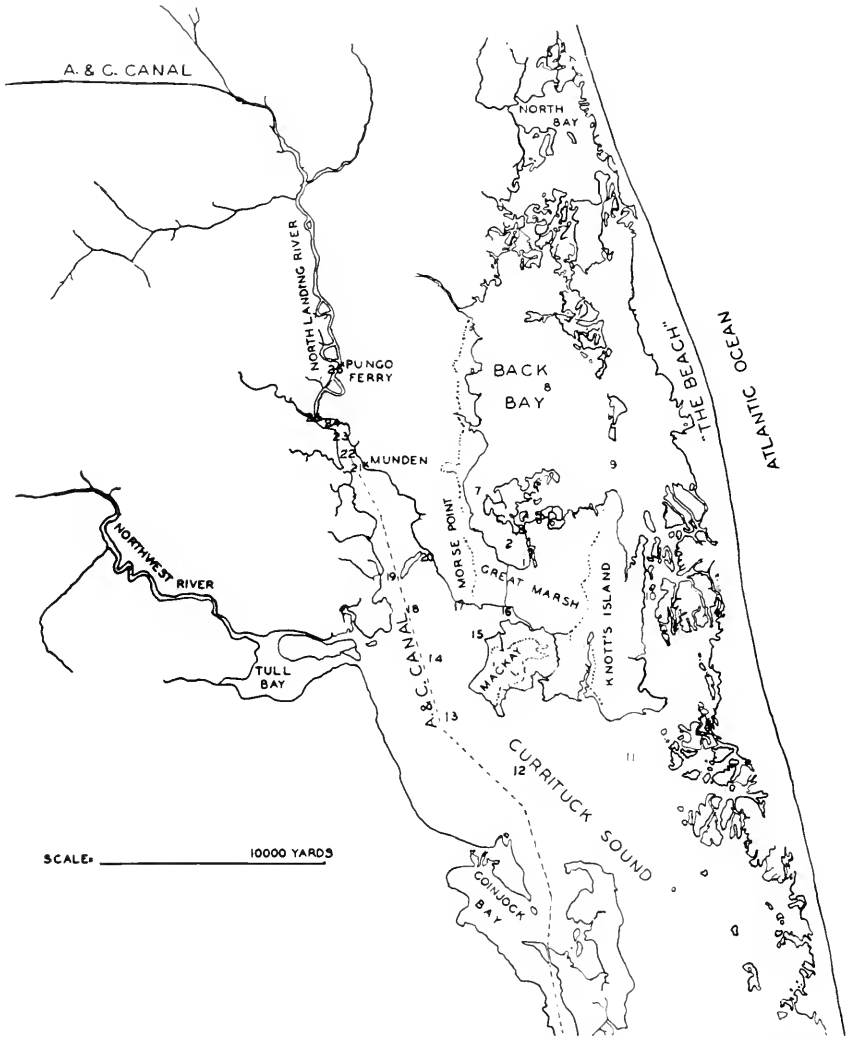

FIGURE 7. Map of the Back Bay, Virginia, and Currituck Sound, North Carolina, region, showing the location of salt testing stations, named and listed according to their order in *Figure 6*.

Norfolk. On the other hand, when the south wind blew during long periods, the salt in Back Bay was as low as 3.2 per cent of sea water. These titration studies proved beyond doubt that considerable salty water was blown from Norfolk region through the Albemarle and Chesapeake Canal and North Landing River into Currituck Sound and from this region it gradually moved through the channel east of Knott's Island into Back Bay. This was the

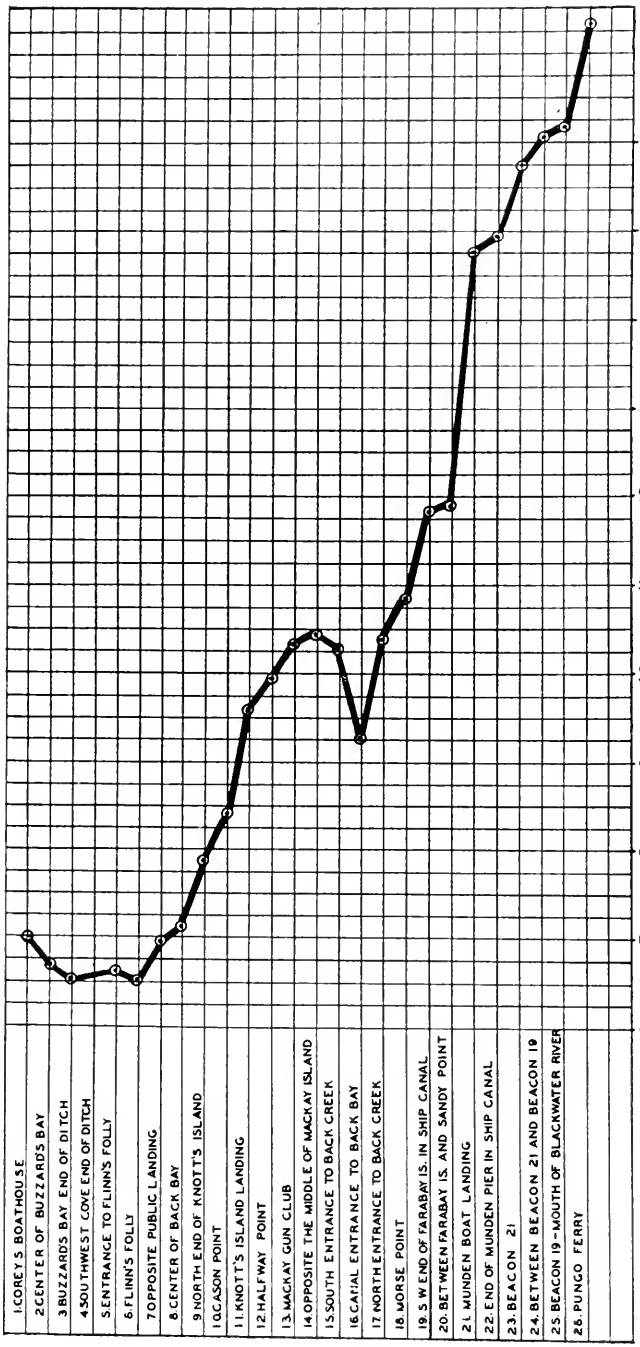

FIGURE 8. The average salt content of the waters of Back Bay, Virginia, and Currituck Sound, North Carolina, for 48 months. The averages are higher in the northern part of Currituck Sound and in the Albemarle and Chesapeake Canal at Pungo Ferry. Dips in the curve are the results of the close proximity of some stations to the mouths of fresh streams.

main source of the fluctuating salt content of these two bodies of water. The decline in duck feeds in these waters from 1918 to 1926 turned out to result from the removal of the sea-level lock mentioned above.

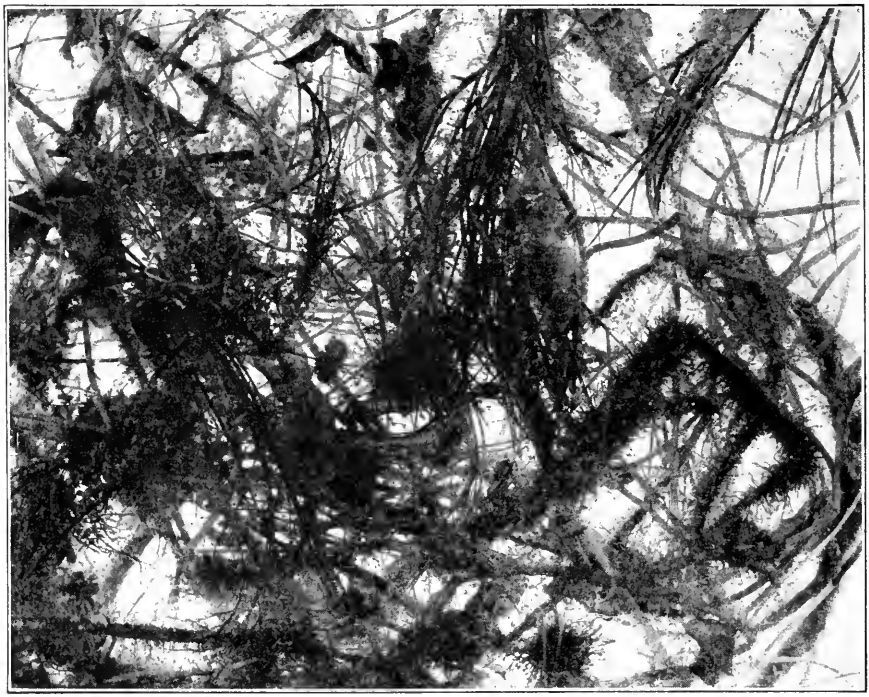

FIGURE 9. Submerged plants covered with colonial growths of the brackish-water hydroid, *Cordylophora lacustris* Allman.

Offhand, one might be inclined to assume that flow of water from the canal injured the plants in Currituck Sound and Back Bay by increasing the salt concentration. Apparently Bourn was early inclined to this view. Culture of the main duck food plants of this region in various dilutions of sea water disproved this. *Potamogeton pectinatus* and *P. perfoliatus* grew better in dilute sea water than in fresh water; 20 per cent sea water proved optimum and 36 per cent showed retardation but not complete inhibition. *P. foliosus* withstood 36 per cent of sea water. *Vallisneria spiralis* could not be grown successfully in concentration above 12 per cent sea water, but did well in 8 to 12 per cent. *Ruppia maritima* thrived in any concentration of sea water from 0 to 80 per cent and lived and stayed healthy in 150 per cent sea water. These facts eliminate salt concentration as any considerable factor of injury to the main duck food plants in these waters. The only place that salt concentration might act as an injurious factor to the dominant plants of the region was in the northerly part of Currituck Sound.

One of the big causes of injury to the plants was a hydroid, *Cordylophora lacustris* Allman, carried down with the brackish polluted waters of the canal

and favored in its growth by these waters. The profusion of growth of this hydroid on leaves and stems of aquatic plants is shown in Fig. 9. Fig. 10 shows the hydroid growing on a leaf of *Potamogeton*, and Fig. 11 shows other stages in the development of the hydroid and accompanying organisms. This hydroid is not a parasite on the plants but feeds instead on the abundance

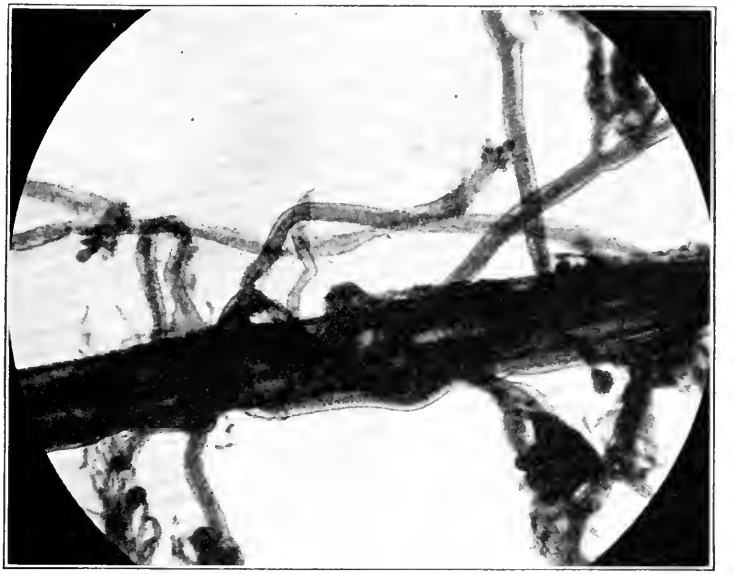

FIGURE 10. Photomicrograph (*about* 50 ×) of *Cordylophora lacustris* Allman entwining a leaf of *Potamogeton pectinatus* L.

of plankton in the polluted water. The hydroid forms a gelatinous sheath about the stems and leaves of the plants and the main injury occurs after the hydroid dies. This gelatinous sheath is also a good cultural medium for other organisms such as larvae, worms, diatoms, rotifers, fungi, and bacteria, which add to the injury. As a result of the smothering activity of all these organisms the plants were partly killed, and were broken from their moorings, after which they sank to the bottom and added to the pollution and turbidity of the water or were carried to the shore by the wind.

Bourn indicates that the biggest factor in denuding these waters of plants was the great turbidity of the water. He made many measurements of the light intensity at different depths in these waters during the growing season. He also determined in cultures the light intensity needed for the growth of the various duck food plants. On the basis of these two sets of measurements he came to the conclusion that during the growing season in many of these waters there was not enough light penetrating three feet below the surface to support plant growth. Although the minimum light requirements for the two important bottom-cover plants, *Chara* and *Nitella*, were not determined, it is probable that light was deficient in the deeper waters for these also. The lack of light was especially destructive to *Potamogeton*

pectinatus and *Vallisneria spiralis*. The former thrives in clear water up to a depth of 8 feet and the latter up to a depth of 12 feet. Recent work [12] indicates that in clear water the latter and perhaps the former may grow at two or three times the depths mentioned. There were two sources of particles of matter causing the turbidity — organic and inorganic. The water

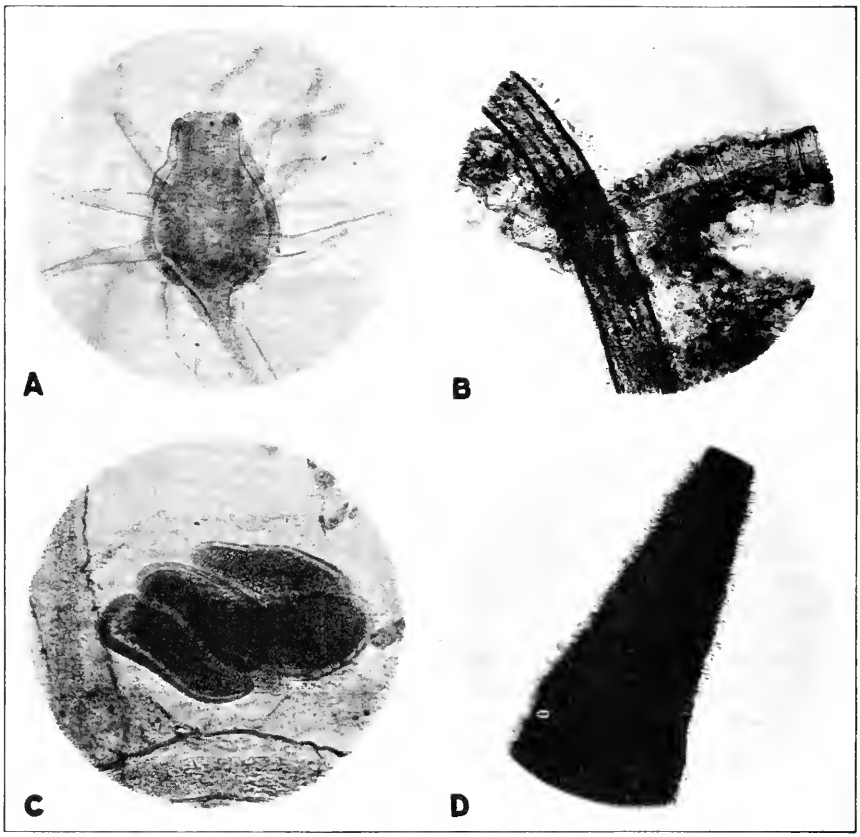

FIGURE 11. Photomicrographs (*about* 50×) of *Cordylophora lacustris* Allman. A, Typical hydranth. B, Stalk of a colony with its gelatinous secretions. C, Embryos. D, Diatoms growing in the gelatinous secretions left by hydroid colonies on a leaf of an aquatic plant.

flowing down the canal had many sewage particles in suspension. This and plants killed by the hydroid formed a sludge on the bottom that was being continually stirred up by wave motion. Inorganic clay particles were kept in suspension by wave action because of the lack of a bottom-cover of *Chara* and *Nitella*.

McAtee [3] reports that at the time of his study of these waters in 1909 there was an almost complete bottom-cover of *Chara* in Currituck Sound. In 1926 to 1930 there were only patches here and there of *Chara* and *Nitella* as bottom-covers. Where they did appear the water was clear; also there

was a good growth of Potamogetons. Potamogetons, especially *P. pectinatus*, require special emphasis in connection with this project, for they are prime duck food plants, furnishing as they do both seeds and tubers; in the days of thriving growth they constituted the greater part of the plants of these waters. McAtee says that in 1909 *P. pectinatus* alone comprised 60 per cent of the abundant duck feed in Currituck Sound.

Bourn found the oxygen content of these waters low and the carbon dioxide content high. This was especially true of the water of the northern part of Currituck Sound and of the canal. He thinks neither of these, as such, significantly limited the growth of green aquatic plants and that both would have changed if other growth conditions had been favorable. The situation was different with fish. The oxygen content of the water was so low that only low-oxygen-requiring herbivorous fish were present in any abundance and these were minor destructive agents to plants. The high-oxygen-requiring, plankton-consuming fish were scarce and consequently did not compete with the hydroid for plankton food.

During his studies in the region, Bourn cut off several coves from the main body of water by means of bulkheads and watched the natural recovery of vegetation in these protected coves or the development of re-set plants or sown seeds in them. In these protected areas, provided the water was not too shallow, the water plants thrived and finally gave a good stand of vegetation. Also the turbidity of the waters in these coves disappeared soon after the construction of the protecting bulkheads. This was true in spite of the fact that the bulkheads were not water-tight but allowed some movement of water, as was shown by the fact that the change of water level in the enclosed areas always accompanied that in the main body of water.

The District Army Engineer stated in 1922 [5, p.32]: "the salinity of Currituck Sound is caused not by accession of salt from the north or through the Albemarle and Chesapeake Canal, but from salt water washing over the beach into Currituck Sound at times of storm, and from Oregon Inlet." The Oregon Inlet through the sand bar opens into the north part of Albemarle Sound. When the evidence was all in, the Army Engineers reversed themselves on this point and expressed themselves as in agreement with the conclusions of the Institute. This is shown by a quotation from the report in 1929 of Major General H. Taylor, U. S. A., Retired, formerly Chief of Engineers. He says [5, p.34]:

"The Division Engineer seems to agree with this view, for he says: 'Considering the possible reasons for the present salinity in these waters, the salt water from Oregon Inlet, the sea washing over the beach, the seepage through narrow parts of the beach and from a salt water table can be disregarded, as there is nothing to show that there has been any increase in salinity from these sources. In fact, there should be a decrease, as Oregon Inlet is said to be smaller than in former years and the beaches have been considerably improved by sand-fence construction.'

"Among the papers submitted in connection with this case is a report sub-

mitted by the Boyce Thompson Institute and filed with the River and Harbor Board under date of February 23, 1929. This report describes present conditions existing in Currituck Sound and particularly refers to the effect of the contamination of these waters by sewage passing through the Albemarle and Chesapeake Canal into them. This report, in my opinion, is a very convincing report as to the detrimental effects caused in Currituck Sound and Back Bay by the removal of the lock. It will be noted that this report was filed in February 1929, while the report of the District Engineer was dated October 11, 1927, the report of the Division Engineer was dated October 20, 1927, and the report of the Board of Engineers for Rivers and Harbors was dated April 3, 1928. The pollution of the waters of Currituck Sound by sewage coming from Norfolk Harbor was discussed by representatives of the Boyce Thompson Institute at the hearing held by the Board of Engineers for Rivers and Harbors, December 14, 1927, but the statements made at that hearing as shown by the record are much less impressive than the carefully prepared paper which was filed after the Board made its report and which was based on studies which were continued after the date of the hearing as well as what had been learned before that date.

"An examination of the Boyce Thompson report shows conclusively that the sewage which is brought through the canal from Norfolk Harbor into Currituck Sound is in itself sufficient to cause destruction of the duck food plants. Whatever difference of opinion there may be as to the possibility of salt water coming into Currituck Sound in other ways than through the canal there can be no difference of opinion as to the sewage, for that can only come from Norfolk Harbor through the canal."

He also says [5, p.35]: "I can come to no conclusion except that the removal of the lock has been the principal cause of the destruction of the duck food plants in Back Bay and Currituck Sound and that it has been detrimental to navigation and to the fishing industry. The history of the Albemarle and Chesapeake Canal shows that the injury to the hunting and fishing interests of Currituck Sound and Back Bay caused by the infiltration of contaminated waters into these waters has been progressive. Every year that the contaminated water from Norfolk Harbor is allowed to freely flow south the damage is increased and the longer the construction of a new lock is postponed the greater the damage will be."

Mr. Bourn and members of the Institute feared that it would take years after the locks were replaced for the natural restoration of the plants in these waters if the process was not accelerated by artificial planting. These fears were based on two facts. (1) There were relatively few seeds, bulbs, and tubers left in the soil of the water bed as a source of new plants. (2) The bottom-cover of *Chara* and *Nitella* had largely disappeared, and with it the best preventive against turbidity.

The following quotations from Bourn in the report referred to by General Taylor [5, p.21] summarize the conclusions reached by the Institute on the basis of this research:

"The damage is not necessarily confined to the direct loss of plants, and the reduction of fish and wild fowl. While the productivity of the region was at its best, hundreds of sportsmen were attracted to the region. More than 40 hunting clubs were established on the shores of Back Bay and Currituck Sound. More than $5,000,000 were invested in marsh lands and properties suited to the sport of gunning, and suited to nothing else. Generous sportsmen contributed sums running into hundreds of thousands toward the erection and maintenance of modern schools, for the construction of roads, and for the general welfare of the community. Fine bus lines have been established and operated at private expense for the conveyance of children to and from the schools, and appreciable sums have been expended in the welfare of these children. Furthermore, there accrues from these investments to the public an annual sum of approximately $500,000 in the way of taxes, licenses, purchases, and other items in connection with the sport of fowling. In addition it is estimated that 5,000 people depend almost wholly for their livelihood and an equal number are partially dependent upon the sport of gunning and fishing. This means of livelihood and the returns from these investments are seriously endangered, for there is now little gunning and fishing and the property values have depreciated greatly in value, some as much as three-fourths."

Bourn also states [5, p.24–25]: "In view of our findings and in view of the fact that the opening of the Albemarle and Chesapeake Canal has been the sole disturbance in the natural conditions of this region we are forced to the logical conclusion that the restoration of the guard-lock in the canal is the only remedy for the present conditions in Back Bay and Currituck Sound and the only remedy that will restore the sole natural resource to this vast region. When we consider the extent of the damage to the region, the economic losses involved, the loss of the sole, large winter-feeding ground on the Atlantic Coast for wild fowl, the number of people and interests concerned, the reduction and threatened extinction of our wild fowl and our fresh water fishes, and our moral obligation to posterity and our treaty obligations to Great Britain for the preservation and protection of our rapidly vanishing wild life, we are further forced to conclude that the application of this remedy should not longer be delayed."

In a letter to the author under date of June 25, 1943, Dr. W. S. Bourn, now of the United States Fish and Wildlife Service, makes the following statements about the recovery of duck feeds after the restoral of the locks:

"You undoubtedly will be delighted to know that the waters in question have returned to a maximum productivity of duck food plants. Last year the peak was reached and sago pondweed seed was washed ashore in windrows. The history of the return of sago pondweed is very interesting. As you will remember, you and myself predicted that first *Chara* and *Nitella* would carpet the bottom. It took about two years for these growths to become appreciable in extent. In the third year these plants covered the bottom and the water became crystal clear. Then by the fifth year sago pond-

weed (*Potamogeton pectinatus* L.), redhead grass (*P. perfoliatus* L.), and wild celery (*Vallisneria spiralis* L.) returned beyond expectation. So by last season the old residents maintained that these plants had returned to abundances that existed before the locks in the canal had been removed. It is regrettable that some scientific agency could not have continued the study of the progressive changes in the flora. About all the opportunity I have had to observe these has been on short periodic visits once or twice a season, but I had no time to make various tests.

"As to the seeding of the depleted areas, this may be attributed to dissemination by waterfowl. On the beach strip between the ocean and Pamlico Sound a few years ago the Fish and Wildlife Service made a large artificial pond by dikes on the sand. This is on what is known as Pea Island, just below Oregon Inlet towards Cape Hatteras, on the sand spit of barrier beach between the two bodies of saltwater. The impoundment filled to a desirable depth with rainwater, as there the normal rainfall exceeds evaporation. Without any seeding except through the agency of waterfowl a good stand of sago pondweed became established the first season. This could have happened in Back Bay and Currituck Sound, but then we knew nothing about the probable continued presence of dormant seeds in the mud of these waters."

In a letter under date of August 9, 1943, Bourn further states:

"It may interest you further to learn that with the recovery of the plant life in Back Bay and Currituck Sound the black bass, the important game fish, also returned. Now on 'blue bird' days during the duck shooting season when the birds are reluctant to fly without a stimulating wind, sportsmen are accustomed to cast from their shooting blinds and catch a limit of these game fish, the commercial seining of which is no longer permitted in Back Bay and Currituck Sound. So the area may again be thought of as a 'sportsman's paradise,' brought about by the restoration of the locks in the Intracoastal Waterway."

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CHAPTER 2

Life Span of Seeds

How long do seeds live? This is a very complex question to answer, for there are many species of seeds varying greatly from one to the other in life span under any one condition of storage.[17] The condition of storage modifies the life span of seeds tremendously. A given storage condition may lengthen the life span of one species and shorten that of another.

In his well known and excellent book, "On the Longevity of Seeds," published in 1908, Alfred J. Ewart [27] gives a rather pessimistic view of the accuracy of the knowledge in this field. He says: "Probably few sections of human knowledge contain a larger percentage of contradictory, incorrect and misleading observations than prevail in the works dealing with this subject, and, although such fables as the supposed germination of mummy wheat have long since been exploded, equally erroneous records are still current in botanical physiology. In addition, there are considerable differences of opinion as to the causes which determine the longevity of seeds in the soil or air. The works of de Candolle, Duvel, and Becquerel are the most accurate and comprehensive dealing with the question, and, in addition, Vilmorin has published very useful data in regard to the seeds of culinary vegetables. The subject is still, however, in an incomplete and fragmentary condition."

Since Ewart's classic work was written, many new data and much evidence have been accumulated in this field, and definite advances have been made in several phases of the subject, including the nature of the changes involved in the degeneration of seeds with age and the effect of storage conditions on their rate of degeneration. Many new records have appeared on life span of seeds of wild plants in herbaria and seed cupboards and of seeds of cultivated plants in storage. We also now have available a good deal of reliable data on the life span of seeds in soil.

On the basis of their life span under optimum conditions, Ewart divides seeds into three biological classes: (a) *microbiotic*, whose life span does not exceed 3 years; (b) *mesobiotic*, whose life span ranges from 3 to 15 years; and (c) *macrobiotic*, whose life duration ranges from 15 to more than 100 years. As we shall see later, we do not have final information on the optimum storage conditions of many kinds of seeds, and in spite of the great amount of research that has been done on seed storage in recent years it is questionable whether anyone can give optimum conditions for the storage of any sort of seed, although one can give good conditions that will greatly

lengthen the formerly assumed life span. Until we possess such information, these terms do not have very definite meaning. As we learn of better and better storage conditions for a given species of seed, it may jump from the microbiotic to the mesobiotic or even to the macrobiotic class.

Seeds of Long Life Span

Let us look at the percentage germination of certain old macrobiotic seeds that have been taken from seed cupboards or herbaria. Becquerel [12] gives a very interesting record. He had access to a batch of old seeds in a storage room in the National Museum of Paris. The time of collection of these seeds varied from 1819 to 1853. He ran germination tests on these seeds in 1906 and again in 1934. For the 1934 test, Humbert and Metman furnished him about 20 seeds of *Cassia multijuga* which were collected in 1776. These seeds were all hard-coated, so they demanded special treatment. They were sterilized, the coats broken, and put to germinate in tubes under sterile conditions at 28° C. The seed stock was considered so precious that only ten of each sort were used for the test. Of *Cassia multijuga* only two seeds were used. Table 1 shows the results obtained for the 13 kinds, showing germination in either the 1906 or the 1934 test. In the last column Becquerel estimates the probable life span of several of the seeds, based on the data for the two tests.

Table 1. Becquerel's Record of Old Seeds

Macrobiotic species	Date collected	Seeds growing in 1906	Seeds growing in 1934	Determined longevity, yrs.	Probable longevity, yrs.
Mimosa glomerata Forsk.	1853	5 out of 10	5 out of 10	81	221
Melilotus lutea Gueld	1851	3 " " 10	0 " " 10	55	—
Astragalus massiliensis Lam.	1848	0 " " 10	1 " " 10	86	100
Cytisus austriacus Linn.	1843	1 " " 10	0 " " 10	63	—
Lavatera pseudo-olbia Desf.	1842	2 " " 10	0 " " 10	64	—
Dioclea pauciflora Rusby	1841	1 " " 10	2 " " 10	93	121
Ervum Lens Linn.	1841	1 " " 10	0 " " 10	65	—
Trifolium arvense Linn.	1838	2 " " 10	0 " " 10	68	—
Leucaena leucocephala Linn.	1835	2 " " 10	3 " " 10	99	155
Stachys nepetifolia Desf.	1829	1 " " 10	0 " " 10	77	—
Cytisus biflorus L'Hérit.	1822	2 " " 10	0 " " 10	84	—
Cassia bicapsularis Linn.	1819	3 " " 10	4 " " 10	115	199
Cassia multijuga Rich.	1776	——	2 " " 2	158	—

All these seeds are of the Leguminosae, except those of *Lavatera* (Malvaceae) and *Stachys* (Labiatae). The seeds of *Cassia multijuga* germinated after 158 years of storage. This exceeds the records of Robert Brown for *Nelumbium speciosum* [12] from the British Museum, which were 150 years; also the records of Ewart for *Goodia lotifolia* and *Hovea heterophylla*, which

were 105 years. Becquerel believes the long life span in all these seeds is made possible by impermeability of the coats, which prevents any exchange of gases or water between the embryo and endosperm and the outside atmosphere, and by the high degree of desiccation, 2 to 5 per cent moisture, and absence of oxygen in which the embryos exist within the hard coats. Late work shows that hard seeds of *Albizzia julibrissin* in the British Museum [25] were alive after 149 years, and seeds of *Nelumbium* (Robert Brown's collection) after 250 years of storage.

Table 2. Turner's Record of Old Seeds

Species	Age, yrs.	Germination
Anthyllis Vulneraria	90	4 per cent
Trifolium striatum	90	14.1 " "
Trifolium pratense	81	2.6 " "
Lotus uliginosus	81	9.6 " "
Melilotus alba	81	163 seeds, 1 germinated
Cytisus scoparius	81	636 " 4 "
Medicago orbicularis	> 78	22 per cent
Ipomoea sp.	43	6 " "

Turner,[57] of Kew Botanical Garden, tested the vitality of old seeds from several sources. All the viable seeds were hard-coated and were treated with sulfuric acid to render the coats permeable. Table 2 gives data on the seeds he found viable.

Schjelderup-Ebbe [50] tested the vitality of 1254 batches and nearly as many species of seeds stored in bottles or paper bags for 34 to 112 years. The oldest living sort found by this author is that of *Astragalus utriger*, 82 years old, with 6 per cent germination. Some kinds that were not so old showed relatively high percentages of germination. The following families are represented by these macrobiotic seeds: Cannaceae, Leguminosae, Euphorbiaceae, Malvaceae, Thymelaeaceae, Convolvulaceae, Solanaceae, and Compositae.

Of more than 1400 sorts (including species and varieties) of old seeds tested, Ewart [27] found 49 that retained their vitality after more than 50 years of storage: 37 Leguminosae, genera *Acacia*, *Albizzia*, *Canavalia*, *Cytisus*, *Eutaxia*, *Galega*, *Gompholobium*, *Goodia*, *Hardenbergia*, *Hovea*, *Indigofera*, *Jacksonia*, *Kennedya*, *Melilotus*, *Mimosa*, *Oxylobium*, *Psoralea*, and *Pultenaea;* 4 Malvaceae, genera *Abutilon*, *Hibiscus*, *Modiola*, and *Sida;* 1 Tiliaceae, genus *Entelea;* 2 Euphorbiaceae, genera *Euphorbia* and *Pseudanthus;* 1 Labiatae, genus *Stachys;* 1 Iridaceae, genus *Watsonia;* 1 Sterculiaceae, genus *Hermannia;* and 1 Polygaleae, genus *Comesperma*. Amongst these were *Goodia lotifolia*, 105 years old with 7.7 per cent germination, and *Hovea linearis*, 105 years old with 17 per cent germination. Seeds of several species show relatively high germination in spite of great age: *Cytisus*

albus, 51 years old, 78 per cent germination; *Entelea arborescens*, 51 years old, 47.3 per cent; *Indigofera cytisoides*, 51 years old, 51.2 per cent; and *Melilotus gracilis*, 58 years old, 28.8 per cent.

Seeds of Short Life Span

Some seeds lose their vitality in a very short time if they are kept in open air after harvest. Until recently this rapid loss of vitality was supposed to be due mainly or solely to the drying effects of the air, that is, the protoplasm of the embryo was killed by partial desiccation. No doubt this is the case with some short-lived seeds, but other factors determine life span in other short-lived seeds. A consideration of some of the later more critical work on various short-lived seeds will show the significance of several factors in the loss of vitality.

According to Jones,[17] the seeds of the river maple (*Acer saccharinum*) are killed by relatively slight drying. When they fall from the tree in June they bear about 58 per cent water. Regardless of the temperature of exposure (0° to 35° C [32° to 95° F]), they were killed when the moisture content reached 30 to 34 per cent. In his experiments it required six days at 35° C (95° F) and 92 days at 0° C (32° F) to reach this water content, or the death point. When these seeds were stored in a closed vessel over water at the freezing point and provision made for preventing carbon dioxide accumulation, they retained full vitality for 102 days, which was the limit of the test. The low temperature prevented germination and reduced the rate of metabolism. The latter is an important consideration, for these seeds are fleshy and have rapid respiration at higher temperatures. They should be sowed as soon as they fall. If this is impossible, because of the necessity of shipping or for any other reason, they should be kept near the freezing point, and water loss prevented. River maple seeds are not dormant but begin germination in nature as soon as they reach the moist ground. The seeds of the fall-fruiting sugar maple (*A. saccharum*) showed very different behavior. They endure complete air-drying and respond to several weeks' low temperature stratification for eliminating dormancy.

Duvel [17] finds that wild rice (*Zizania aquatica*) seeds lose their vitality if they are allowed to dry in the air for even a few days, but that they retain their vitality perfectly until spring if stored in water at 0° to 1° C (32° to 36° F). In the spring they must be transferred from storage water to the water in which they are to grow, without being allowed to dry. Seeds of wild rice are dormant when mature, and storage in water near the freezing point after-ripens them as well as maintains their vitality.

According to Barton,[10] various citrus seeds endure only partial drying in the air. Grapefruit and sweet orange seeds are injured by drying to 52 and 25 per cent moisture content, dry weight basis, respectively, at laboratory temperatures but grapefruit seeds retain their full vitality for more than a year when stored in the open at 5° C (41° F), where the moisture falls

to 17 per cent. Rate of drying as well as degree may be important, or it is possible that the temperature at which the drying occurs is a determining factor. Sour orange and rough lemon seeds also retain their vitality well when stored open at 5° C (41° F). They also endure more drying than the other two sorts of citrus seeds. Storage at −5° C (23° F) was injurious to all four sorts of citrus seeds because of their necessarily high water content.

Other seeds of the temperate zone that lose their vitality readily when stored in open air are oaks, beeches, horse chestnuts, walnuts, hickories, and chestnuts. These are generally stratified with moisture at a low temperature in the fall, which prevents germination until spring and after-ripens such as are dormant. Delavan,[17] working on seeds of three species each of hickories and of the white oak and the black oak groups, found that the seeds kept well until the following spring in an ice box and in a pit outside, but lost their vitality in a few months in dry storage. They also gradually after-ripened at the low temperatures, as shown by quicker germination with the lengthening of the period of storage. Barton [17] found low-temperature stratification necessary for the after-ripening of seeds of hickory, walnut, and butternut.

Seeds of willows also lose their vitality quickly when exposed to the air. This has been assumed to be due to excessive drying, but Nakajima's work disproves this assumption. He found [17] that seeds of *Salix opaca*, *S. japonica*, and *S. Reinii* retained their vitality much better in closed tubes over a solution of 50 per cent by volume of H_2SO_4 in water than they did in open air. In later work he found that seeds of *Salix Pierotii* and *S. japonica* in open air lost their vitality completely within a week, but when stored over the sulfuric acid solution mentioned above and kept in an ice chest they still gave 53 per cent germination after 360 days of storage. Such a solution gives a relative humidity of only 13 per cent, which is much lower than the average humidity of the atmosphere at ripening time of the seeds. Evidently the injury in open air is not caused by excessive drying. Valuable information might have been obtained if he had also tried low oxygen pressure and absence of oxygen, as Busse did for aspen seeds.

It is a well known fact that poplar seeds lose their vitality within a few weeks when left in the air. Busse [17] believes this is due to the injurious action of oxygen, also that higher temperatures hasten the degeneration. Storage of these seeds in a vacuum in a cellar preserved 90 per cent viable after 22 months.

Seeds of the English elm lose their vitality almost completely within six months of open storage and seeds of the American elm keep little better.[17] Barton [6] finds that sealed storage of American elm seeds at low temperatures, 5° and −5° C (41° and 23° F), prolongs the life of these seeds greatly. There seems to be little difference whether the moisture is 2, 3, or 7 per cent. In sealed low-temperature storage these seeds retained full vitality for five years, with the experiment still running.

Sugar-cane seeds degenerate rapidly when stored in open air. This makes

it impossible to ship them with assurance from one sugar region of the world to distant regions where seedlings are desired for breeding. Verret [17] found that vitality could be lengthened materially by taking the seeds from the thoroughly air-dried heads, placing them in cans with 9 grams of $CaCl_2$ to 1 liter of space, displacing the air with carbon dioxide, hermetically sealing, and storing at the freezing point. In these seeds, low and perhaps constant water content and absence of oxygen seem to be necessary for retention of vitality. It is possible also that carbon dioxide plays a positive role rather than merely displacing oxygen. Kidd [17] finds that the life span of seeds of *Hevea brasiliensis* can be greatly lengthened by sealing them in 40 to 45 per cent carbon dioxide. He assumes that this gas acts as a narcotic and that it induces dormancy.

There are many other seeds that retain their vitality for only a few weeks or less than a year when stored in the air. For most of these there is little information on the effect of the several factors involved in atmospheric storage. It is generally assumed, however, that the seeds are injured by drying, but the possible error in such a conclusion is made evident by the discussion above. Among the seeds of tropical plants that have a short life span, *Hevea* and sugar cane have been mentioned already. Others are *Boea, Thea, Cocos, Oreodoxa, Sabal, Attalea, Mauritia, Thrinax,* and *Acrocomia.*

De Candolle [17] says seeds of most species of the families Rubiaceae, Myrtaceae, and Lauraceae lose the germinative capacity soon after being detached from the mother plants. There are, however, exceptions in the first and second families. *Coffea* (Rubiaceae) seeds are used for planting up to three years. Ewart [27] reports several Myrtaceae that retain their vitality for considerable periods. Nearly all of 35 species of *Eucalyptus* reported upon show some vitality in the seeds after 10 years of storage. *E. calophylla* seeds 10 years old gave 96 per cent germination, and those 32 years old, 5 per cent. *Leptospermum scoparium* 10 years old gave 8.2 per cent, and those 16 years old, 2.4 per cent. *Callistemon lanceolatus* seeds 16 years old gave 75 per cent germination, and *C. rigidus* seeds 22 years old, 2.8 per cent. A thorough study would probably show many other exceptions to de Candolle's statement. The seeds mentioned by de Candolle as short-lived generally are sown soon after harvest.

While the studies mentioned above have thrown much light on the factors determining the life span of short-lived seeds, our knowledge would be much fuller and more conclusive if the life span of each seed had been studied under a wide range of intensity of each of the effective atmospheric variables, singly and in combination.

Life Span of Seeds in Soil

In the literature there are hundreds of records of seeds that are supposed to have lain in the soil for decades, still dormant but capable of germination. These determinations are based on the appearance of plants, not common

in the region, on recently excavated soils, or similar phenomena on recently plowed meadows or pastures of long standing. Peter [17] studied the seed content of soils of the forest that had been planted on meadow, swamps, or pastures for known periods and kept free from open land plants by deep shading. In general, as the age of the forests increased, seeds of field plants became more scarce and those of forest plants more abundant in the soils of the forest. He found seeds of the following in deeper layers of soils of forests 100 years old: *Hypericum humifusum*, *Stellaria media*, and *Juncus bufonius*. In soils of forests 20 to 46 years old, he found seeds of a large number of open-land plants belonging to various genera, such as *Thlaspi*, *Plantago*, *Sinapis*, *Juncus*, *Stellaria*, *Stachys*, *Anagallis*, *Polygonum*, *Chenopodium*, etc. Peter concludes that seeds of some meadow and swamp plants may lie in the soil more than fifty years, still capable of germination.

Ewart [27, p.182–183] has the following to say about the reliability of Peter's conclusions: "Peter's observations are good evidence of the readiness of dispersal of certain seeds, but as evidence of their longevity are quite untrustworthy. They contain a grain of truth buried in a mass of inaccuracy. The same applies to all similar records of supposed old seed in soil or under water being germinable, from the classical case of Mummy Wheat downwards. Here and there long-lived seed has accidentally been hit upon, but in the great majority of cases the records are incorrect." Later accurate records of Beal and the United States Department of Agriculture on the life span of seeds buried in the soil lead one to conclude that the claims of Peter, and others mentioned above, may be true in the main and that Ewart's criticism of their claims is far too severe. Ewart was especially concerned because the seeds that Peter claimed had long life in the soil were mainly small. The buried seed results, as we shall see later, show that very small seeds as well as larger seeds may live for long periods in the soil.

There is little doubt of Ohga's [47] claim of great age of the *Nelumbo nucifera* seeds he excavated from a naturally drained lake bed in Manchuria. The seeds were buried about 1.5 meters deep in a layer of gray mud covered in turn by a layer of peat and a layer of loess. The eroding river which drained the lake has now cut a channel through the lake bed about 13 meters deep. Since there were no *Nelumbo* plants growing in the region, and the seeds were buried so deep, Ohga concludes that the seeds were from plants growing in the lake before it was drained. Judging from the rate at which the river is eroding its bed, the age of the trees growing on the land since drainage of the lake, and the record of a family that has been farming the drained lake bed for several generations, Ohga concludes that the seeds have been buried for at least 120 years and more likely for 200 to 400 years. They still give perfect germination after treatment of coats.

United States Department of Agriculture buried seed project. The most extensive buried seed project on which considerable data have already accumulated is that of the Seed Testing Laboratory [17] of the United States Department of Agriculture, designated hereafter as U.S.D.A. These seeds

were buried by Duvel in 1902. Thirty-two sets of 107 species of wild and cultivated plants were placed in sterile soil in flower pots covered with porous clay lids and buried outside at three different depths, 8, 22, and 42 inches. Records of the tests for 1, 3, 6, 10, 16, and 20 years are now available. The tests for the 30-year period were made in 1933, but unfortunately there has been great delay in publication.

Seeds of a number of sorts of wild plants showed little or no germination after shorter burial periods, but considerable to excellent germination after longer burial periods. This may have resulted either from the use of less favorable germination conditions in the earlier tests, or from the gradual after-ripening of the seeds in the soil. At any rate, the conditions used for the later tests were fairly favorable for the germination of seeds of wild plants on the surface of flats of sterilized soil in a greenhouse. This provided light, fluctuating temperatures, and the stimulative effect of the soil. It is regrettable that samples of the seeds were not placed in dry storage under a variety of conditions, including sealed storage in the soil. This would have answered the interesting question of the relative life span of the seeds in several dry storage conditions and in the moist soil.

After 20 years' burial, some seeds of 51 of the 107 species were still alive. The following gives the families, genera, and the highest germination for each genus of those seeds still alive after 20 years.

Family	Genus	Per cent germ.	Family	Genus	Per cent germ.
Gramineae	*Chaetochloa*	26	Malvaceae	*Abutilon*	57
"	*Phalaris*	11.5	"	*Hibiscus*	57.5
"	*Phleum*	12.5	Onagraceae	*Oenothera*	87.5
"	*Poa*	18.5	Umbelliferae	*Apium*	10.5
"	*Sporobolus*	74.5	Convolvulaceae	*Convolvulus*	43
Cyperaceae	*Cyperus*	17	"	*Ipomoea*	57
Urticaceae	*Boehmeria*	71	"	*Cuscuta*	25
Polygonaceae	*Polygonum*	55	Verbenaceae	*Verbena*	90
"	*Rumex*	82.5	Solanaceae	*Datura*	78
Chenopodiaceae	*Chenopodium*	65.5	"	*Nicotiana*	56
"	*Beta*	1	"	*Solanum*	94.5
Phytolaccaceae	*Phytolacca*	75	Scrophulariaceae	*Verbascum*	92.5
Portulacaceae	*Portulaca*	38	Plantaginaceae	*Plantago*	83.5
Cruciferae	*Brassica*	38	Compositae	*Ambrosia*	83.5
"	*Thlaspi*	0.5	"	*Arctium*	29
Rosaceae	*Potentilla*	91	"	*Carduus*	4.5
Leguminosae	*Cassia*	2	"	*Chrysanthemum*	48
"	*Lespedeza*	48	"	*Onopordon*	37
"	*Robinia*	31	"	*Rudbeckia*	56.5
"	*Trifolium*	15.5			

Seeds of cultural plants that were dead after 20 years in the soil (and most of them died even after one year in the soil) were: oats, meadow fescues, barley, rye, wheat, corn, onion, asparagus, hemp, buckwheat, cabbage, turnip, pea, cowpea, bean, pepper, tomato, watermelon, muskmelon, cucumber, lettuce, sunflower, and pine. Seeds of cultural plants that showed some live seeds after 20 years in the soil were: timothy, Kentucky

blue grass, beet, bush clover, three clovers, tobacco, celery, and black locust. In the main, seeds of the first group have only transient dormancy and as a result soon germinate and perish under the soil, while those of the second group have a long dormant period in the soil: the leguminous seeds, because of hard coats; and blue grass, tobacco, and celery, because of the need of light for germination. The beet seeds gave only 1 per cent germination after 20 years and only 7 per cent and 9 per cent, respectively, after three and six years.

In discussing the results after the 20-year test, Goss [29] draws the following conclusions. Depth of burial has little effect on the longevity of the seeds. Seeds of cultivated plants, especially of cereals and garden legumes, perish quickly in the soil, while seeds of wild plants, especially persistent weeds like dock, lamb's quarter, plantain, daisy, poke, purslane, Jimson weed, and ragweed, retain their vitality well. Persistent weeds cannot be controlled by plowing the seeds under, for the seeds outlive any crop rotation.

Beal's buried seed project. In 1879 Dr. Beal [17] of East Lansing, Michigan, buried 20 sets of seeds as follows: 50 seeds each of 20 kinds of plants were mixed with sand and placed in pint bottles, and the 20 uncorked bottles were buried in sandy soil 20 inches deep with the mouths tilted downward to avoid filling with water. It was originally planned to take up a bottle every five years for vitality tests so that the experiment would

Table 3. Beal's Buried Seeds — Results of All Germination Tests to Date. Plus Sign Indicates Germination

Name of species tested	5th yr. 1884	10th yr. 1889	15th yr. 1894	20th yr. 1899	25th yr. 1904	30th yr. 1909	35th yr. 1914	40th yr. 1920	50th yr. 1930	60th yr. 1940
Amaranthus retroflexus	+	+	+	+	+	+	0	+	0	0
Ambrosia elatior	0	0	0	0	0	0	0	+	0	0
Brassica nigra	0	+	+	+	+	+	+	+	+	0
Bromus secalinus	0	0	0	0	0	0	0	0	0	0
Bursa Bursa-pastoris	+	0	+	+	+	+	+	0	0	0
Erechtites hieracifolia	0	0	0	0	0	0	0	0	0	0
Chamaesyce maculata	0	0	0	0	0	0	0	0	0	0
Lepidium virginicum	+	+	+	+	+	+	+	+	0	0
Agrostemma Githago	0	0	0	0	0	0	0	0	0	0
Anthemis Cotula	+	+	+	0	+	0	?	0	0	0
Malva rotundifolia	+	0	0	+	0	0	0	0	0	0
Oenothera biennis	+	+	+	+	+	+	0	+	+	+
Plantago major	0	0	+	0	0	0	0	+	0	0
Polygonum Hydropiper	0	+	+	+	+	?	0	0	+	0
Portulaca oleracea	0	+	+	+	+	0	0	+	0	0
Rumex crispus	+	?	+	+	+	+	+	+	+	+
Chaetochloa lutescens	+	+	+	0	+	+	0	0	0	0
Alsine media	+	+	+	+	+	+	0	0	0	0
Trifolium repens	0	0	0	0	0	0	0	0	0	0
Verbascum Thapsus	+	?	+	+	0	0	+	0	0	0
Verbascum Blattaria									+	+

extend over a period of 100 years. So many seeds were still alive at the 40-year period that it was decided to test the other 12 samples at 10-year periods, thus extending the experiment over a total period of 160 years.

Table 3 gives the results of the tests for periods up to the sixtieth year. The plus signs in the table show the kinds of seeds that still germinated [18] at the various periods. The seeds still germinating after 40, 50, and 60 years' burial along with the percentage for each and the percentage of all those buried that still germinated are given in Tables 4, 5, and 6, respectively.

Table 4. Seeds Alive after 40 Years' Burial

Name of plant	Number of individuals	Percentage of germination
Brassica nigra	9	18
Oenothera biennis	19	38
Rumex crispus	9	18
Portulaca oleracea	1	2
Plantago major	5	10
Amaranthus retroflexus	1	2
Amaranthus graecizans	33	66
Lepidium virginicum	1	2
Ambrosia elatior	2	4

Percentage of all seeds buried still germinating: 8.2%.

Table 5. Seeds Alive after 50 Years' Burial

Name of plant	Number of individuals	Percentage of germination
Rumex crispus	26	52
Oenothera biennis	19	38
Verbascum Blattaria	31	62
Brassica nigra	4	8
Polygonum Hydropiper	2	4

Percentage of all seeds buried still germinating: 8.2%.

Table 6. Seeds Alive after 60 Years' Burial

Name of plant	Number of individuals		Percentage of germination	
	1930	1940	1930	1940
Rumex crispus	26	2	52	4
Oenothera biennis	19	12	38	24
Verbascum Blattaria	31	34	62	68

Percentage of all seeds buried still germinating: 4.8%.

It will be noted that nine of the 20 kinds of seeds buried were still germinating at the 40-year period, five at the 50-year period, and three at the 60-year period, and that 8.2 per cent of the seeds were still alive at the first two periods and 4.8 per cent at the 60-year period. Darlington [18] states that *Verbascum Blattaria* seeds were evidently placed in the bottles tested for the 50- and 60-year periods instead of *V. Thapsus*, which appeared in the earlier records.

Not a single species of seed germinated at every test. This may be explained either on the basis that certain seeds kept better in some bottles than in others, or perhaps the germination conditions used in the various tests were not equally effective in forcing those that were dormant to germinate. The following did not give any germination for any of the periods: *Bromus secalinus*, *Erechtites hieracifolia*, *Chamaesyce maculata*, *Agrostemma Githago*, and *Trifolium repens*. Either these seeds do not live five years in the soil or the germination conditions used were not suitable for overcoming the dormancy. In the case of *Trifolium repens* the latter may be the explanation, for after 20 years of burial in the U.S.D.A. experiments, some hard viable seeds were recovered. Another possible explanation is that Beal may have buried mainly soft seeds. Considering all the seeds that lived in the soil in Beal's experiment for 40, 50, or 60 years, the following families are represented: Onagraceae, Portulacaceae, Plantaginaceae, Amaranthaceae, Cruciferae, Compositae, and Scrophulariaceae. All these families are represented in the U.S.D.A. list of seeds that lived 20 years, except Amaranthaceae. Every genus that showed live seeds in Beal's list after 40 or 50 years' burial also showed live seeds in the U.S.D.A. list after 20 years' burial, with the exception of *Amaranthus*, which did not survive in the latter's record, and *Lepidium* which was not represented. Also the long life span species, so far as they appear in both records, agree closely.

The size of the seed is evidently of no significance in determining life span in the soil. *Nelumbo* seeds, which have the longest record for life duration in the soil, are rather large, as are *Robinia* seeds. On the other hand, many of the seeds that survive well in the soil are small: tobacco, celery, timothy, evening primrose, mullein, and others. Depth of burial, at least within the range used by the U.S.D.A. experiments, also has no effect on life span.

Leguminous seeds and others that will not absorb water are ideally adapted for long life in the cool soil; the hard coats assure dormancy and the low water content prevents waste of stored foods by respiration. Many of the seeds with long life span in the soil are not hard; these are maintained in the dormant condition in the soil by lack of light, as in the case of celery and tobacco seeds, or by seed coat or embryo characters. It is harder to explain, however, why the stored foods are not soon exhausted by respiration. This is especially true in the light of measurements of the initial respiration shown by dormant seeds that absorb considerable water.

Sherman [17] found that dormant imbibed *Amaranthus retroflexus* seeds have an initial rate of respiration which, if maintained, would exhaust all the stored starch (47 per cent of the dry weight) within 160 days at 20° to 25° C (68° to 77° F). On this basis, even at 10° C (50° F), the stored starch would last no more than a year. In spite of this, Beal's experiments show that *Amaranthus retroflexus* seeds remain alive in the soil for 40 years. Atwood [17] found that dormant seeds of *Avena fatua*, which had lain dormant in the germinator for a few days, showed great reduction in oxygen consumption.

Denny [23] finds that gladiolus corms can be kept in moist soil in the dormant fresh condition for much more than a year if, immediately after digging, they are placed in soil at 20° to 27° C (68° to 80° F) and kept at that temperature. In this condition they have very low respiration, using up less than 20 per cent of their sugars and starches in a year. This leaves out of consideration other stored foods, such as glucosides. When they are removed from the soil the respiration intensity rises 5- to 30-fold or more in a few hours, then gradually falls back to the original rate. The curtailed respiration in the dormant corms [22] would make the life duration in this condition perhaps five years, so far as food is concerned. Other factors, however, limit the life under the conditions mentioned above to two years or less. Barton [10a] finds that dormant seeds of *Amaranthus retroflexus* in a germinator at 20° C (68° F) show a gradual fall in respiration with lapse of time. After 125 days in the germinator the CO_2 output has fallen to about one-sixth the original rate. Evidently imbibed dormant plant organs in nature have a means of conserving the food by curtailing respiration. The mechanism of this curtailment is no doubt very complex and the details of it are not known. The curtailment of respiration must be enormous, however, in a case like *Amaranthus* seeds, in order to conserve a year's food supply so that it cares for the needs of the embryo at least for 40 years. Poor oxygen supply in compact soil may also lower respiration. There is need for a detailed study of the course of change in the respiratory intensity of such seeds as they lie in the soil, as well as the mechanism by which the respiratory intensity is reduced.

Ewart,[27] Becquerel,[12] and others have assumed that long life span in seeds is necessarily connected with hard-coatedness. The buried-seed work of Beal and the U.S.D.A. brings to light the fact that many seeds without hard coats have long life span in the soil, and it is probable that many retain their vitality much longer in the soil in the imbibed condition than in ordinary dry storage in the air.

Table 7 was assembled by selecting seeds of species that show long life span in the soil, as reported by Beal and the U.S.D.A., and that at the same time appear with a number of records for life span in ordinary dry storage in Ewart's assembled tables. In some cases Ewart's tables do not show adequate records on the particular species of a genus with which Beal and the U.S.D.A. worked, but do give many records for other species

of the same genus. In such cases, column 2 shows the number of species of the genus reported by Ewart and the maximum longevity for any species of the genus.

Table 7. Comparative Life Span of Certain Seeds in Dry Storage and in Soil

Species of seeds	Life span in dry storage according to Ewart	Life span in soil and per cent germination according to Beal and U.S.D.A.
Amaranthus graecizans	7 spp., less than 15 yrs.	Beal, 40 yrs., 66%
Apium graveolens	Less than 10 yrs.	U.S.D.A., 20 yrs., 10.5%
Chenopodium album	Less than 20 yrs.	U.S.D.A., 20 yrs., 65.5%
Datura Stramonium	Less than 15 yrs.	U.S.D.A., 20 yrs., 78%
Nicotiana tabacum	6 spp., less than 10 yrs.	U.S.D.A., 20 yrs., 56%
Oenothera biennis	Less than 15 yrs.	Beal, 50 yrs., 38%; U.S.D.A., 20 yrs., 87.5%
Plantago major	Less than 10 yrs.	Beal, 40 yrs., 10%; U.S.D.A., 20 yrs., 83.5%
Poa pratensis	5 spp., less than 12 yrs.	U.S.D.A., 20 yrs., 18.5%
Polygonum Hydropiper *Polygonum persicaria*	15 spp., less than 15 yrs.	Beal, 50 yrs., 4% U.S.D.A., 20 yrs., 55%
Portulaca oleracea	Less than 15 yrs.	Beal, 40 yrs., 2%; U.S.D.A., 20 yrs., 38%
Verbascum Blattaria	Less than 15 yrs.	Beal, 50 yrs., 62%
Verbascum Thapsus	Less than 15 yrs.	U.S.D.A., 20 yrs., 92.5%

While Ewart's data on longevity in dry storage cannot be considered adequate to make the claim certain, still the evidence indicates strongly that the seeds reported in Table 7 have much longer life span in the soil than in ordinary dry storage. The life span of grains of timothy in the U.S.D.A. buried-seed tests exceeds the records in dry storage, and much other similar evidence could be cited to show that some seeds live longer in moist soil than in ordinary air storage. Recent work by Kjaer [40] confirms this conclusion. In five-year tests he found that the seeds of the following retained their vitality in soil better than in dry storage: *Polygonum tomentosum*, 20 vs. 0 per cent; *Thlaspi arvense*, 87 vs. 1 per cent; *Vicia hirsuta*, 50 vs. 5 per cent; *Daucus carota*, 43 vs. 10 per cent; *Plantago major*, 30 vs. 0 per cent; *Cirsium arvense*, 55 vs. 0 per cent. He reports Dorph-Petersen's results with seeds of *Sinapis arvensis:* buried 10 years, 87 per cent germination; dry-stored, 21 per cent; buried 18 years, 17 per cent; dry-stored 0 per cent. Avery and Blakeslee [2] state that *Datura* seeds stored in the laboratory are all dead after nine or ten years, but *Datura* seeds buried in the soil outside (U.S.D.A. buried seeds) still show 97.5 per cent germination after 39 years.

The fact that seeds of many wild plants remain in the soil for long periods in a dormant viable condition means that the soil is always well stocked with seeds which are capable of germination when the soil is disturbed. This assures the persistence of the species. It also makes the task of the farmer and gardener in fighting weeds difficult, for when the soil is once

well stocked with seeds it takes years of cultivation for the complete germination and final destruction of the weeds.

While the old imbibed seeds in the soil are of necessity dormant — or else they would have germinated — the dormancy in the main is due to a rather delicate equilibrium that is overcome by exposure to light, by fluctuating temperatures of the top soil, or even by mechanical disturbance or better oxygen supply. Disturbing dormant weed seeds in soil starts them germinating.[30]

Barton[10a] finds that seeds of *Amaranthus retroflexus* that have remained dormant in a germinator at 20° C (68° F) for more than a year can be induced to germinate quickly by several different treatments: raising the temperature to 35° C (95° F) gives prompt, complete germination; rubbing them in the palm of the hand with the finger for three minutes and placing them back at the same temperature induced 83 per cent germination; partial desiccation and fluctuating temperatures are also effective. Denny [23] finds that dormant corms of gladiolus that have remained in moist soil at room temperature for a year or more can be thrown out of their dormancy and caused to grow by only a few hours of exposure to 5° C (41° F), whereas freshly harvested corms require several weeks at 5° C (41° F) to after-ripen.

Dormancy and Delayed Germination in Seeds of Wild Plants

In connection with the statements about buried seeds it has been mentioned that seeds of wild plants are much more commonly and more persistently dormant than those of cultivated plants, and consequently their germination is much more delayed. There are many records of long-delayed germination in seeds of wild plants. Nobbe and Hänlein [46] placed 400 seeds each of 31 species of wild plants, mainly weeds, in Petri dishes as germinators at room temperature and kept records on the germination for 1173 days. All the species of seeds used in this experiment were said to be soft-coated, that is, absorbed water. Table 8 shows the number of germinations of the different kinds of seeds after various numbers of days in the germinator, also the total germination and per cent germination for each after 1173 days. It will be noted that there was no germination, also no decay, of the *Phyteuma spicatum, Primula elatior,* and *Verbascum nigrum* seeds. Lack of decay indicates that the seeds were alive, and the failure to germinate indicates that room temperature in the germinator failed either to furnish the conditions for after-ripening or for germination. The evidence in the next chapter will indicate that it was the former. Five other sorts of seeds gave less than 1 per cent germination; likewise, these showed no decay, and the low germination is undoubtedly explained on the same basis as the three mentioned above. In several of the seeds that showed moderate or considerable germination, the germination is

Table 8. After Nobbe and Hänlein; 400 Seeds of Each Species

Species	No. of seeds germinated after days																Total	Per cent
	4	5	6	7	8	9	10	16	36	72	145	351	519	714	874	1082	1173		
1. *Aquilegia vulgaris*	—	—	—	—	—	—	—	3	—	—	—	—	—	—	—	—	—	3	0.75
2. *Campanula rotundifolia*	—	—	—	11	8	1	1	1	1	—	—	—	11	13	10	1	—	58	14.50
3. *Campanula persicifolia*	—	—	—	—	3	8	14	112	56	1	—	11	9	2	8	2	6	232	58.00
4. *Campanula Trachelium*	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	—	1	0.25
5. *Chaerophyllum temulum*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	3	3	0.75
6. *Chenopodium album*	—	—	2	—	—	2	3	2	—	1	19	53	27	—	2	3	3	117	29.25
7. *Capsella Bursa-pastoris*	—	3	—	3	—	—	—	—	—	—	4	4	20	—	24	10	7	75	18.75
8. *Chelidonium majus*	—	—	—	—	—	—	—	—	59	8	2	—	—	3	90	1	32	195	48.75
9. *Digitalis purpurea*	—	—	—	9	45	102	47	159	24	1	—	—	—	—	rest decayed			387	96.75
10. *Hypericum montanum*	—	—	—	3	19	61	31	171	46	—	—	—	—	1	—	—	—	332	83.00
11. *Hypericum hirsutum*	—	—	—	2	1	4	13	132	58	—	—	2	—	—	—	—	—	212	53.00
12. *Hypericum perforatum*	—	—	—	1	—	—	2	20	28	2	—	—	—	1	3	1	—	58	14.50
13. *Jasione montana*	—	—	17	98	141	81	30	28	2	—	—	—	—	—	rest decayed			397	99.25
14. *Lithospermum arvense*	2	12	32	36	52	18	8	52	42	28	22	12	22	6	rest decayed			344	86.00
15. *Lysimachia vulgaris*	—	—	—	—	—	—	—	—	—	—	—	—	—	1	—	—	—	1	0.25
16. *Myosurus minimus*	—	—	16	160	103	19	2	5	—	1	—	11	11	4	13	—	2	347	86.75
17. *Oxalis corniculata*	—	—	—	—	—	—	—	3	5	1	—	1	—	—	2	—	—	12	3.00
18. *Papaver Argemone*	—	—	5	65	116	16	8	34	30	15	4	26	17	—	—	—	—	336	84.00
19. *Papaver dubium*	—	—	2	216	169	—	—	1	—	—	—	—	—	—	—	—	—	388	97.00
20. *Phyteuma spicatum*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	0.00
21. *Plantago major*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	1	1	0.25
22. *Plantago media*	—	5	8	4	4	2	1	—	—	—	—	1	—	9	1	6	2	43	10.75
23. *Polygonum persicaria*	—	3	33	15	2	—	—	2	—	—	—	—	—	—	—	—	—	55	13.75
24. *Primula elatior*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	0.00
25. *Potentilla argentea*	—	—	3	15	57	67	58	74	7	—	—	2	3	3	3	2	7	301	75.25
26. *Scrophularia nodosa*	—	—	1	5	27	96	42	47	6	—	—	—	—	—	7	—	—	231	57.75
27. *Thlaspi alpestre*	—	—	—	—	—	—	—	—	—	—	—	—	all decayed			—	—	0	0.00
28. *Thlaspi arvense*	—	—	—	—	1	—	—	2	—	—	—	2	10	11	11	15	35	87	21.75
29. *Verbascum nigrum*	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	0	0.00
30. *Veronica Beccabunga*	—	—	—	—	—	—	—	1	1	1	6	25	42	103	—	4	—	183	45.75
31. *Veronica officinalis*	—	—	—	—	—	8	56	332	—	—	—	—	—	—	rest decayed			396	99.00

distributed over much of the 1173-day period: *Campanula rotundifolia, C. persicifolia, Chenopodium album, Capsella Bursa-pastoris,* and several others. Delayed and time-distributed germination of seeds of wild plants is striking in the results reported in this table. Nobbe and Hänlein used room temperature with relatively little fluctuation in temperature. In the soil out-of-doors in Germany there would be much greater fluctuation in temperature during more than three years of the experiment.

Table 9. Germination Experiments with Seeds of Various Wild-Growing Plants (From Dorph-Petersen)

Species	Seed harvested	Put into germinator	Per cent germination after years												
			1	2	3	4	5	6	7	8	9	10	11	12	Total
Stellaria nemorum	July 5 '97	Aug. 16 '97	1	2	34	19	3	26	2	—	—	—	—		87
Sisymbrium sophia	Sept. 20 '98	Oct. 8 '98	0	0	54	15	—	—	—	—	—	—	—		69
Sisymbrium officinale	Aug. 24 '96	Sept. 11 '96	6	15	47	23	2	—	—	—	—	—	—		93
" "	Sept. 20 '98	Oct. 8 '98	14	32	2	3	39	1	—	—	—	—	—		91
Thlaspi arvense	July 27 '96	Aug. 22 '96	1	0	9	7	1	0	29	36	4	—	—		87
" "	Aug. 28 '97	Sept. 1 '97	73	4	1	0	16	—	—	—	—	—	—		94
" "	Aug. '04	Sept. 9 '05	96	—	—	—	—	—	—	—	—	—	—		96
Sinapis arvensis	Aug. 11 '99	Sept. 2 '99	12	11	15	18	26	3	1	—	—	—	—		86
" "	Sept. 21 '03	Oct. 5 '03	35	12	10	22	1								80
Geranium molle	July 31 '96	Aug. 22 '97	35	11	16	13	11	2	4	0	1	—	—	—	93
Malva vulgaris	Aug. 24 '96	Sept. 11 '96	29	10	5	7	3	2	10	22	3	2	0	1	94
Cynoglossum officinale	Aug. 10 '99	Sept. 4 '99	0	0	0	0	52	10	13	7	—	—	—		82
Ballota nigra	Aug. 8 '96	Aug. 27 '96	15	20	2	12	12	3	9	—	—	—	—		73

Dorph-Petersen [24] carried on similar experiments with weed seeds at Copenhagen, using the Jacobsen germinator, but ran them in duplicate for a period of 11 years. One set of germinators was kept in the laboratory and the other on an open porch without heat. In one set of these experiments he got much earlier and much higher percentage of germination in the outdoor germinators for *Anthriscus silvestris, Primula elatior,* and *Galeopsis tetrahit,* and somewhat earlier and higher for *Potamogeton natans,* while *Datura stramonium* gave 99 per cent germination after eight years (98 per cent during the eighth year) inside and only 2 per cent after 11 years outside. In another set of experiments he showed that in the germinator on the porch with the wide fluctuations in temperature, there was marked delay and distribution in the germination of weed seeds (Table 9).

Why Do Seeds Remain Dormant in a Germinator or in the Soil?

Considering both the indoor and outdoor germinators, Dorph-Petersen had a range of temperatures that cared for one of the very important factors in the after-ripening and germination of dormant wild seeds. As we shall see in later chapters, many seeds require a period in a germina-

tion medium just above freezing in order to after-ripen the seeds preparatory to germination. Some seeds require low temperatures for germination, while others germinate only at high temperatures, and finally others germinate well if the temperature fluctuates rather widely from day to night. There are other factors active in the soil that might either stimulate the germination of dormant seeds or prolong their dormancy. Nitrates and other nitrogen compounds generally present in the soil promote the germination of certain seeds. This is especially true of many of the light-stimulated seeds.[16] Deep burial in the soil excludes light. Light-stimulated seeds, such as celery, tobacco, and timothy, have been mentioned above as remaining in the soil for several years in the dormant condition.

Why do seeds of so many wild plants lie in a germinator or in the soil in conditions that are favorable for germination of seeds of our cultural plants and fail to germinate even after years? The answer to this question is very complex and involved. The next chapter, which deals in large part with types of dormancy in seeds, will throw much light upon it. At this point we shall discuss only two factors that may play a part in keeping seeds of wild plants dormant in the soil: (1) inhibiting chemicals in the fruits, seed coats, or seeds themselves, and (2) unfavorable germination conditions in the soil that throw seeds into secondary dormancy.

Many investigations [14, 26, 28, 48, 52, 53, 58, 60] show that fleshy and dry fruits, seed coats, and even the living parts of seeds contain chemicals which inhibit germination of seeds. For instance, the fleshy fruits of the tomato, cucumber, pawpaw (*Carica papaya*), and many other plants contain chemicals that inhibit the germination of the seeds while they are within the fruits, and this without any permanent injury to the seeds. The woody material of the seed balls of beets and the coats of lettuce seeds contain chemicals that inhibit germination. This becomes very noticeable if batches of either are germinated repeatedly on the same moist filter papers so that the inhibiting chemicals become concentrated. The embryos and endosperms of some seeds also contain inhibiting chemicals. Mostly these chemicals are not specific in their action but inhibit other species of seeds as well as the species in which the chemicals develop. In some cases the chemicals are more effective as inhibitors on seeds of other species than on those of the species producing the chemicals. Some of these chemicals are volatile and others are not. They are mostly heat-stable, although some workers claimed to find inhibitors that are destroyed at the boiling point of water. Some of the substances that have been found in fruits and seeds that act as germination inhibitors are ammonia, hydrocyanic acid (from amygdalin of rosaceous seeds), essential oils, alkaloids, glycosides, and an unidentified substance, "Blastokolin." [41] These inhibitors are more effective in ordinary germinators than in the soil, for the soil moisture allows them to diffuse away from the seeds; also soil, like animal charcoal, adsorbs the inhibitors and removes them from action on the seeds.

For such inhibiting chemicals to be effective in holding seeds dormant in the soil for years, two conditions will have to prevail: (1) the inhibiting chemicals must be very stable in the soaked-up seeds, and (2) they must be prevented from diffusing out of the seeds by semipermeable membranes of either the living protoplasm or of the non-living seed coats. Both these conditions may be met by alkaloids and glycosides. On the whole, however, much more investigation will be needed before we can attribute to inhibiting chemicals an important role in holding many seeds of wild plants dormant in the soil for years. We have learned already that many of them do stay dormant for years in the soil in the imbibed condition.

Secondary dormancy. It has long been known that unfavorable germination conditions often throw seeds into dormancy so they will not germinate when shifted to a favorable condition. Kinzel [16] showed that seeds of *Nigella sativa*, which are prevented from germinating by light, if placed in an illuminated germinator, soon change so they will not later germinate in darkness. They become "light-hard." This holds for other light-inhibited seeds. Many seeds that need light for germination when placed in a dark germinator become "dark-hard," so they will not germinate later in light. Kidd [38] has shown that high partial pressures of carbon dioxide, 24 per cent more or less, will inhibit germination of certain seeds and in time throw them into dormancy. Reduced pressures of oxygen render the carbon dioxide effective in lower concentrations. There are many other cases of bad conditions in a germinator that throw seeds into dormancy. Most of these conditions are active in the soil and may, in part, account for the long rest period of seeds in nature. In 1916 the author [15] spoke of dormancy in seeds induced by bad conditions in a germinator as "secondary dormancy."

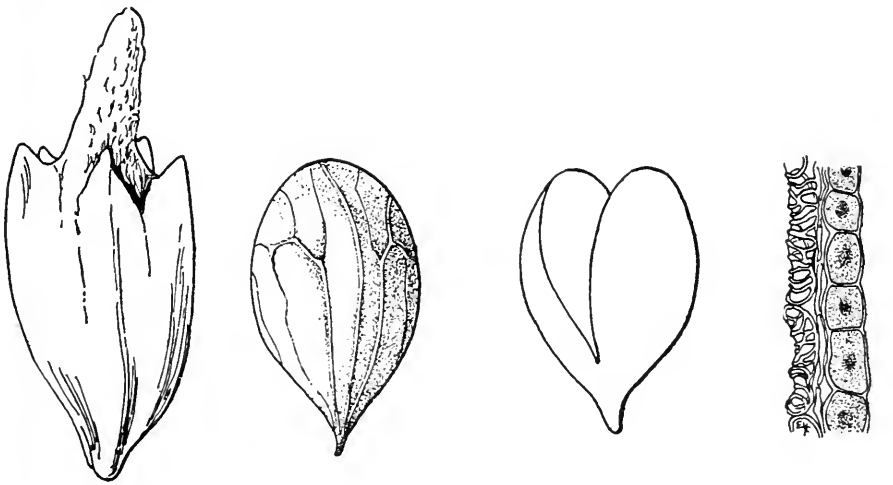

FIGURE 12. *Ambrosia trifida* fruits and seeds: A, the fruit; B, the seed; C, the embryo; D, section of the two-layered seed coat.

The most thorough study in inducing dormancy in seeds is that of W. E. Davis [19, 20] on seeds of *Ambrosia trifida* and *Xanthium*. This was a joint contribution of this Institute and the Department of Botany and Plant Pathology of Kansas State Agricultural College. *Ambrosia* seeds

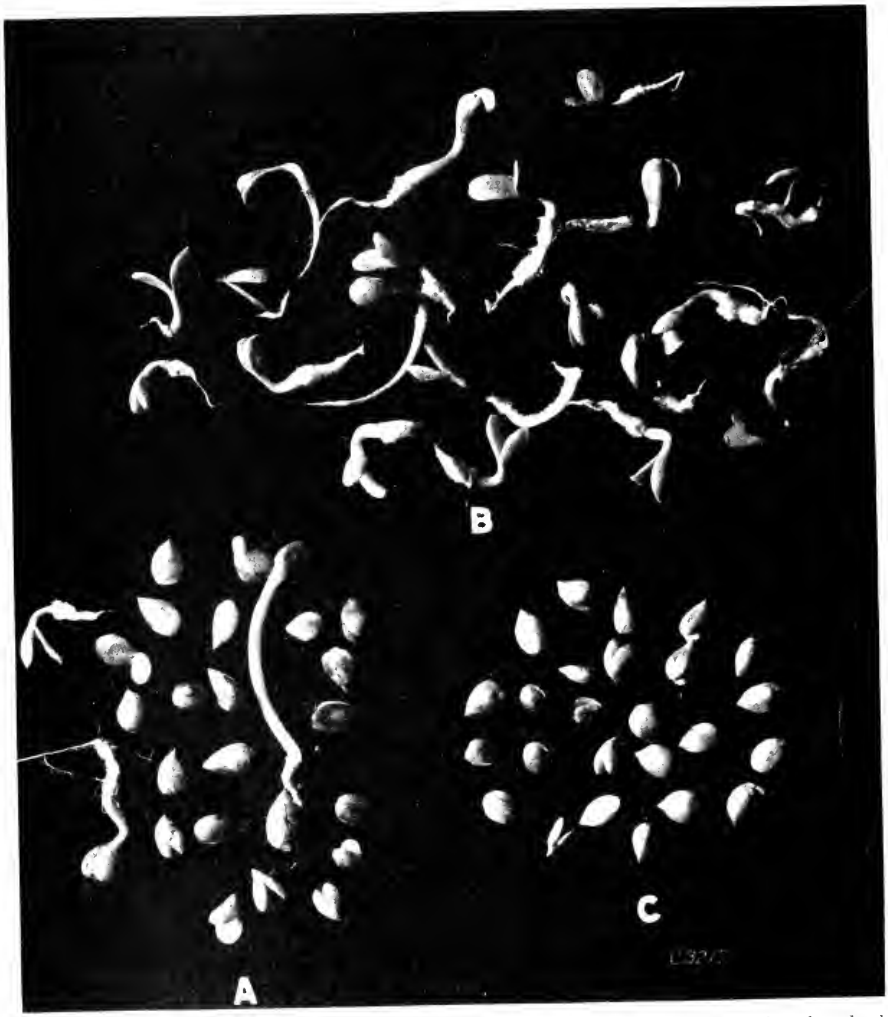

FIGURE 13. Germination of embryos of *Ambrosia trifida* taken from fruits that had been stored for three months as follows: A, dry; B, in a germinator in a refrigerator; and C, in a germinator at 27° to 30° C (80° to 86° F).

consist of an embryo covered with a paper-thin transparent seed coat. The seed is enclosed in a thick, woody, indehiscent fruit coat (Fig. 12). In controlling germination and dormancy the seed coat and not the fruit coat is important. *Ambrosia* seeds (embryos) are dormant and they afterripen slowly and incompletely after several months of dry storage and

completely after three months in a germinator at 1° to 10° C (34° to 50° F). After the seeds are after-ripened they will germinate slowly but nearly completely in a germinator at 20° C (68° F) and more rapidly but more sparingly in a germinator at 30° C (86° F). At the higher temperature many of the seeds go back into the dormant condition. The secondary dormancy is induced by the seed coats restricting the oxygen supply to the embryo in a germinator at a high temperature.

Fig. 12 pictures the fruit, seed, embryo, and seed coat structure of *Ambrosia*. Fig. 13 shows the behavior of isolated embryos of *Ambrosia* when

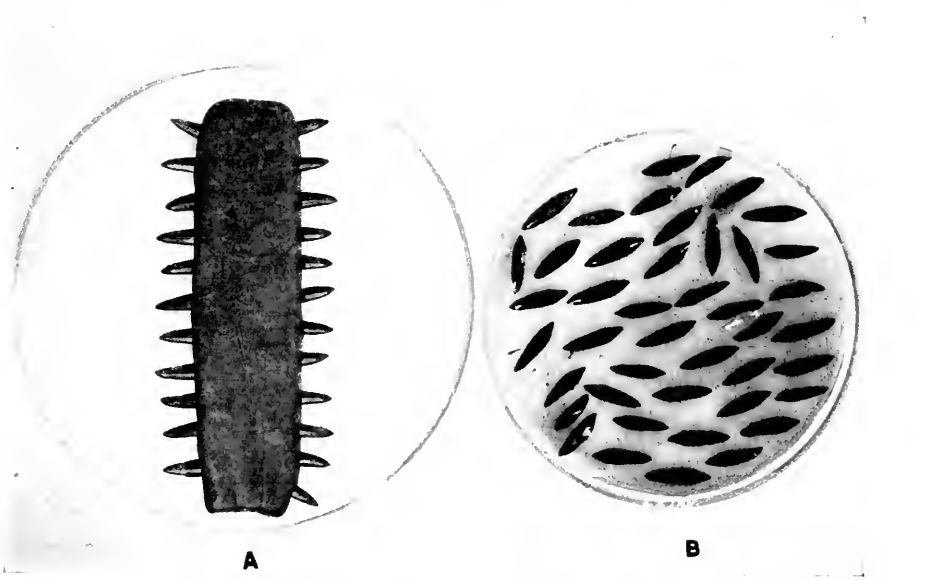

FIGURE 14. A, seeds of cocklebur in clay in a high temperature germinator to produce dormancy; B, imbedded in agar for the same purpose.

placed in a germinator for three to four days at room temperature after three months' storage of fruits in three different conditions. Note that the embryo of intact fruits in a germinator at low temperature, 1° to 10° C (34° to 50° F), after-ripened so that they germinated promptly when the fruit and seed coats were removed and the embryos were placed in a germinator at room temperature. Those which had been kept in a germinator at a low temperature gave prompt, complete germination; those in dry storage a small percentage germination; and those in a high-temperature germinator no germination. The embryos of the latter were in deep dormancy.

The embryos of the cocklebur [20] are also enclosed in very thin seed coats that interfere with oxygen diffusion to the embryos. They differ from *Ambrosia* in three ways: the involucral fruit coat (bur) encloses two seeds, an upper and lower; the embryos are not dormant in the mature seeds, but the thin seed coats hinder germination by limiting oxygen supply to the embryos at certain temperatures, that is, below 20° C (68° F) for lower

seeds and below 30° C (86° F) for upper seeds. We shall describe more fully the behavior of the two cocklebur seeds in the next chapter. To throw the embryos of cockleburs into the dormant condition, the intact seeds were placed in a germinator at a high temperature, 28° C (82° F) or above, with greatly reduced oxygen pressure. The reduced oxygen pressure can be obtained by displacing some of the oxygen in the air with a gas such as nitrogen or hydrogen, or by imbedding the whole seed in agar jelly or the radical end in modelling clay in a germinator as shown in Fig. 14. With the intact seeds in a high-temperature germinator at reduced oxygen pressure the embryos become dormant in from two to several months. Thornton [56] confirms Davis' work on the cocklebur. The dormant seeds can be thrown out of dormancy by placing the seeds in a germinator at 5° C (41° F). While the intact seed in secondary dormancy will not germinate, the embryo can be made to germinate if the seed coat is removed, but the growth is very slow and sluggish and the seedling is dwarfish with abnormal leaves. Fig. 15 shows that the non-dormant embryo gives a much bigger plant after 14 days' growth than the embryo from the dormant seed gives after 32 days' growth. Likewise, the non-dormant embryo gives much more growth after 21 days of growth than the embryo from the dormant seed gives after 47 days. As we shall see later, so-called dormant embryos are not generally incapable of growth if the coats are removed, but they have so little vigor of growth that they cannot overcome the slight resistance of even so thin a coat as the cocklebur seed bears. In general, also, dormant embryos, if forced to grow, produce dwarfish plants so far as the above-ground portion of the plant is concerned. The root system is not dwarfish but is large in comparison with the top. In the next chapter we shall have much more to say about the effect of oxygen pressure on germination and dormancy induction, about the importance of seed and fruit coats in dormancy of seeds, and about dormant embryos.

Davis was able to throw seeds (embryos) into and out of dormancy repeatedly and at will. Nature induces dormancy in embryos of some species of plants when they mature. Many seeds after-ripen in nature in the soil during the cool weather of fall, winter, and spring. If Davis and nature use the same methods in inducing and overcoming embryo dormancy, embryos in nature are thrown into dormancy as the seeds mature because of the relatively high temperature during maturing and because of limited oxygen supply to the embryo caused by seed coats and other tissues about embryos. As we shall see in the next chapter, many seeds in the temperate zone after-ripen in soil at temperatures slightly above freezing. Do many seeds in the soil after-ripen in nature in the cool weather of fall, winter, and spring, which gives an abundance of germination in early spring? Does the hot weather of summer throw many of the seeds in the soil back into the dormant condition to be after-ripened again the

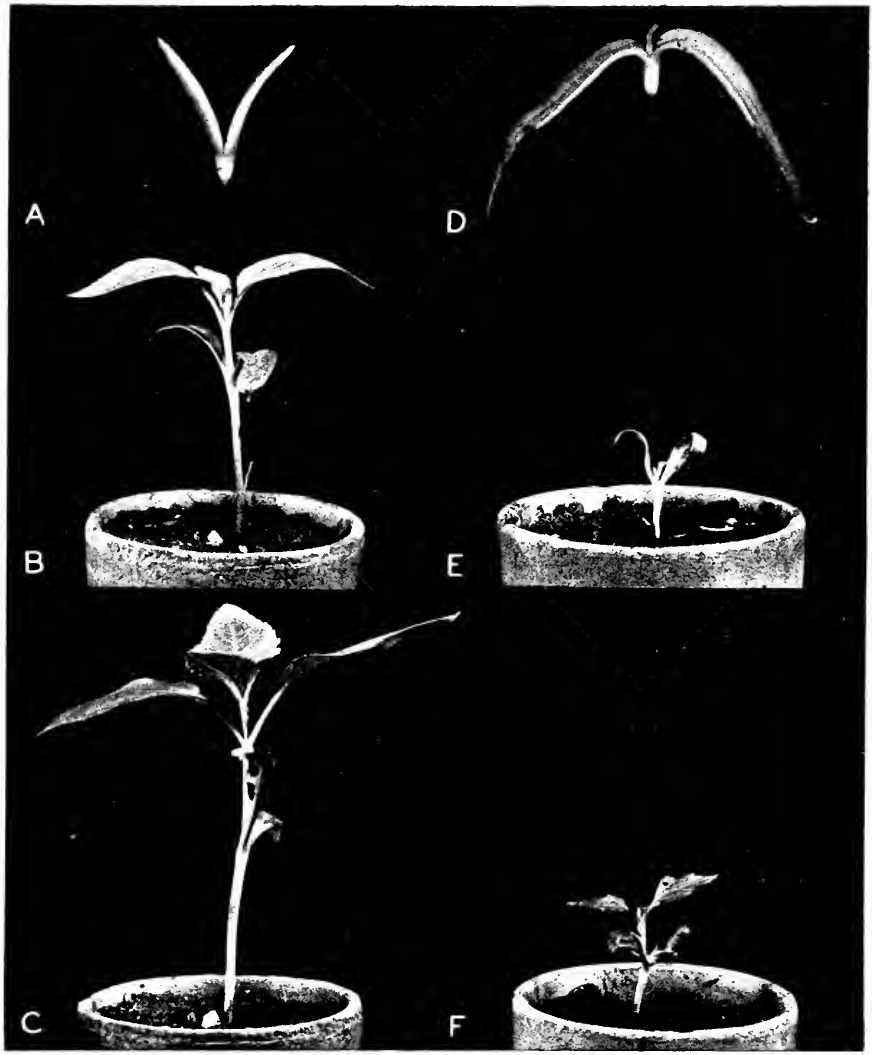

FIGURE 15. Embryos of lower seed of cocklebur. A, Imbibed embryo of non-dormant seed; B, same 14 days after planting; C, same 21 days after planting. D, Embryo of dormant seeds after 18 days in germinator; E, same as D after 18 days in germinator, plus 14 days in soil, or 32 days in germinating conditions; F, same after 18 days in germinator, plus 29 days in soil, or 47 days in germinating conditions.

following winter? In any case, Davis has thrown much light upon the dormancy and after-ripening rhythm of seeds in nature.

HARD SEEDS BEST ADAPTED FOR LONG LIFE SPAN

We have been discussing the life span of soft seeds (seeds that absorb water) in the soil and find that some of them stay alive for 60 years or

more, but the seeds best adapted for long life in the soil are certain hard seeds, *i.e.*, seeds that do not absorb water. This is true only if the hard coats offer great resistance against softening in the soil. The most striking example of this is the old seeds of the East Indian lotus (*Nelumbo nucifera*)

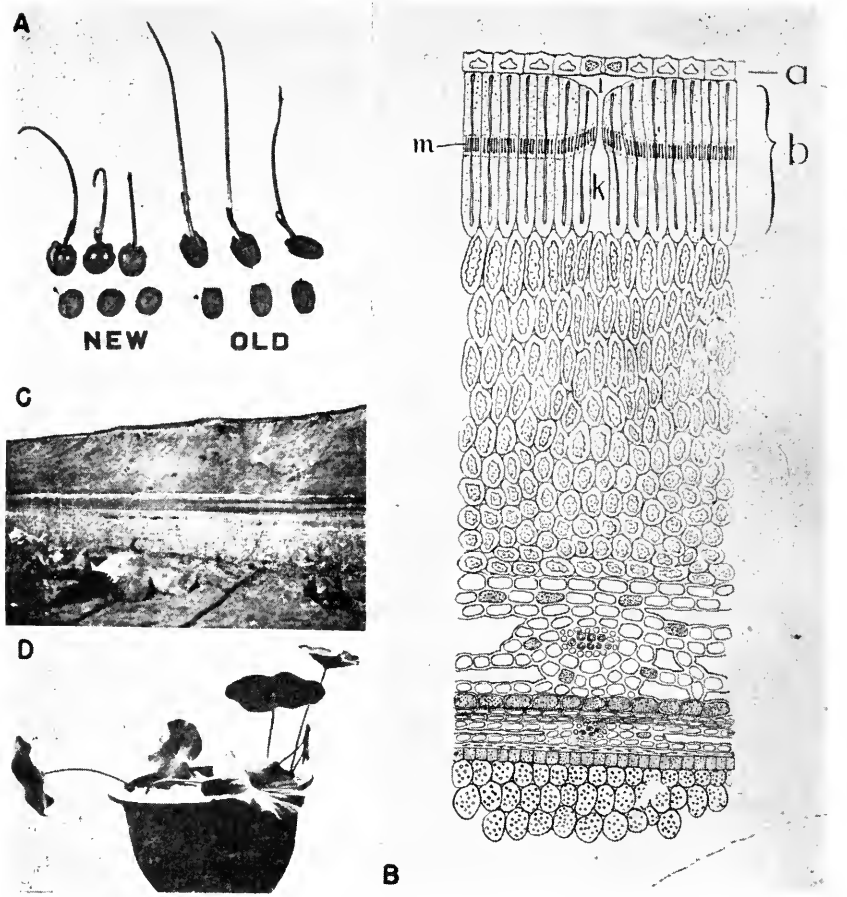

FIGURE 16. A, Relative rate of growth of freshly-harvested and century-old seeds. Coats must be broken to permit water absorption and germination. B, Structure of coats showing water-resistant layers. The water-resistant layers are (*a*) epidermis, and (*b*) outer end of palisade layer, down to (*m*) the "light line." C, 30–60 ft. bank left by the cut of the river. Seeds located in strata 3–6 ft. from top of this precipice. D, Plant grown from one of the old seeds.

that Ohga [47] dug from a naturally drained lake bed in Manchuria. He offers evidence that these seeds, many of which were still hard and 100 per cent viable, had been in the soil for two centuries or more. Fig. 16 shows the vigor with which the old seeds germinate when the coat is made permeable by sulfuric acid treatment, also the several layers of the seed coat, indicating the parts that prevent water absorption. In the old seeds that had been in the soil the epidermis and much of the outer end of the pali-

sade cells had been eaten away by bacteria and fungi during the long period in the soil. No doubt Ohga recovered only a small remnant of the seeds originally buried. During the centuries the bacteria and fungi had eaten the coats of most of them down to the "light line," the seeds had swollen and germinated, and the seedlings were killed due to deep burial. Many leguminous seeds are hard-coated, and no doubt many of them remain alive in the soil for years because of this fact. Goss [29] found seeds of several species of clovers viable after 20 years of burial. Allers [1] claims that hard yellow lupine seeds lay in the soil 40 years unswollen and fully viable.

Nobbe found that some seeds of various species of Papilionaceae, Mimosaceae, Cannaceae, and Ranunculacae remain hard and viable after being soaked in water for years. Different crops of seeds, red clover from various parts of Europe, varied greatly in percentage of seeds remaining hard after 10 days' soaking at room temperature as well as in percentage remaining hard after 12 years of soaking; one sample had 5.33 per cent still hard after 12 years in water and a sample of very small seeds gave 53.33 per cent [45] hard after 37 years of soaking. After 9 years in water,[44] white sweet clover had 48.93 per cent hard, *Vicia cracca* 43.36 per cent, *Laburnum vulgare* 94.5 per cent. Two samples of black locust showed 18.5 per cent and 5.5 per cent hard after 15½ years of soaking. Davis [21] found that some velvet-leaf (*Abutilon Theophrasti*) seeds remained hard after 20 years in water. Kondo [43] found some seeds of *Astragalus sinicus* still hard and alive after soaking in water for 21 years and [42] 5.50 per cent of black locust seeds were hard and alive after soaking 14 years. Rees [49] found that seeds of *Albizzia lophantha* that had been in the soil at least 23 years were still hard and viable. The conditions in the soil with variations in temperature, moisture supply, and presence of organisms would probably decrease the resistance to swelling. Yet it is likely that very hard leguminous seeds such as palo verde,[55] Kentucky coffee tree,[61] and certain Albizzias, Acacias, and Cassias may lie in the soil under favorable conditions hard and viable for as long periods as the East Indian lotus seeds.

The only chance in the main for leguminous seeds to remain in the soil alive for a long time is their failure to swell. Once they swell, the coats in most species split and swell up into massive gelatinous tissue very subject to attack by pectin bacteria [32] and the embryos are left unprotected and subject also to bacterial attack unless good germination conditions prevail. Bier [13] indicates that some yellow lupine seeds bear substances that offer considerable anti-bacterial action. The same thing is true of many other hard seeds. The seeds that lie in the soil viable and swollen for years have coats that do not rupture or otherwise decompose markedly. They furnish good protection to the embryo and endosperm.

In the next chapter, under types of dormancy, we shall have occasion to discuss the anatomical, environmental, and genetical factors that deter-

mine the hardness of leguminous seed coats as well as factors that overcome hardness.

Storage of Seeds

Conditions for storing seeds so that they will maintain their full vitality for a considerable period are not only of academic interest but of great economic importance. Some plants give big crops of seeds only on alternate years, or in some cases only on occasional years, with several years of poor crops between. The latter is true of red pine. Such seeds must be stored so that nurseries may have an ample supply of good seeds to plant every year. No doubt many seeds could be produced more cheaply if a larger acreage were grown only alternate years or even less frequently. Seed producers must plant sufficient acreage to supply the need for the following year even when the season proves poor for production. Consequently, in good cropping years there is a great excess of production over the needs for the following year. We have been asked to determine good storage conditions for *Cinchona* seeds so that the greater part of seeds of various selections and crosses can be held viable until samples are grown and the quinine yield of the resulting plants determined.

We have already discussed seeds that are ordinarily short-lived because they will endure only slight drying in the air. Most seeds will endure complete drying in the air and considerable additional desiccation. What are the best storage conditions for such seeds? We have noticed that the seeds that live longest in seed cupboards and in the soil are of the hard-coated type. Nature may give us a hint at best storage conditions for seeds that stand moderate to considerable drying. Hard coats prevent any exchange of moisture and air between the outside atmosphere and the living parts of the seeds. The hard coats hermetically seal the embryos individually. Becquerel [12] has determined that the percentage of water is low, 2 to 5 per cent, in hard-coated seeds of legumes and of course aerobic respiration is prevented except for oxygen within the coats. The storage of such seeds can be made almost perfect by placing them at low temperatures. Perhaps it might prolong the life of these hard seeds if two impossible changes could be made in them, namely, withdrawal of the last trace of oxygen and most or all of the water from within the coats. The recipe for prolonging the life of seeds that endure drying is to remove as much water from them as possible without injury, and seal them so as to hold the moisture low and constant in absence of oxygen at a low temperature. Let us examine the effects of these three storage factors — moisture content, temperature, and oxygen.

Seeds with soft coats stored in the air fluctuate in water content with the relative humidity of the air. Barton [8] determined the water content of tomato, pine, and lettuce seeds stored open in the laboratory at Yonkers eight different months of the year. Fig. 17 shows the results. During the

winter months in the heated laboratory the water content was low. During the moist, hot weather of summer the water content was high, reaching a maximum in August when the water content was about twice that of the winter months. It is also evident that different kinds of seeds vary considerably in their water content when stored under identical atmospheric conditions. Seeds in which the storage substances are mainly fats absorb

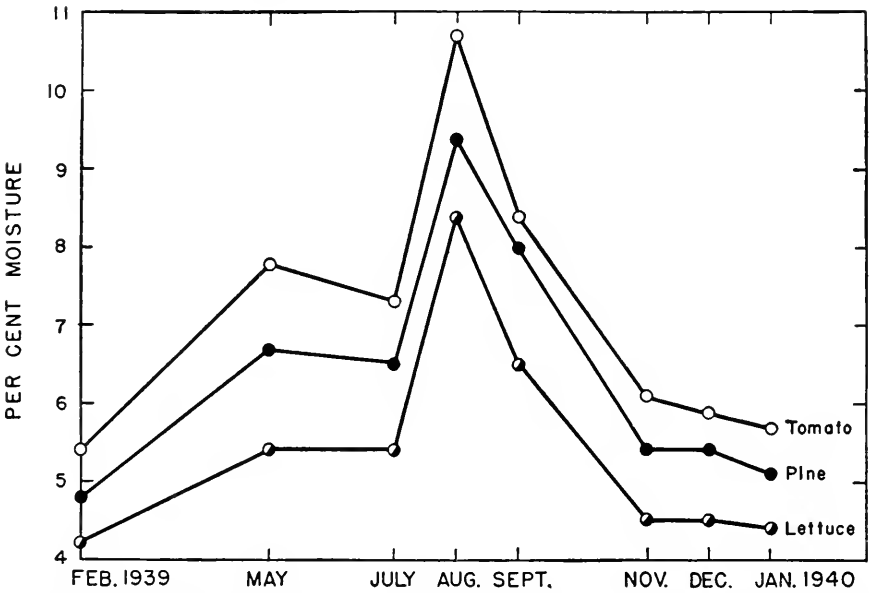

FIGURE 17. Moisture contents at various times of the year of seeds stored open in the laboratory. Moisture expressed as percentage of dry weight of seeds.

ess water than those in which the storage substances are mainly carbohydrates. Peanut and pine seeds are high in fats, tomato seeds low, and flax seeds intermediate. No complete analyses for onion and lettuce seeds are available, but microchemical tests show them rich in fats. None of these six kinds of seeds bear carbohydrates as the main storage material. All store their foods mainly in the form of proteins and fats. The nature and thickness and chemical nature of the coats and other factors modify the amount of water absorbed. The pine seeds absorb a higher percentage of water than lettuce, although both are fatty seeds. Barton [9] has shown not only that is high moisture content injurious to the keeping quality of seeds but that fluctuation in moisture content is also detrimental. Fig. 17 shows the wide variation in water content in open storage. This is avoided by sealed storage or by hard-coated seeds.

Another interesting observation made by Barton [8] is the fact that with different temperatures under the same relative humidities the amount of moisture held by the seeds varies. Fig. 18 shows this variation. This is especially marked at the higher humidities, 76 per cent and 55 per cent,

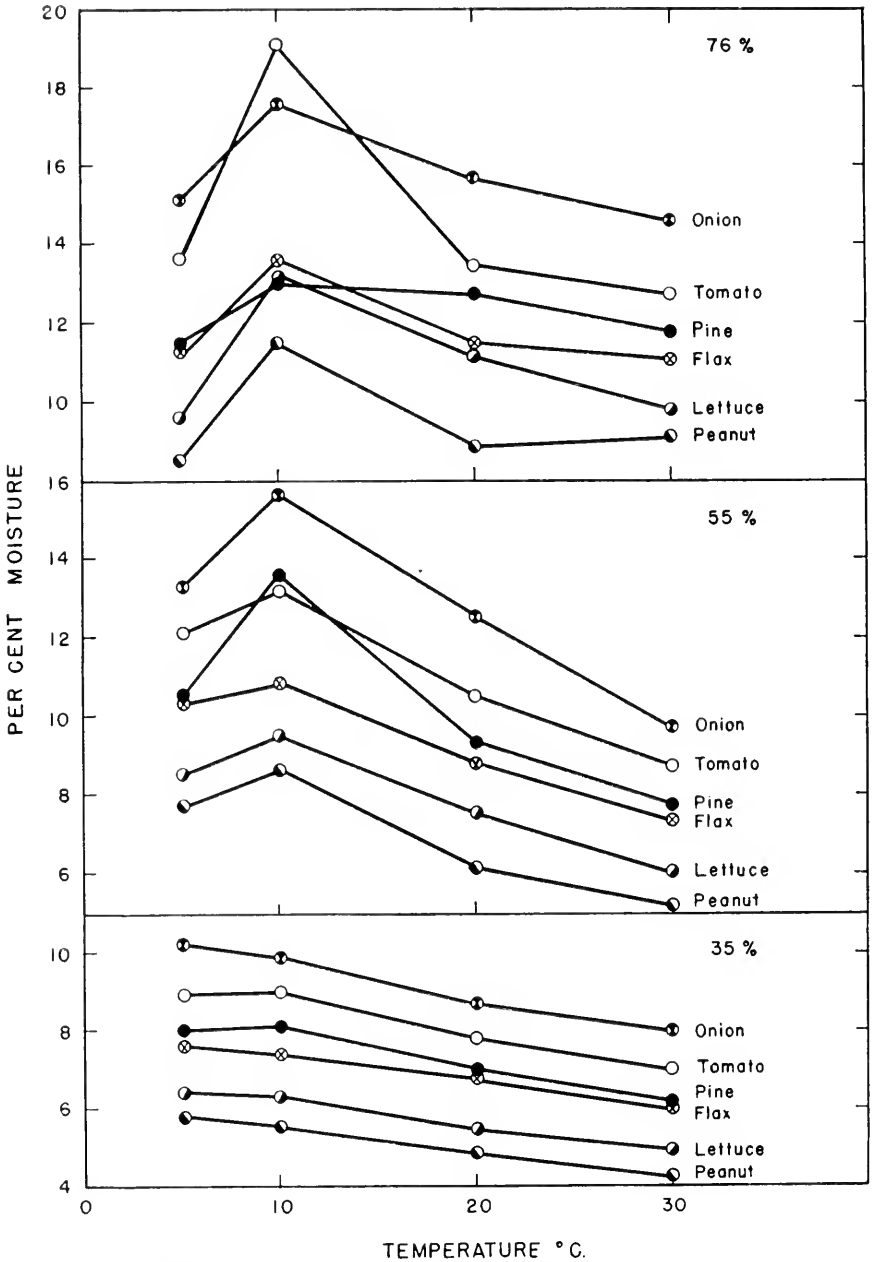

FIGURE 18. Moisture content of seeds after 43 days of storage at relative humidities of 76, 55, and 35 per cent.

where the maximum amount of water is held at 10° C (50° F) with less at 5°, 20°, and 30° C (41°, 68°, and 86° F). It is less marked at 35 per cent relative humidity. The explanation for the difference is not known. Perhaps the colloidal condition, and consequently the water-holding power of the seeds, varies with the temperature. Why seeds have maximum water-retaining or absorbing power at 10° C (50° F), especially at high humidities, is an interesting academic question for an investigator to answer in the future. It is of little significance in practical seed storage. The curves in Fig. 18 show again the great difference in the amount of water that different kinds of seeds will hold when they are in equilibrium with the atmosphere at various humidities and temperatures. Here again the fatty seeds, peanut, lettuce, flax, and pine, are relatively low in water content at all temperatures and humidities as compared with tomato and onion seeds, which are also fatty.

If low water content is required for storage of seeds, how low should it be? Probably the lower the better, provided the drying itself does not injure the seeds. We have already seen that some seeds endure little drying and also, in the case of citrus, that seeds will endure more drying at low temperatures with slow drying than at high temperatures with fast drying. It would be well if we had data for all commercial seeds that can be stored dry, on the best conditions for drying, as well as the degree to which they can be dried without injury. We do not have such data, but we do have enough to conclude that complete removal of water is injurious to most seeds and that the degree of drying endured without injury varies with different seeds. Ewart [27] states that seeds will not endure drying below 2 to 3 per cent moisture. Waggoner [59] dried Icicle radish seeds to 0.4 per cent moisture, and Joseph [35, 36] dried parsnip seeds to 0.4 per cent moisture and paper birch seeds to 0.6 per cent moisture without injury. Kiesselbach [39] dried maize grains to 5 per cent moisture without injury but did not find that the maximum drying endured without injury. Gray birch seeds [35] were injured by drying to 5.2 per cent moisture and some pine seeds [4] are injured by over-drying.

If we are interested in the water content necessary to keep seeds perfectly for a few years in sealed storage using other good storage conditions, the answer is easy. Fatty seeds should be reduced to 4 to 5 per cent moisture and starchy seeds to 5 to 6 per cent moisture before sealing.

The second important storage condition affecting the life span of seeds is temperature. In the case of seeds which contain so much water that they soon perish at 20° or 30° C (68° or 86° F), lowering the temperature to near the freezing point will prolong the life markedly. With such seeds, lowering the temperature far below the freezing point may prove injurious due to freezing. As the moisture is reduced, lower and lower temperatures can be used, until in seeds having very low moisture the temperature of liquid air is not injurious.[11]

Guillaumin [17] found that soybeans stored in air lost their vitality com-

pletely in six years, while those stored in nitrogen gas or in a vacuum retained their full vitality for the same period. Some other fatty seeds seem especially sensitive to oxygen during storage; flax seeds, on the other hand, seem to endure air storage very well. Dillman and Toole [17] found that the latter stored in the dry air of Mandan, North Dakota, still showed 58 per cent germination after 18 years of storage. We have mentioned other cases above where short-lived seeds were benefited by being stored in absence of oxygen. In seeds with high moisture the complete removal of oxygen may cause injury due to anaerobic respiration. No doubt oxygen is generally injurious to long life in seed storage, but its ill effect is largely overcome by proper drying of the seeds. Drying may counteract the ill effects of oxygen, because dried seed coats are impervious to gases; also drier protoplasm may be more resistant to whatever oxygen remains in the intercellular spaces of the dried seeds. Lowering the temperature of storage also minimizes the ill effects of oxygen in storage. The three factors, moisture content, temperature, and oxygen, are interactive: placing one near the optimum lowers the ill effects of the other two that are not near the optimum.

Suppose that, as a matter of academic interest, somebody wanted to find out how long the several farm and garden seeds could be kept fully viable. He would have to learn the best method and the proper degree of drying each, and the best temperature of storage, whether a little below freezing or much lower, possibly even as near absolute zero as possible. He would proceed to dry each sort of seed, hermetically seal it in a vacuum or in an atmosphere free of oxygen, and to store it at the proper temperature. With all this his troubles would just begin. For some of the seeds, at least, he would have to arrange with his great-great-grandchildren or later progeny to see the end of the experiment.

Now let us examine a few experimental results to see how much fair storage conditions, probably far from optimum, will lengthen the life of some short-lived seeds.

Delphinium seeds degenerate rapidly in open air storage. Table 10 shows the results [3, 7] of storing annual and perennial delphinium seed under various conditions. The best storage conditions used in these experiments were probably far from optimum. These seeds were not dried beyond the drying in the laboratory in December, which gives a medium low water content, as is seen in Fig. 17. The seeds were corked in small vials with paraffin over the cork. This is not as good as sealing in glass tubes with a flame so far as holding the moisture content is concerned. Finally, the seeds were sealed in air rather than in a vacuum or in absence of oxygen. In spite of only moderately good conditions, annual delphinium seeds retained their full vitality in sealed storage at the 8° and 5° C (46° and 41° F) combination for 143 months, while in open air at room temperature they had degenerated noticeably in 11 months, and nearly half had lost vitality in 22 months. The perennial delphinium seeds had retained their

Table 10. Viability of Delphinium Seeds Stored under Various Conditions

Seed	Storage conditions	Germination percentages after months of storage								
		11	22	46	69	111	123	143	168	193
Annual, 72% germinated when stored	Open room temp.	57	44	0	0	—	—	—	—	—
	Sealed room temp.	75	80	50	15	0	0	—	—	—
	Open 8° C (46° F) *	50	41	31	5	0	0	—	—	—
	Sealed 8° C (46° F) *	70	67	66	80	76	71	71	48	43
Perennial, 43% germinated when stored	Open room temp.	11	0	0	—	—	—	—	—	—
	Sealed room temp.	35	21	0	0	—	—	—	—	—
	Open −15° C (5° F) †	44	45	37	27	8	6	8	3	—
	Sealed −15° C (5° F) †	42	53	57	44	49	50	45	45	33

* After 7 years the temperature was changed to 5° C (41° F).
† After 7 years the temperature was changed to − 5° C (23° F).

original vitality after 168 months in sealed storage at −15° C (5° F) for 7 years followed by −5° C (23° F) for the rest of the storage period, although nearly all had lost their vitality in 11 months in open storage at room temperature, and all the seeds were dead in 22 months under this condition. Even after 193 months in sealed storage at the −15° and −5° C (5° and 23° F) combination of temperatures, two-thirds of the seeds were still alive.

The Italian population of the United States commonly grows dandelions for greens. Dandelion seeds are very short-lived in open storage. Fig. 19 shows the effect of temperature, moisture content, and sealed storage upon retention of vitality by these seeds.[7] In open storage they keep perfectly for three years at −5° C (23° F), but degenerate rapidly at room temperature and 5° C (41° F). In sealed storage with 7.9 per cent moisture they keep perfectly at −5° C (23° F) and 5° C (41° F) and fall nearly 50 per cent in vitality at room temperature for three years. The situation is similar in sealed storage with 6.2 per cent water content, except that the fall in vitality at room temperature is considerably less. Finally, with 3.9 per cent water content the seeds keep their vitality almost equally well at all three temperatures, with only a slight fall at room temperature. Barton has found that repeated opening and resealing of sealed seeds results in more rapid degeneration than occurs if the seeds are kept sealed continuously for the whole period. She attributes this to slight fluctuations in water content due to opening and exposing to the air.

Let us look at another set of storage experiments (Fig. 20) where only one storage factor approached the optimum, namely, reduced and constant moisture content with no reduction in oxygen pressure and laboratory temperature for storage.[5] These seeds at the beginning bore from 8.2 to 12.5 per cent moisture, about the amount held by seeds in equilibrium with the air in mid-summer at Yonkers. The seeds that were

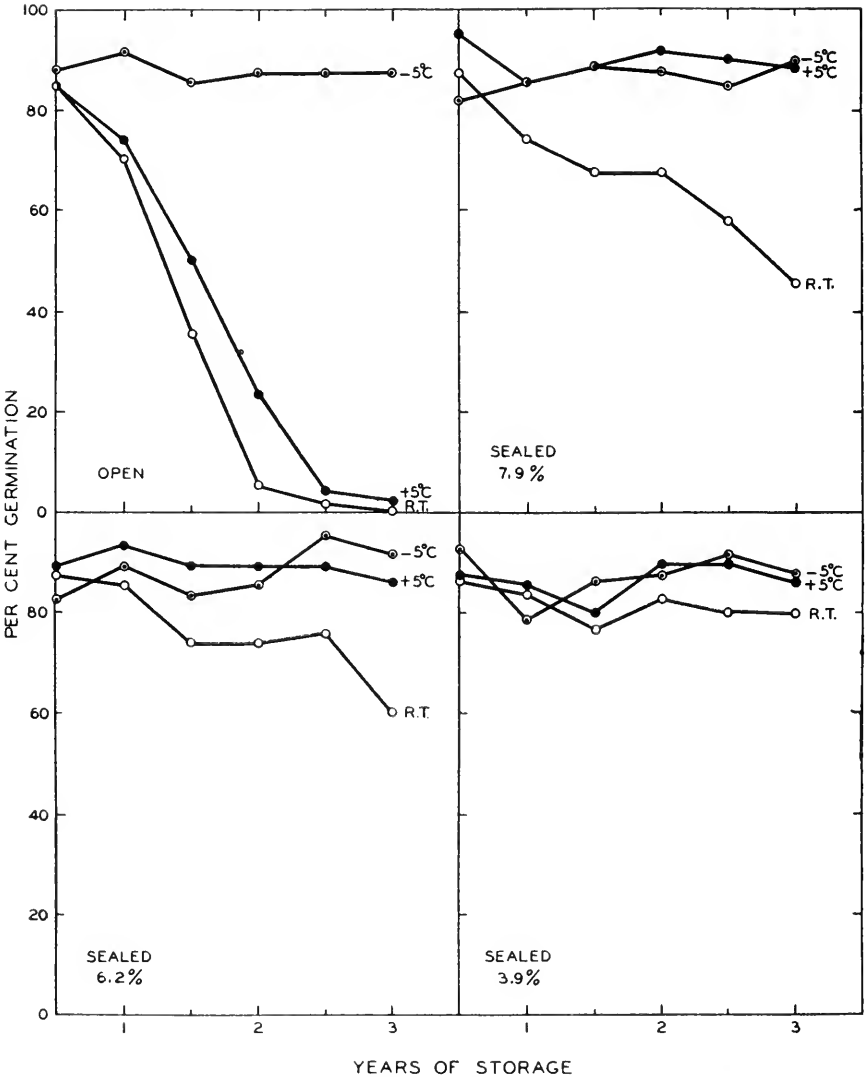

FIGURE 19. Germination of dandelion seeds on moist filter paper after storage for various periods at room temperature (R.T.), + 5° C (41° F), and − 5° C (23° F) in open containers (*upper left*) or sealed in glass tubes (*upper right and lower half*) with moisture contents of 7.9, 6.2, and 3.9 per cent. "Open" lots moisture at start was 7.9 per cent.

dried with CaO for sealing had one-third of the moisture removed before sealing. It will be noted that the seeds sealed without drying degenerated most rapidly. The moisture was too high for sealed storage at such a high temperature. All the seeds kept well for six years in sealed storage after the rather slight reduction in moisture, except pepper seeds, and even they were improved by drying and sealing. More thorough drying prob-

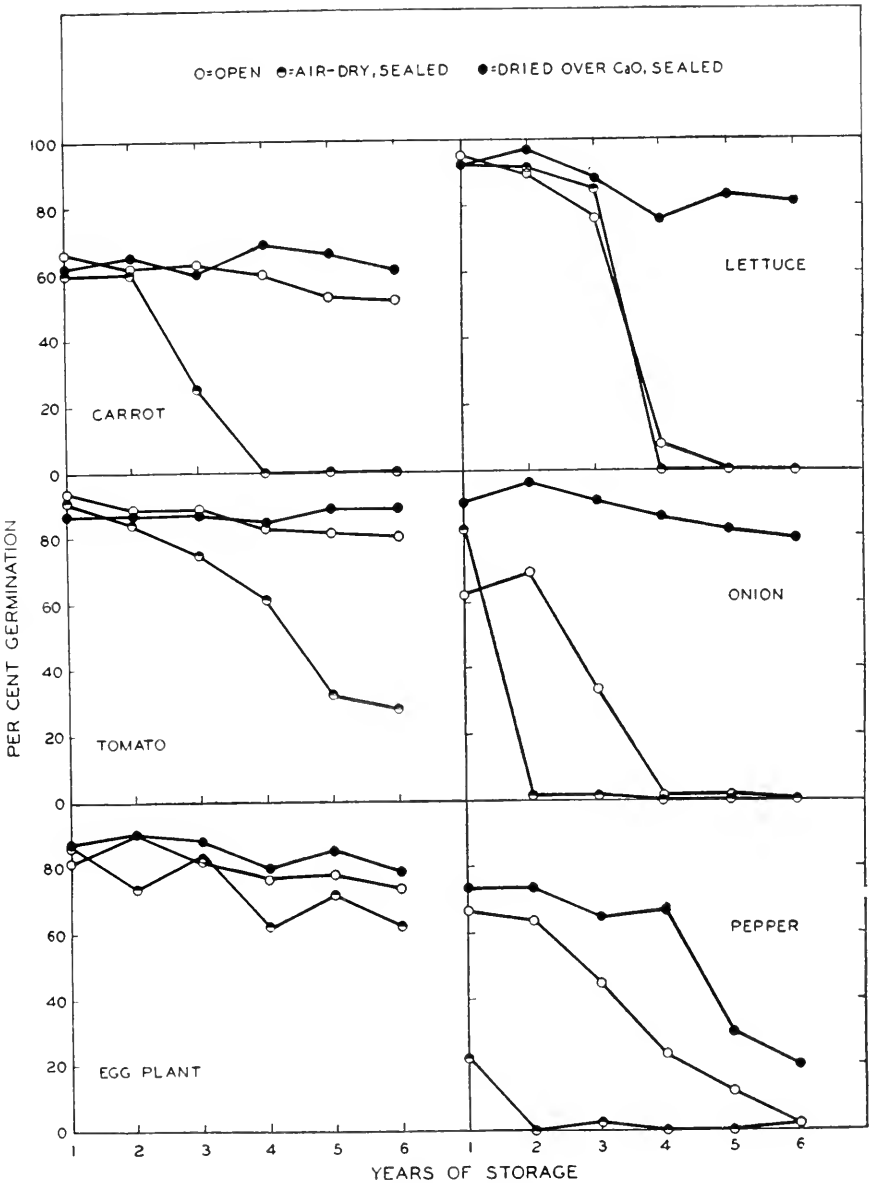

FIGURE 20. Deterioration of seeds at room temperature storage. Germination tests made in ovens on moist filter paper.

ably would have improved their keeping qualities. At the same time samples of all these, not shown in the curves, were stored at −5° C (23° F). At this temperature those in open storage and sealed storage without drying kept almost perfectly for six years, as of course did those in sealed storage after drying.

Air conditioning — control of temperature and humidity of the atmosphere — is now used extensively and on a large scale in the United States. The data given above indicate the importance of low constant moisture content of seeds and of low temperatures in conserving the vitality of seeds in storage. Already big seed firms are building large, air-conditioned seed-storage houses, which can be used for drying the seeds by putting them in loose fabric bags and cording the bags up, as well as to provide low constant moisture and temperatures after the seeds are dried. It might be desirable to use separate rooms for drying, so that higher temperatures with low humidities could be used for quickly drying the seeds to the desired moisture content. Some rooms should also be run below freezing for storage of certain seeds after they have been properly dried. One large seed firm has consulted the Institute on the desirable specifications for such storage houses. With the storage houses described above available, there need be no trouble in holding most farm, garden, and flower seeds in full vitality for two, three, or four years.

Why Do Seeds Degenerate with Age?

Many theories have been offered to explain the degeneration of seeds with age. Most of the explanations offered to date are highly theoretical and have relatively little factual substantiation. We shall consider only the more prominent of them and finish by giving the more probable one along with the facts that tend to confirm it.

It has been suggested that the enzymes in seeds degenerate with age, with the result that the seeds become incapable of germination. This is not at all probable, for dry seeds are relatively low in enzymes, and the latter, both hydrolyzing and respiratory, are formed largely by the protoplasm of the embryo in the initial and later stages of germination. The failure of enzymes to form in adequate amounts in older seeds must be sought in changes in the protoplasm itself. Auxins persist [37] in *Zea mays* seeds after 26 to 38 years of storage.

It has been suggested that stored foods disappear in seeds with long aging, and that the embryos do not get sufficient nourishment in old seeds. Most old seeds kept in dry storage contain large amounts of stored foods long after vitality has been lost. For seeds in the soil under natural conditions, if they absorb water readily, it is possible that exhaustion of the foods by respiration determines the life span. Hard-coated seeds in the soil will, of course, use little stored food in respiration and after many years in the ground will have an abundance of stored foods. Jones and Gersdorff [33, 34] find that three different types of changes occur in the proteins of the grains of wheat and corn and the seeds of soybeans in storage: (*a*) a decrease in solubility, (*b*) a partial breakdown of the proteins indicated by decrease in true protein content, by decrease in the amount of nitrogen precipitable with trichloroacetic acid, and by increase

in amino nitrogen, and (*c*) a decrease in digestibility. These changes were rapid at first and had slowed down considerably after two years of storage. They were also much more rapid at 24.5° C (76° F) than at −1° C (30° F) and in open as against sealed storage. Even in the favorable storage conditions the changes were easily measurable in two years. We do not know to what extent these changes occurred in the living germ or scutellum, and to what extent in the more inert endosperm and aleurone layer; but the fact that the proteins of white flour showed very much greater changes than those of the intact grains suggests that the storage proteins are involved in these storage changes. The changes were thought to be due to enzyme action and oxidation. The latter change may be greatly reduced in intact grains by the low oxygen permeability of the dry grain or seed coats. While stored foods are not used up to any great extent in dry stored seeds, no doubt they are being slowly denatured.

Ewart [27, p.184] offers the following explanation: "Longevity depends not on the food materials or seed coats, but upon how long the inert proteid molecules into which the living protoplasm disintegrates when drying, retain the molecular grouping which permits of their recombination to form the active protoplasmic molecule when the seed is moistened and supplied with oxygen." This explanation is not satisfactory because it is highly speculative and not capable of experimental proof.

Crocker and Groves [17] have suggested that the degeneration of seeds in dry storage may be due to the gradual coagulation of proteins of the embryo. They applied Buglia's time-temperature formula for coagulation of proteins to the degeneration of wheat grains at various temperatures and at two different moisture contents, and found that the calculated longevities agreed well with the determined longevities for high temperatures. The calculated longevities for lower temperatures were, however, many times as long as the life span of wheat grains in ordinary storage, but, as we shall see later, controlled storage prolongs the life span of seeds tremendously. This explanation has the fault of being very general. There are many different kinds of proteins in an embryo, and this work does not throw any light upon the particular proteins that coagulate with time. Furthermore, it throws no light on the possibility of the degeneration of some particular mechanism of the living cells.

It is possible that in seeds in dry storage the greatly curtailed respiration leads to the accumulation of intermediate products of respiration that are toxic to the delicate mechanism of the cell nucleus. Stubbe [54] has suggested this as one possible cause of the degeneration of seeds in storage and of the increased mutation shown by old seeds. Schwemmle [51] offers some experimental evidence of the accumulation of inhibiting or toxic substances in seeds as they age. In certain hybrids of *Oenothera berteriana* the seeds germinated more slowly as they became older, and from the old seeds he could extract substances that greatly inhibited the germination of fresh seeds that showed prompt and complete germination in absence

of the extract. Seeds grown on the meal of the old seeds were more inhibited than seeds grown on the extract. The inhibition on meal was so great that many of the embryos of the fresh seeds could not free themselves from the seed coats. This work suggests that we need to examine aging seeds of many sorts of plants to learn whether accumulation of inhibiting or toxic substances increases with age and leads to degeneration, or whether Schwemmle's findings apply to the particular seeds he studied due in part to their hybrid nature. If accumulation of metabolic products leads to the death of long-stored seeds, the good storage conditions are those that reduce the rate of formation and accumulation of such products. Undoubtedly low moisture content and low temperatures will fulfill these conditions. Perhaps many seeds last longer soaked up in the soil than they do in dry storage, either because such intermediate products are not formed in imbibed seeds or because they diffuse out through the soil water.

The later work indicates that the fall in the vitality of seeds in dry storage is due to a gradual degeneration of the nuclei [31] of the cells of the embryo, which results in disorder in the delicate mechanism of mitotic division. This work shows that aging, heating, and x-ray treatment of dry seeds all cause a similar degeneration. Seeds partially injured by any one of these conditions produce seedlings showing an increased number of irregularities in chromosome distribution during mitosis, chromosome mutation, and an increasing number of mutants in the resulting plants. Navashin [17] found that fresh *Crepis tectorum* seeds produced plants in which only 0.1 per cent showed chromosome irregularities in mitosis, and a corresponding percentage of mutants among the plants. On the other hand, more than 80 per cent of the plants grown from five-year-old seeds showed cytological mutations in the roots, and the seedlings grown from such seeds showed many abnormalities. Many of the plants died before they were large enough for transplanting, and others at later stages. Many albino plants appeared. Some of those that reached maturity were partially or wholly sterile. Peto [17] has found similar changes in plants produced from old maize grains, as well as in plants produced from barley grains subjected to high temperatures. By heat treatment he produced a tetraploid barley plant. Avery and Blakeslee [2] find that *Datura* seeds mutate much more slowly when buried in the soil than when stored in the laboratory, just as they degenerate much more slowly, as mentioned above. In a summary of the literature, Goodspeed [17] shows that irradiation of plants causes chromosome and plant mutations very similar to those produced in seeds by aging or heat treatment.

The literature is accumulating rapidly on the chromosome and plant mutations caused by aging, heating, and irradiating of seeds, and the results are in general agreement. Let us summarize briefly the effects these treatments produce, realizing of course that the degree of change increases with the aging under a given set of storage conditions or with

the intensity of the treatment: (*a*) on the mitotic divisions of the embryos; (*b*) on the resulting seedlings. The following are some of the chromosomal and mitotic modifications brought about by these treatments: fragmentation of chromosomes with fragments attaching to other chromosomes or remaining unattached and not entering into the constitution of daughter nuclei; change in the number of chromosomes in the daughter nuclei, sometimes resulting in polyploidy; giant nuclei; two nuclei in one cell; globules of chromatin in the cytoplasm; and ring chromosomes. The aged, heated, and irradiated seeds showed the following changes in the resulting seedlings: polyploid plants or parts of plants; new forms of plants, some of them larger and more vigorous than the parents; slower germination; slower growth after germination; death of many seedlings in early stages; greatly increased number of chlorotic seedlings and sterile plants; as well as many other morphological abnormalities. In these treatments, as the nuclear or chromosome abnormalities increase, the morphological abnormalities also increase.

Aside from the delicate mechanism of nuclear division we should not forget that some of the most complex organic compounds enter into the make-up of the cell nuclei. These may decompose in storage and in turn upset the nuclear mechanism. Also it is possible that toxic or inhibiting substances accumulated in seeds during storage may upset the nuclear mechanism. The remarkable fact is not that the delicate nuclear mechanism or the complex compounds of the nuclei degenerate with time, but that for many seeds under good storage conditions they stay intact for centuries.

Sure it is that in nature some plants are produced from old seeds, seeds that have lain in the ground for 10, 50, or in some cases, 100 years or more. Since aging seeds produce more and more mutations as they age, we have here one of the means by which nature produces new forms of plants, or carries forward evolution.

This conception of the degeneration of seeds in storage has the virtue of concrete evidence in its favor; it localizes the significant changes in the nucleus and ties the change up with one of the most delicate cell mechanisms, mitotic division. If this explanation of seed degeneration is correct, then the best storage conditions for seeds are those that best preserve complex organic compounds of the nuclei and the mitotic mechanism of the embryo cells.

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CHAPTER 3

Dormancy in Seeds

Significance of Dormancy in Seeds

In the previous chapter we showed that seeds of many wild plants will lie in a germinator or in moist soil for years without germinating, or in cases where some germinate, the germination of a single planting of a given crop may spread out over a period of years, with now and then a few seeds germinating. In short, in seeds of wild plants, delayed or distributed germination is very common both in a germinator and with nature in the soil. While this is markedly true for seeds of wild plants, it is also true to a lesser degree of seeds of many cultivated plants. In the latter, the dormancy is often transient and extends over a period of days or weeks rather than years. The seeds of some cultivated plants do have a long period of dormancy. This is true of some leguminous and other hard-coated seeds that are slow to absorb water.

With the knowledge available during the last part of the past century the explanation of dormancy and delayed germination of seeds was much simpler than it is today; and it was correspondingly more vague. Hard-coated seeds, of course, did not germinate because they did not absorb water. Seeds that absorbed water and did not germinate were supposed to need some special stimulus besides the three conditions ordinarily thought necessary for germination — proper temperature, oxygen, and water supply. The stimuli were supposed to act promptly and to give immediate germination. This was thought of as a release response, and workers sought for stimuli that gave such immediate release. True, during the latter part of the last century, many workers showed that light was necessary for the germination [20] of some seeds and accelerated the germination of others, and early in this century some seeds were found to be prevented from or hindered in their germination by light. Since the response to light is rather quick and the light need not act for a long time, it was interpreted as a stimulus or release response. Now it seems probable that even light brings about biochemical or biophysical changes that lead to germination, and that its effect can be interpreted on the basis of definite chemical and physical changes rather than on the basis of the vague conception of release or stimulus response.

We have already seen how long some seeds must be held in a low-temperature germinator to after-ripen the dormant embryos, and also in a high-temperature germinator with restricted oxygen pressure in order

to induce dormancy in embryos. Later we shall discuss some of the physical and chemical changes occurring in embryos during after-ripening and during dormancy induction. As we shall see later, many other changes involved in overcoming dormancy also take much time and involve known biochemical changes and, like those mentioned above, can be interpreted on the basis of definite physical and chemical changes rather than by the vague term "stimulus."

Later in this chapter under the topic "Categories of Dormancy" we shall point out many ways in which nature maintains dormancy in a moist soil, and several means by which seeds are thrown out of dormancy. Contrasted with the old explanations of seed dormancy caused by hard-coatedness on one hand and lack of a release stimulus on the other, the new data and explanations afford a much greater richness of concept and precision of conclusions.

Advantages to Plants of Delayed Germination

Delayed germination is advantageous to many wild plants in nature. It carries the plant over the winter in the seed stage and the young plant grows in the spring. In the Dakotas, Minnesota, Saskatchewan, and adjoining regions wild oats are a bad weed because the grains are sufficiently dormant to carry over intact until spring, when they germinate. Cultivated oats and false wild oats are not troublesome weeds in these regions because the dormancy of these grains is so temporary that the grains after-ripen and germinate in the fall and the cold winter kills the seedlings. In the previous chapter we learned that seeds of some wild plants lie dormant in the soil for years, or even centuries, and germinate only when the soil is stirred up or burned over. This is no doubt helpful in the persistence of the species.

Advantage to Man of Dormancy in Seeds

It would be a calamity for mankind if all at once seeds of cultivated plants ceased to have at least a temporary dormant period. Mangelsdorf [79, 80] found a number of types of maize, produced either by inbreeding or by crossing, in which the grains had no dormant period, but instead the embryos continued to grow in the green ear and to form seedlings. Professor William H. Eyster [33] has kindly furnished an illustration of such an ear of corn which is shown in Fig. 21. Pope and Brown [94] forced young embryos in heads of normally very dormant varieties of barley to continue to enlarge and form seedlings in the green head by placing moist filter paper on the embryo portion of the immature grain. Apparently water deficit in the green head is a factor in inducing dormancy. Fig. 22 shows viviparous heads of barley, the photograph for which was very kindly supplied by the authors. Evidently appearance of dormancy in grains of cereals, or its failure to appear, is determined either by genetic characters, as shown by Mangelsdorf and by Eyster, or by conditions of

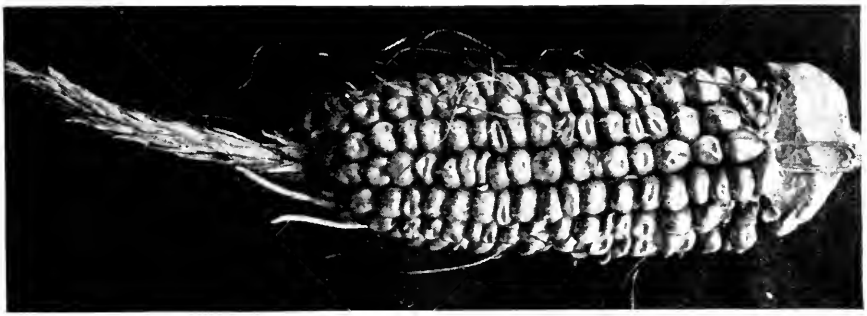

FIGURE 21. An ear of maize homozygous for vivipary showing the viviparous embryos in various stages of development. (*Photograph furnished by Dr. William H. Eyster, Botanical Laboratory, Bucknell University, Lewisburg, Pa.*)

FIGURE 22. Viviparous seedlings in the spikes of one spring and three winter varieties of barley. 1, Manchuria (C. I. 2330) 6 rowed, white, spring; 2, Dentil (C. I. 1260) 2 rowed, white, winter; 3, Abyssinian (C. I. 1222) 6 rowed, black, winter; 4, Meliton (C. I. 1456) 2 rowed, black, winter. (*Photograph furnished by Drs. Merritt N. Pope and Edgar Brown, U. S. Dept. Agriculture, Beltsville, Md.*)

growth, as shown by Pope and Brown. If seeds of all cultivated plants failed to have a dormant period as in this illustration, man and domestic animals would be without any food in the form of dry seeds and grains, which form a large proportion of the food of both. Such a failure of dormancy would also bring about a great disturbance in plant propagation by making difficult or eliminating propagation by seeds.

It is a common experience that during rainy weather grains germinate in the shock. Different varieties and strains of cereals vary a good deal in the length of dormancy of the grains [18, 28]; also the weather conditions during ripening produce considerable variation in length of dormancy. Length of dormancy of the grains [62] is one of the factors being studied in breeding desirable new varieties of wheat. Grain dormancy will become less and less significant as the combine more and more displaces the binder in harvesting, for the grains seldom retain enough water to germinate while still standing unharvested in the field. Recently the swather has been introduced. It leaves the grain in a swath to dry and is followed after drying by the pick-up combine. Here again a period of dormancy will be important in preventing germination in the swath in case of rain.

Seed Dormancy Inconveniences Man

Dormancy in seeds causes man a great deal of inconvenience in at least three ways. (1) Seeds of species of plants that are persistently dormant need pretreatment to induce after-ripening, so that they will come up promptly and all at the same time when planted. As we shall see later, it takes a long time to after-ripen some seeds. In some cases two or more very different sets of conditions must be used for after-ripening, and in others two long-time, low-temperature treatments must be applied to after-ripen the seed and later to after-ripen the epicotyl. Until one knows the conditions necessary for after-ripening any particular sort of dormant seed, he may not be able to produce any seedlings; and after he knows how, he may have a two-, three-, or four-phase job in producing seedlings. These difficulties are met in their most complicated form when one undertakes to grow certain wild flowers.

(2) We have seen that some weed seeds lie in the ground for 60 years, dormant and still capable of producing plants. Fighting weeds is one of the heavy tasks of both the farmer and gardener. It is probable that if a piece of land were cultivated so thoroughly that no weed seeds matured, and weed seeds were prevented from coming in from outside, the land could be freed of all weeds far short of 60 years, because cultivation stirs up the dormant seeds and throws them out of dormancy.

(3) In farm and garden operations it is customary to test seeds in advance of planting to be sure that only viable and vigorous seeds are sowed. Even winter cereals are sometimes sufficiently dormant to give trouble in testing previous to fall sowing. Harrington [61] found that dormant cereal grains could be hastened in germination by various means

such as using 12° C (54° F) germinators instead of 20° C (68° F) or higher, by breaking the grain coats near the embryo, by using high oxygen pressure, etc. Grains are more persistently dormant when they ripen during rainy weather. The Germans repeatedly report difficulty in converting "rain barley" promptly into malt because of delayed germination of the grains. Even for many seeds that have very long dormant periods, including those with dormant embryos, there are means of making prompt vitality and vigor tests, as we shall see later in the discussion of quick vitality tests.

Categories of Dormancy

Now let us consider the types of dormancy in seeds based on the mechanism by which the dormancy or delayed germination is secured. As we shall see, nature has several devices for securing delayed and distributed germination of seeds.

Hard Coats

We have already shown the extreme importance of hard-coatedness in seeds in increasing their life span in storage as well as in the soil. Hard coats maintain a low constant moisture content in the embryos — an effective storage condition — by hermetically sealing them individually. There are several families, some species of which produce seeds that will not absorb water. Leguminosae [59] are outstanding in this respect, but the Malvaceae, Cannaceae, Geraniaceae, Chenopodiaceae, Convallariaceae, Convolvulaceae, Solanaceae and other families have species that bear hard seeds. Hard-coatedness is primarily determined genetically, but the appearance or degree of hardness is also modified by environmental factors. In white sweet clover [115] either hard or soft strains can be developed by selection and inbreeding. The same is true of hairy vetch.[64] On the other hand, the author has observed at Yonkers that more than 98 per cent of hand-hulled, white sweet clover seeds are hard when they ripen during hot, dry weather and that 100 per cent are soft when they ripen during rainy weather.

Many investigators agree that the outside layer of cells of the coats which are palisade in form prevent the entrance of water, and some claim that, of this layer of cells, only the outer half or the portion outside the "light line" is impermeable. This layer of cells in *Nelumbo* was illustrated and described in the previous chapter. White [128] claims that in small leguminous seeds the cuticular layer over the palisade cells determines the impermeability, while in the larger leguminous seeds the outer portion of the palisade cells is involved as well. The question has also been raised whether the physical character or chemical composition of this thin layer gives it the remarkable resistance to water absorption. Raleigh [96] concludes that as the seeds of the Kentucky coffee tree harden in ripening, pectic substances change into water-resistant substances. Shaw [106] germi-

nated American lotus seeds by treating them with acetone and then placing them in water. She assumed that fat-like substances were dissolved out of the stomatal cavities, which extended deeper than the palisade layer, allowing water to enter. Ether as the solvent made the coats permeable also, but killed the embryos. Shaw also maintains that the palisade cells are impervious throughout their length.

Hamly [58] speaks of suberin caps over the palisade cells as causing the water-resistance in sweet clover seeds. McKeever [77] found that treatment of black locust seeds for 10 to 120 minutes with several wax solvents (ether, xylene, acetone, and others) was effective. All removed considerable amounts of wax. The main effect seems to be hastening the germination by 10 or 15 days rather than increasing it after 27 days. Very early Höhnel [63] claimed that soaking yellow lupine seeds in ether with immediate transfer to water softened the coats. Perennial lupine seeds did not respond to this treatment. Verschaffelt,[126] working mainly with hard honey locust seeds, found that placing them in ethyl alcohol and transferring them immediately to water led to swelling. He assumed that the alcohol filled the rifts or interstices in the hard coats and furnished a channel by which water could travel to the deeper layers of the coats. Other simple alcohols were effective, but higher alcohols, ether, and other fat solvents were not, because water is not sufficiently soluble in them to reach the deeper layers; also some of them failed to fill the rifts in the seeds. Many hard seeds of Caesalspinaceae and Mimosaceae acted like honey locust seeds, but alcohol was less effective with Papilionaceae. The first two groups have minute rifts all over the surface of the seeds, while the latter has one rift at the hilum. According to Verschaffelt's interpretation, the alcohol does not act as a wax or fat solvent but as a bridge for conveying the water to the deeper layers of the coats.

Some regions of the hard seed surface seem to be rendered water-permeable more easily than others. Hutton and Porter [65] showed that dry, hard seeds of *Amorpha* and *Lespedeza*, when shaken in a bottle, become water-permeable at the hilum. Hamly [58] found that hard *Melilotus* seeds were made water-permeable by moderate heating or mechanical impact by producing a rift at the strophiole. This long and much worked problem of what physical or chemical characteristics make hard seed coats resistant to entrance of water evidently still needs thorough research attention. The chemical or physical method of water-proofing may vary with different kinds of hard seeds. This is made probable by the contradictory explanations offered above; also we must remember that little attention has been given to the mechanism of hard-coatedness in several of the families of plants. In some, not even the layer of the coat involved is known.

There is another reason for learning the mechanism or mechanisms of hard-coatedness in seeds. Hard-coatedness is the world's best example of highly effective water-proofing by thin layers. Man can well devote some

time to learning the method or methods of this water-proofing in the hope of applying it to his needs. The seeds of any crop vary in perfection of water-proofing, as we have already seen. This insures time-distributed germination of any crop.

The embryos of most kinds of hard seeds germinate readily and grow vigorously when the hard coats are broken and germination conditions furnished; but the embryos of redbud, *Cercis canadensis*, need several weeks' after-ripening in a low-temperature germinator or stratification in the swollen condition to prepare them for growth.[1] The embryos of hard seeds of the beach pea, *Lathyrus maritimus*, grow much more promptly after the seeds have a period in dry storage.[29]

In red clover seeds Săulescu [101] finds that percentage hardness increases with diminution in size and with darkness in color from yellow to purple. Grimm [57] finds the same size-hardness relation for several clovers, but believes that lighter-colored seeds have a higher percentage of hard seeds. *Lespedeza stipulacea* seeds [82] show the same relations between size and hardness. In alfalfa seeds [83] percentage hardness seems to decrease with range in color from bright yellow to green to brown. Hardness in *Vicia sativa* seeds [70] increases with depth in color. In commercial alfalfa and clover seeds the percentage of hard seeds varies greatly with varieties, conditions during ripening and storage, and with conditions in machine-hulling such as closeness of cylinder and concave, and moisture content. The persistence of hardness [98, 117, 130, 131] increases in the following order: alfalfa, red, white, and alsike clover. With spring sowing, hard alfalfa seeds swell and germinate mostly the first season with few or none carrying over. In the clovers some seeds carry over until the second spring and a few still longer. In storage, hard alfalfa seeds [50] soften faster than the clover seeds, and low temperatures and high moisture favor softening during storage.

Lupines [31] and hairy vetch seeds [123] become hard in high-temperature, low-humidity storage, and soften in low-temperature, high-humidity storage. Even a few days in dry laboratory air increases the degree and percentage of hardness in yellow and blue lupine seeds.[32] Careful attention must be given to storage condition of these seeds during the winter to avoid hard-coatedness and failure to germinate when sown in the spring. Temperatures at the freezing point or lower favor softening of hard clover and alfalfa seeds whether wet or dry.[50, 59, 83] Busse [17] found that freezing dry hard seeds of sweet clover and alfalfa to −190° C (−310° F) softened the coats without injury to the seeds. Even repeated freezing at this temperature did not injure dry alfalfa seeds. High temperatures, 60° C (140° F) for 2 hours or 75° C (167° F) for 0.6 hour,[75] soften hard alfalfa seeds. Both high and low temperatures are probably factors in softening hard seeds in the soil. This is especially true of low and variable temperatures during the winter.

High hydraulic pressures [23, 24, 99] have been used to soften hard coats of

seeds. The pressures used varied from 20 to 60,000 pounds per square inch. Higher pressures softened the coats but injured the seeds. Lower pressures softened the coats without injury. Small seeds required higher pressure to soften the coats and also endured higher pressures without injury. In the soil such factors as weathering, bacterial and fungal action, and abrasion of agricultural instruments modify hardness.

Several methods have been developed for overcoming hard-coatedness in seeds before sowing. Soaking the seeds in hot or boiling water has been long used. In the nineties Rostrup,[100] a Swedish botanist, discovered that the outside layers of the hard coats could be eaten away with concentrated sulfuric acid followed by thorough washing to remove all acid. The length of time for either of these treatments varies greatly with different kinds of seeds and to a degree with different crops of the same kind. This is especially true of the sulfuric acid treatment. Many different sorts of scarifying machines have been invented and used commercially. In such machines the seeds are thrown against sandpaper, needle points, etc., to scratch the hard outside surface of the seeds. Some of these have proved useless, either because the impact broke the embryos or injury laid the seeds open to infection. Porter and Brown [95] have shown that shaking hard, black locust seeds in a bottle for 20 minutes makes them water-permeable. In small-seeded commercial leguminous seeds one will generally find a much smaller percentage of hard seeds in those threshed by a mechanical huller than those hulled by hand. Even rubbing the seeds through a sieve to get rid of the hulls softens some of the seeds. In short, the threshing machine acts as a more or less effective scarifier. We have discussed only a portion of the important data on factors that induce or overcome water-impermeability of hard seeds in practice or in nature as well as in storage and in the soil; but space does not permit a fuller discussion.

Light as a Factor in Dormancy

Some seeds require light for germination and many others are favored by light, while other seeds are completely or partially inhibited in their germination by light. Light-favored seeds may remain dormant when covered by soil to such a depth as to exclude the necessary light, or light-inhibited seeds may fail to germinate if they are sown with little or no cover. The first type of seeds should be sown on or near the surface of the soil or otherwise treated to overcome the light need, and the second type should be sown deep enough to prevent the inhibiting effect of light.

Relatively little time can be given to this topic. Consequently, the author will quote a summary of an article which he wrote in 1936 entitled "Effect of the visible spectrum upon the germination of seeds and fruits" [20, p.820–822] and published in the two-volume treatise "Biological effects of radiation."* This quotation will be supplemented by a brief

* McGraw-Hill Book Co., Inc., New York, N. Y., 1936. Permission to quote this material is gratefully acknowledged.

statement concerning a few of the more recent and more significant contributions to the subject.

"(*A*) Light favors the germination of a large number of seeds and fruits. Among these are *Viscum album* together with many other Loranthaceae and epiphytes, all Gesneriaceae studied to date, many grasses, various species of *Oenothera* and *Epilobium, Ranunculus sceleratus, Lythrum salicaria,* and *L. hyssopifolia. Viscum album* and *Arceuthobium oxycedri* will not germinate at all without light. The former is killed in darkness within a few weeks, while the latter endures darkness for a longer period. Of 964 species of seeds studied by Kinzel, 672 or about 70 per cent were favored by light under the [very limited] conditions used in his experiments.

"(*B*) Light interferes with the germination of many seeds and fruits. Among these are several species of *Phacelia* and other Hydrophyllaceae, 3 species of *Nigella,* several species of *Allium* and most other Liliaceae. Of 964 species of seeds and fruits tested, Kinzel found 258 inhibited by light under the conditions of his experiments.

"(*C*) Some seeds and fruits germinate equally well in light and dark. This is true of the small grains, *Zea mays,* beans, clover, and many other legumes. Of the 964 species investigated by Kinzel, 35 were indifferent to light.

"(*D*) Several conditions partly or entirely displace the effect of light in light-sensitive seeds and fruits.

"(*a*) After-ripening in dry storage reduces or entirely eliminates the need for light in various light-favored seeds. *Poa* achenes [caryopses] kept in dry storage for one year germinate almost as well in darkness as in light. After-ripening partially eliminates light need in *Chloris, Ranunculus, Epilobium,* and *Oenothera* achenes or seeds. The inhibiting effect of light on *Phacelia* seeds falls with period of dry storage.

"(*b*) Seed or fruit coats, or the hulls of grasses, increase the necessity for light in the germination of some light-favored seeds. The hulls render *Chloris* achenes light-obligate and increase the need for light in *Poa.* Pricking the seed coats of *Oenothera* increases germination in darkness. The coats also modify the action of light on light-inhibited seeds. Removal of the seed coats from *Phacelia* seeds overcomes the inhibiting effect of light. Pricking the coats causes 'lichthart' seeds of *Nigella* to germinate in part.

"(*c*) A full atmosphere of oxygen forces the light-obligate *Chloris* achenes with hulls intact to full germination in darkness, and the light-inhibited *Phacelia* seeds to full germination in light.

"(*d*) Knop's solution substitutes for light in a number of light-favored seeds. The nitrate of the solution is effective. The other salts of the solution are not effective. Nitrites, nitric acid, ammonium salts, and urea are also favorable. Nitrates entirely displace the light need of *Chloris* achenes with hulls intact at temperatures above 22° C. They also increase greatly the germination at temperatures below 22° C, where light inhibits. Nitrates

favor the germination of the following light-favored fruits and seeds in darkness: *Poa, Ranunculus, Epilobium, Lythrum,* and the Gesneriaceae. The light-inhibited seeds of *Phacelia* and *Nigella* are not favored by nitrates.

"(*e*) Weak acids substitute for light in part in the light-favored seeds of *Lythrum, Scrophularia, Verbascum,* and *Epilobium.*

"(*f*) Either daily intermittent or high constant temperatures substitute for light in various light-favored seeds. The most favorable intermittent temperatures give better germination of *Poa* achenes than light with any constant temperatures. Light and nitrates increase the germination of *Poa compressa* achenes somewhat at the most favorable intermittent temperatures. Intermittent temperatures replace light with after-ripened *Chloris* achenes with hulls intact, but not with non-after-ripened or 'dunkelhart' achenes. With seeds of *Epilobium, Oenothera,* and others intermittent temperatures substitute fully for light.

"(*E*) When light-favored achenes of *Chloris* are kept for a time in a dark germinator, they are changed in a manner that makes them incapable of germination later even in light. Such seeds are said to be 'dunkelhart.' 'Dunkelhart' achenes can be forced to germinate by breaking the coats, increasing oxygen pressures, and other treatments. When light-inhibited seeds of *Nigella* are kept for a time in a light germinator at a temperature above 20° C, they are changed in such a manner that makes them incapable of germination later even in darkness. Kinzel spoke of such seeds as 'lichthart.' 'Lichthart' seeds can be forced to germinate by breaking the coats, or still better by other treatments. Imbibed *Phacelia* seeds also become 'lichthart' when exposed to light.

"(*F*) If imbibed *Ranunculus sceleratus* seeds are exposed to light, dried, and later placed in a dark germinator with intermittent temperatures, they still show the favorable effect of the light exposure. *Chloris* achenes also show this latent light effect. Since the light exposure of the seeds during ripening in the capsules varies with the weather, the rate of drying of seeds in the capsules, and the position of the capsule on the plant, Wieser concluded that the latent light effect may account in part for the great variation in the amount of light required for the germination of different collections of the same species of light-favored seeds.

"(*G*) Several theories have been offered to explain the favoring or inhibiting action of light upon the germination of seeds and fruits. Most of these theories postulate that the action of the light is upon the living endosperm or embryo, but some of them assert that the action is upon the non-living coats. None of these theories has adequate evidence for even a single species of seeds. It is not improbable that light has its effective action upon the endosperm and embryo of some seeds, upon the coats of others, and upon both in still others. There is need of a very thorough and detailed chemical, microchemical, and physiological study of the effect of light upon the coats and living portions of several light-favored and light-inhibited seeds and fruits. There is also need of a similar study of

the changes brought about in seeds and fruits by agents and conditions which substitute for light."

The recent findings of Flint and associates [45, 46, 47, 48] on the effect of different portions of the spectrum on the germination of lettuce seeds are of great interest. These findings deserve special consideration because of the excellence of technique on which they are based. In batches of freshly imbibed lettuce seed that required light for germination, a few seconds' exposure to light induced germination. The sensitiveness approached that of a photographic plate. The region 5200 to 7000 Å (red, orange, and yellow) was stimulative, with the critical wave length at about 6600 Å; the region 4200 to 5200 Å (green, blue, and violet) was inhibitive, with the critical wave lengths at about 4400 and 4800 Å; and the band 7000 to 8600 Å (mainly infrared) was even more inhibitive, with the critical wave length at about 7600 Å. The critical regions for inhibition of germination in the visible spectrum are about the same as those for induction of phototropic curvature and for the inhibition of growth of plant organs. The critical inhibitive region in the infrared was not associated with assimilation or temperature effects, and 7600 Å did not induce phototropic response in lettuce seedlings. Keeping seeds in a dark germinator at 5° C (41° F) for several weeks did not modify their sensitiveness to light; but keeping them in a dark germinator at 25° C (77° F) for 24 hours did so alter their sensitiveness to light that they would not respond to standard illumination. As we shall see later, a germinator at 5° C (41° F) is an excellent condition for after-ripening many dormant seeds, while a germinator at 20° to 25° C (68° to 77° F) maintains many dormant seeds in status quo.

It is well established that many fern spores require light for germination. Orth [88] finds two groups of fern spores in respect to their response to light: those that germinate in various bands within the region 550 to 710 mμ, and have brown exospores, and those that germinate as above and also in ultraviolet light, and have colorless exospores and much carotene in the cells. Within the generally favoring band there are hindering and favoring regions. In the short end of the spectrum various bands of inhibiting rays appear. The same is true in the infrared around 800, 1000, and 2400 mμ. Fern spores germinate in light that passes through green leaves, unlike light-sensitive seeds. This is due to the strongly favoring action of green-yellow overcoming the inhibiting action of the infrared. Because of the several bands of favoring or inhibiting action in the spectrum, Orth concludes that the action of light on germination of fern spores cannot be explained on the basis of quanta, as Kommerell [71] has attempted to do for seeds, but on the basis of the specific effects of various bands of the spectrum on the spores. Flint's results on lettuce seed would seem to justify the same conclusion for seeds. Raleigh [97] showed that thiourea forced the germination of dormant lettuce seeds (*Lactuca sativa*) in darkness, and in *L. Serriola* it increased the germination in both light and darkness.

Muenscher [86] shows that light is necessary for the germination of *Lobelia inflata* and that other factors will not substitute for light. These seeds gave no germination when covered with 1 cm. of soil. *L. cardinalis* and *L. siphilitica* seed also require light, while *L. tenuior* and five forms of *L. Erinus* seed germinate equally well in light and dark. Funke [49] has recently confirmed the findings of Wiesner and others on the role of light in maintaining the life and inducing the germination of *Viscum album* seed. The seeds are injured by two days of continuous dark. Most rapid germination is produced by removing the endosperm and subjecting the embryo to continuous illumination, artificial light at night, and sunlight during the day. The failure to germinate in Belgium during the winter, he feels, is due to low light intensity and daily duration rather than low temperatures.

Sprague [113] further confirms the fact that dry storage overcomes the need for light; *Poa pratensis* seed six months after harvest no longer required light or alternating temperature for germination. Jensen [66] suggests that exposure of seeds to artificial light lengthens their viability in dry storage. Various workers [76, 78, 87] have shown that certain light rays modify enzyme content, metabolism, and growth substances in germinating seeds, but in no case does this work explain how light induces or hinders germination.

Oxygen Deficiency and Dormancy

Growing plants with their intracellular aeration systems connected with stomates and lenticels are much better equipped to get the needed oxygen supply from the air than are embryos of seeds which are, in the main, completely sealed within seed coats and often additionally covered with fruit coats and other structures.

Using common cultivated species of plants (common bean, broad bean, cress, savory, and *Hydrangea*) in a special growing chamber, Schaible [102] grew the plants and germinated the seeds in one-fourth of an atmosphere of pressure with a continuous change of the atmosphere; in one case he drew air through the chamber, thereby giving one-fourth the partial oxygen pressure of a full atmosphere of air; and in the other he used oxygen-enriched air so that the partial oxygen pressure in the chamber was equal to that in a full atmosphere of air. Plants in the reduced pressure grew much faster than plants in a full atmosphere, regardless of whether the partial oxygen was normal or one-fourth normal. The atmospheric pressure determined the rate. Seeds germinated a little better in the reduced pressure of oxygen-enriched air than in a full atmosphere of air, but very much worse in the reduced atmosphere without oxygen enrichment. In other words, the oxygen content of the air is far above that needed for the fastest growth of plants, but it is not so far above that needed for the germination of seeds. The latter is conditioned by the slow passage of oxygen through the seed and fruit coats.

Brown [16] estimates from his experiments with barley grains that embryos in partially imbibed grains floating on water are in equilibrium with 10 per cent of oxygen, whereas excised embryos are in equilibrium with the full percentage of oxygen of the air. The rate of uptake of oxygen and release of carbon dioxide was much higher in the excised embryos. This no doubt is partly due to the higher water content of the excised embryos as well as to higher oxygen pressure. Brown [15] has also shown that the imbibed seed coat of *Cucurbita pepo* permits carbon dioxide to diffuse through it several times as fast as oxygen. Stålfelt [114] shows that the amount of oxygen taken up by white mustard seeds in a germinator is increased if the partial pressure of oxygen is increased from 20 to 50 per cent. The increase is much greater for the cotyledons than for the root.

The researches just cited, as well as a number of others that might be cited, show that some imbibed seeds are limited in their use of oxygen from the air because of the low permeability of the coats to oxygen. Are there cases in which seeds stay dormant because oxygen does not pass through the coats sufficiently fast to permit germination of the embryos even when they have good aeration? If this is true, two conditions must be fulfilled in such seeds: (1) the embryo must have a certain oxygen pressure demand in order to grow, and (2) the low permeability of the coats must limit the oxygen pressure to the embryo below the minimum necessary for growth.

It is well established that embryos of various kinds of seeds vary greatly in the oxygen pressure or oxygen supply needed for germination. One might expect seeds of water plants that normally germinate under water to have low oxygen requirement for germination. Crocker and Davis [21] found that embryos of seeds of *Alisma Plantago*, with coats broken, will germinate in absence of oxygen. They heated the seeds in water in special flasks at 35° C (95° F) for 30 minutes under reduced air pressure of 0.1 mm of mercury and sealed the flasks at this temperature and vacuum. In the vacuum cultures in water the embryos grew in length 1100 to 1200 per cent in 21 days, while in the checks in water the growth in length was 1800 to 2200 per cent. In the vacuum cultures no leaf branches and no chlorophyll formed. Both developed in the controls. About 5 mm of air pressure were required for chlorophyll formation, and more than 5 cm of air pressure for leaf differentiation. While germination occurs in absence of oxygen, the growth is limited and differentiation and chlorophyll formation do not occur. Takahashi [118] claims that the plumule in rice, another water plant, will grow in absence of oxygen, but the root will not. Taylor [119] determined the effect of various oxygen pressures on the germination of rice, as a water plant, and wheat, as a land plant. He says (p. 736): "In the absence of O_2 the germination of rice seeds was reduced less than 10 per cent below that in air and was accomplished at more than half the normal rate. No germination of wheat occurred under similar conditions. Significant reduction in the extent and rate of germination of wheat

occurred when the O_2 tension was lowered to 5 per cent, and considerably less than half of normal germination resulted in O_2 concentrations below 1 per cent. At O_2 tensions of 5 per cent or less, rice seedlings made approximately twice as much growth as wheat seedlings (on the basis of increment in dry weight of embryo in air). Reduction in O_2 tension inhibited roots of both seedlings more than shoots, and it had least effect on rice shoots." He attributes the ability of rice seeds and seedlings to grow in very low oxygen tension to a well-developed anaerobic, energy-liberating fermentation system within the seed, which is lacking in wheat; in rice the energy liberation in absence of oxygen was $\frac{1}{6}$ that in air and in wheat $\frac{1}{15}$. In both grains root growth was more limited by low oxygen pressure than plumule or stem growth.

Morinaga [84] made a study of the germination of many seeds of land plants under water. He listed 34 species that will not germinate under tap water, 25 species that germinate better on moist filters than under water, 18 that germinate well under water, and 21 that germinate under boiled water sealed with paraffin oil. In none of these was there total absence of oxygen, for the seeds themselves contained some oxygen within the intracellular spaces and even the boiled, paraffin-sealed water permitted some diffusion from air. Among the seeds germinating in boiled water were: timothy, Bermuda grass, Canadian blue grass, lettuce, wormwood, celery, alfalfa, and *Petunia*. From this work it is evident that oxygen requirements for germination vary greatly even among seeds of land plants.

Now let us consider seeds that will not germinate in full air pressure because the low permeability of the coats to oxygen reduces the oxygen supply to the embryo below that needed for growth. From all that has been said above, such species of seeds might be thought to be rather rare, and they probably are. It is a different story, however, when the restriction offered by coats is further increased by environmental factors, such as submersion in water, in water-logged or packed soil, or in soil rich in carbon dioxide.

The best understood case of seed coats restricting the oxygen supply to the embryo below the minimum needed for germination is the cocklebur. Farmers had claimed that one of the two seeds in the cocklebur germinates the first season after maturity and the other the second season. Arthur [3] investigated this claim of the farmers and in the main confirmed it, although in some cases both seeds in a bur germinated during the first season and in some burs the second seed did not germinate until the third or a still later season. He found that the two seeds differed in their size, shape, and position in the bur. One was borne higher up in the bur, was smaller and convex on its outer face and concave on the inner face. He termed this the "upper seed." The other was borne lower in the bur, was concave on the outer face and convex on the inner face, and was called the "lower seed." The lower seed is the one that germinates in the first season

after maturing. Both seeds absorb water readily, and neither the bur nor the old dried ovary wall seems to play any considerable part in the delay. Fig. 23 shows the bur, the two seeds, and the arrangement of the seeds in the bur. Without evidence except that enzyme differences were used to explain many plant responses, Arthur concluded that enzymes were more abundant or developed faster in the lower seed, and consequently it germinated more promptly.

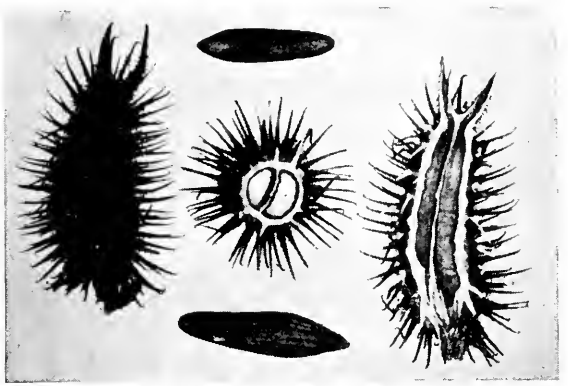

FIGURE 23. The cocklebur. *Upper row:* upper seed. *Middle row:* intact bur, cross section of bur showing the two seeds, longitudinal section of the bur showing the two seeds. *Lower row:* lower seed.

Crocker [19] attempted to explain this delay in the germination of the upper seed of the cocklebur. He observed the following facts: Germination failure of the upper seed, with the coat intact and either in or out of the bur, is due to the seed coat and not to the bur or ovary wall; this is true in spite of the fact that the three-layered seed coat is very thin, about 0.034 mm at the cotyledon end and 0.145 mm at the radical end of the seed. When the excised seeds or the seeds in the bur are placed in a germinator in air at 22° C (71° F) the lower seed only germinates. When placed in a germinator with a full atmosphere of oxygen at 22° C (71° F) both seeds germinate, but the growth in the upper seed starts in the cotyledons where the coat is thin. When placed in a germinator at 33° C (91° F) with air, both seeds germinate. When the seed coats are removed, both embryos germinate promptly, even at 18° or 20° C (64° or 68° F). Pricking the coat of the upper seed with a pin causes it to grow in a germinator at 20° C (68° F) in air, but the growth starts in the region of the prick.

From these results Crocker [19] concluded that both seeds would germinate in the first season after harvest if the seed bed reached a temperature as high as 33° C (91° F) at a time of adequate water and air supply; that the failure of the upper seed to germinate at lower temperatures was due to the fact that the thin seed coat reduces the supply of oxygen to the embryo below the minimum needed for germination; and that the oxygen supply to the embryo of the lower seed was restricted by the coats, for it too germinated at a somewhat lower temperature when the coat was removed. Naturally, one inquires why the upper seed germinates during the second or later seasons. Crocker suggested that the delicate semiper-

meable membrane is slowly decomposed by organisms in the soil. In other types of seed dormancy to be discussed later, we shall see that microörganisms in the soil play an important part in eliminating coats as factors in seed dormancy; moreover, Thornton [120] shows that the seed coat of *Xanthium* is rendered permeable by very slight injury. Whether the higher temperature increased the permeability of the coats to oxygen, or reduced the oxygen supply or pressure needed for the germination of the embryo of the upper seed and thus forced the germination, was later partly answered by Shull and still later by Thornton.

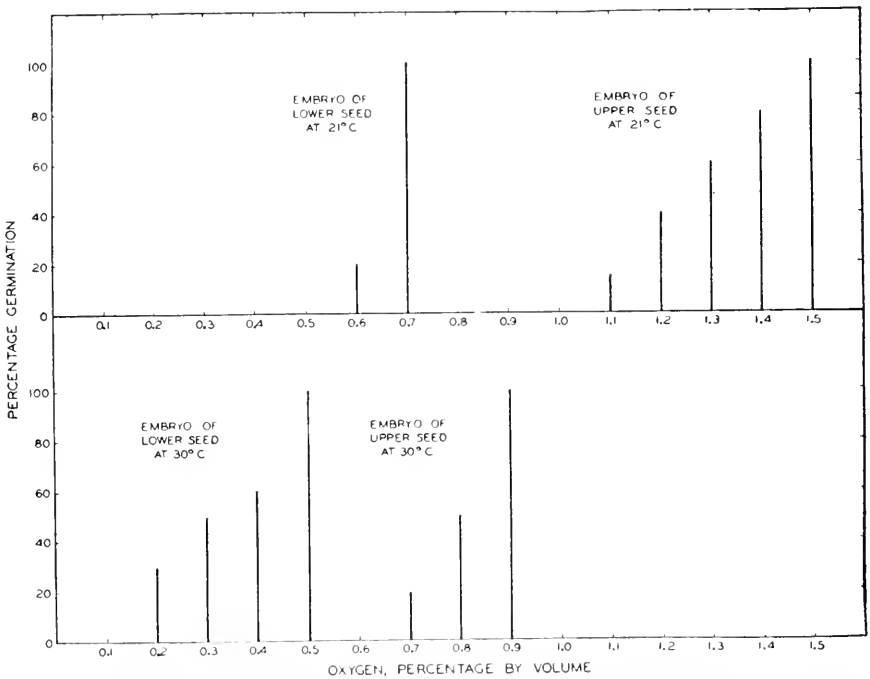

FIGURE 24. Minimum oxygen required for germination of the naked embryos of cocklebur during six days at 21° and 30° C (70° and 86° F).

Shull,[109, 110] using Schaible's method of reduced atmospheric pressure, determined the minimum O_2 pressures under which the naked embryos of the upper and lower seeds of *Xanthium* would germinate at 21° and 31° C (70° and 88° F); for the upper embryos it is 12 mm at 21° C (70° F) and 7 mm. at 31° C (88° F) and for lowers 9.5 and 3 mm at the respective temperatures. The thin coats are extremely effective in reducing the O_2 absorbed by the embryos in upper seed at 21° C (70° F); 12 mm of O_2 pressure is required with naked embryos and 760 mm, or 63 times as much, for the intact seeds. Naked lowers as against intact lowers absorb 2½ times as much O_2 and naked uppers 5 times as much as intact seeds.

Thornton,[120] using full atmospheric pressure with reduced or increased

percentages of O_2, determined the percentage germination of naked embryos and intact seeds of both seeds of the cocklebur at 21° and 30° C (70° and 86° F) at a range of O_2 percentages. Upper naked embryos (Fig. 24) at 21° C (70° F) require 1.5 per cent and at 30° C (86° F) 0.9 per cent O_2 for 100 per cent germination; lowers under like conditions require 0.7 and 0.5 per cent O_2. Note that complete germination of the lower embryos occurs at percentages of O_2 that give no germination in the upper embryos. Higher temperature in both lowers and uppers reduced the O_2 percentage needed for germination. Fig. 25 shows percentage germination of both

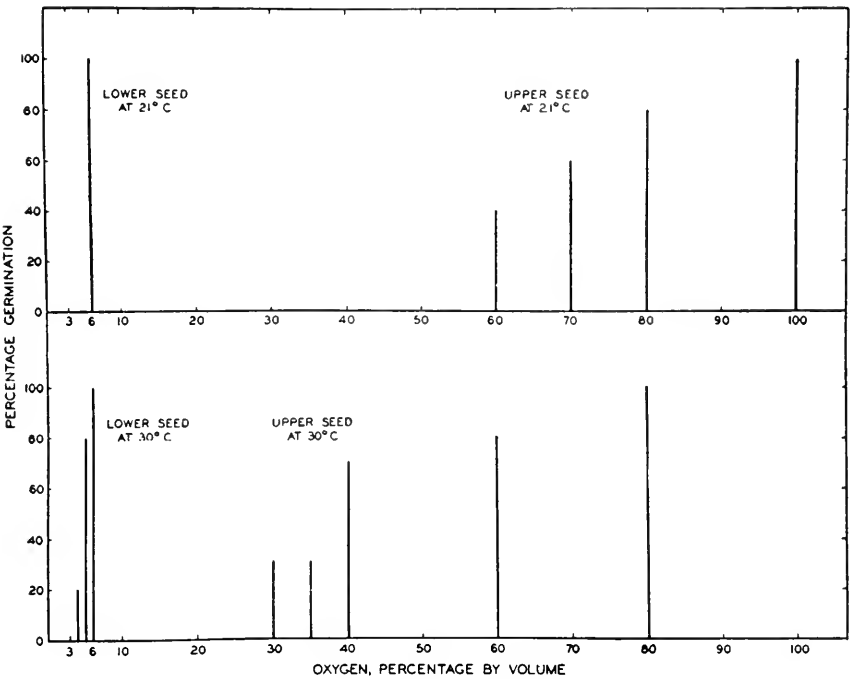

FIGURE 25. Minimum oxygen required for germination of the intact cocklebur seed during six days at 21° and 30° C (70° and 86° F).

upper and lower intact seeds at like temperatures and range of O_2 percentage. Intact seeds require higher percentage of O_2 for germination than do naked embryos. The naked embryo of the upper seeds gives 100 per cent germination in 1.5 per cent O_2, the uppers with coats intact require 100 per cent, or 66 times the O_2 pressure. Exact quantitative comparisons between Shull's and Thornton's data are impossible since they worked with different species and used somewhat different temperatures and duration of experiments. They do, however, agree on all essential points: naked embryos of both upper and lower seeds need definite, easily measurable minimum O_2 pressures for germination at various temperatures; minimum pressure needed for the upper embryo is always higher than that

for the lower; that the minimum oxygen pressure needed for the germination of the naked embryos falls decidedly with a rise of 9° to 10° C (16° to 18° F) in temperature; that the intact coat of the upper seeds, through its low permeability, increases the required oxygen pressure for germination more than 60-fold; and finally, that one factor leading to the germination of the intact upper seed in air at higher temperatures is the lower minimum oxygen pressure required for the germination of the embryo. Crocker, Shull, and Thornton all agree that the failure of the upper intact seed to germinate in air at temperatures below 30° C (86° F) is due to the low permeability of the seed coat to oxygen; hence the upper seed remains dormant in the soil at lower temperatures. It is of interest but of no known significance in dormancy that on an equal dry weight basis the embryo of the lower seed is richer in catalase [111] than that of the upper seed. On the basis of his data, Shull agrees with Crocker in concluding that oxygen has its function in the germination of cocklebur seeds in producing sufficient aerobic respiration to furnish the necessary energy for growth and that it does not act merely as a stimulus, as Becker, Lehmann, and others have assumed. The cocklebur embryos probably rank high among seeds in their need for the energy from aerobic respiration for growth.

A few other dormant imbibed seeds have been found in which increased oxygen pressure will force them to grow. Atwood [5] and Johnson [67] have found that dormant, recently harvested wild oat grains are forced to germinate by increased oxygen pressure, and Harrington [61] has found the same for freshly harvested cereals. Spaeth,[112] for American basswood, and Stier,[116] for freshly harvested potato seeds, find that the portion of the seed coat derived from the nucellus inhibits the passage of oxygen. A number of other similar cases could be mentioned. For years we have been studying the mechanics of dormancy in seeds in the seed laboratory at this Institute and we always try increased oxygen pressure for forcing. We have found very few cases where this is effective in contrast to the many kinds of seeds that are dormant because of hard coats, because of dormancy of the embryo, or because of the coats limiting the absorption of water. The findings for the cocklebur, as interesting and definite as they are, may not explain the dormancy of any considerable number of different kinds of seeds.

There is another way in which oxygen pressure is involved in dormancy of seeds. As described in a previous chapter, Davis [26] and Thornton [120] have shown that *Xanthium* and *Ambrosia* embryos can be thrown into dormancy by keeping them in germinators at higher temperatures with subminimal oxygen pressure for germination. In the case of after-ripened seeds of *Ambrosia*, the thin seed coats reduced the oxygen supply to the embryos sufficiently at high temperatures to induce dormancy. In *Xanthium* reduced oxygen pressure was necessary, in addition to the intact coats. As mentioned in the last chapter, other unfavorable factors in the germinator also induce secondary dormancy.

The oxygen pressure relation to germination is not as simple as it might seem from the statements above. Thornton found that high percentages of carbon dioxide, especially 40 to 80 per cent, lowered greatly the minimum oxygen pressure needed for the germination of intact upper seeds at 25° C (77° F). He did not determine whether this increased the permeability of the coats to oxygen or lowered the oxygen pressure needed by the embryo for germination as does a rise in temperature. Harrington [60] found that 60 to 80 per cent carbon dioxide is effective in forcing dormant Johnson grass seeds in which oxygen supply is not a limiting factor. Later, Thornton [121] found that similar concentrations of carbon dioxide were effective in forcing the germination of intact lettuce seeds at 35° C (95° F), a temperature many degrees above the maximum germination temperatures in absence of carbon dioxide.

As we have seen above, the upper seed of *Xanthium* is almost unique among seeds, in that the thin coat reduces the oxygen supply below the minimum needed for germination at lower temperatures. It is likewise peculiar in that it prevents the germination at low temperatures but not at high temperatures, while in the other two seeds studied in this respect, *Ambrosia* and lettuce, the thin coats prevent the germination at higher temperatures but not at lower temperatures.

Dormant Seeds that Respond to a Single Period of Moist Low-Temperature Stratification

There is probably no other condition — unless it is breaking the coats, which is mainly impractical — that will overcome the dormancy of as many different kinds of temperate-zone seeds as placing them in germination conditions at a low temperature for periods varying from a few days to many months, according to kind and condition of the seeds to be treated. In the earlier and century-old practice, the seeds and sand were laid down in successive horizontal layers and the stratified mass exposed to low temperatures during the winter; hence the term "stratification." In recent practice the seeds are mixed with moist sand, granulated peat, or other medium and exposed to low temperature for the desired time. Many workers still use the term "low-temperature stratification" for the newer practice. For seeds of many water plants, which need little oxygen, water is a good stratification medium. Low-temperature stratification imitates nature's methods of after-ripening seeds in the temperate zone; the seeds fall to the ground in the fall and are more or less covered in the cold soil during winter. Artificial stratification has the advantage of making possible the holding of the several stratification factors (temperature, moisture, and oxygen) at the optimum, which produces the quickest possible results. In nature these factors, especially temperature, are at the optimum only a portion of the time. Fall, winter, and early spring sowing of many seeds that respond to low-temperature stratification is a fair substi-

tute for stratification and has advantages over nature's method in that the seeds are well covered with soil.

For a long time it was assumed that after-ripening in stratification was brought about by freezing or by freezing and thawing. This is almost, although not quite, entirely wrong. The changes involved in low-temperature after-ripening of seeds occur in the main at temperatures above freezing, ranging from 1° to 15° C (34° to 59° F), for various sorts of seeds.

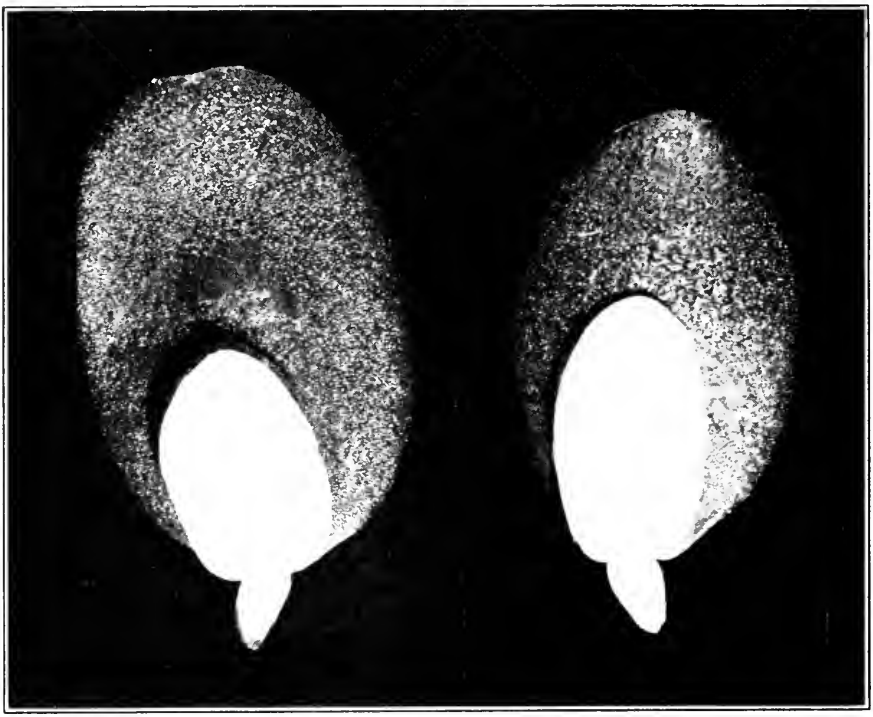

FIGURE 26. Excised embryos of control seeds of *Sorbus aucuparia* showing the development of the cotyledon in contact with the moist filter paper for 21 days.

These are temperatures at which essential metabolic changes occur within the living tissues of the seeds. The exceptions which may involve freezing or freezing and thawing are seeds of certain water plants, like *Alisma*,[103] as well as some others in which the seed is held dormant entirely by the coats and in which freezing and thawing rupture or weaken the coats. Even such seeds are generally after-ripened by low-temperature stratification a little above freezing. It will be best to discuss the two different physiological groups of seeds that respond to low-temperature stratification under separate headings: (*a*) seeds with dormant embryos, and (*b*) seeds with non-dormant embryos held dormant solely by the coats.

Seeds with Dormant Embryos. Because the embryos are dormant in this type of seed it must not be assumed that the coats are not important

in their dormancy. Indeed, one cannot determine whether the embryos are dormant until they are removed and put into a germinator. The dormancy in the embryo does not manifest itself by complete inability to grow when the embryo is removed from the coats and placed in a germinator, but rather by a marked sluggishness in early growth and by a dwarfishness in the part of the seedling derived from the epicotyl. Once the hypocotyl starts to germinate, it forms a normal vigorous root system, just as does the hypocotyl of an after-ripened embryo.

A number of investigators [19, 27, 30] early showed that embryos of certain rosaceous seeds are dormant. They also showed that these embryos are

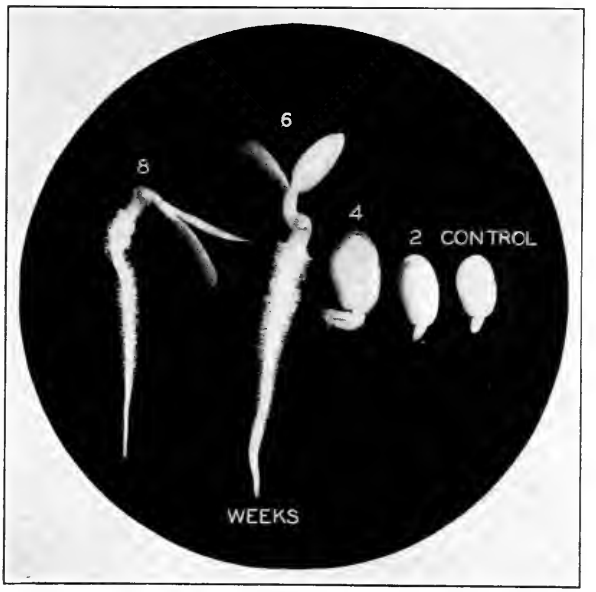

FIGURE 27. Excised embryos of seeds of *Sorbus aucuparia* which had been stratified for various weeks at 1° C (34° F) after three days on moist filter paper.

after-ripened by low-temperature stratification and that the time required for after-ripening the embryos in stratification is shortened if the pericarps and seed coats are removed.

Sluggish growth of dormant embryos. It was left, however, to Flemion to make a thorough-going study of the physiology of dormant embryos. The sluggishness of the dormant embryo of *Sorbus aucuparia* [34] is shown by Fig. 26. After the excised dormant embryos have lain on moist filter papers in the light for 21 days, only the cotyledon in contact with the paper has grown and become green. This indicates great resistance to the movement of water through the embryo, which results in an insufficient supply of water to the other cotyledon and the hypocotyl for growth. Fig. 27 shows the great increase in vigor of growth of the embryo caused by stratifying the intact seeds at 1° C (34° F) for two months and then

excising the embryo and placing it on moist filter paper. The non-after-ripened embryo showed growth only in the cotyledon lying against the moist filter paper after 21 days, while the after-ripened embryo showed some enlargement of both cotyledons and a spreading apart of the cotyledons and an elongation of several centimeters of the hypocotyl after only three days. One is struck by the enormous increase in growth vigor of this embryo induced by six weeks of low-temperature stratification. In this same figure, one seed that had four weeks' stratification partially removed the dormancy since there is some growth after three days. There is no growth in the embryo from a seed stratified only two weeks.

FIGURE 28. The degree of after-ripening attained by seeds of *Sorbus aucuparia* which had been stratified for two months at various temperatures, as shown by the changes in the excised embryos after being on moist filter paper for two days.

Correlation in the growth of the several organs of the dormant embryo is changed by after-ripening at low temperatures. In after-ripened embryos and embryos of non-dormant seeds the hypocotyl and radical grow first, and a root is formed with abundant root hairs. Later the cotyledons begin to grow and spread apart, and still later the epicotyl develops. In dormant embryos not only is the growth much slower but the cotyledons grow first — often only one of them if it alone is in contact with water; also, the epicotyl often elongates before the hypocotyl and radical. Fig. 28 shows the effectiveness of various low temperatures in after-ripening the embryos of intact *Sorbus* seeds in two months: 1° C (34° F) gives greatest vigor, 5° C (41° F) next, while −5° C (23° F) and weekly alternations at −5° and +5° C (23° and 41° F) are ineffective. It is evident that temperatures above freezing are effective, whereas freezing temperatures and

freezing and thawing are not. Although the optimum temperature for after-ripening of *Sorbus* seeds is nearer 1° C (34° F) than 5° C (41° F), that for other rosaceous seeds with dormant embryos such as some species of *Rosa* (Fig. 29) is about 5° C (41° F). The best optimum temperature for the stratification of *Rhodotypos* seeds [35] is about 5° C (41° F). Fluc-

FIGURE 29. *Rosa rubiginosa* seeds: check stored dry; the others in moist sand for six months at the temperatures designated and then planted in a flat in the greenhouse. The picture shows the effective temperature for stratification, that is, 5° C (41° F).

tuating temperatures within a certain range are effective; for instance, for *Rhodotypos* seeds daily alternating (16 hours at low temperature and 8 hours at high temperature) or weekly alternating temperatures (half time at each temperature) of 1° and 10° C (34° and 50° F), 1° and 15° C (34° and 59° F), and 5° and 10° C (41° and 50° F) proved about as effective as, and in some cases better than, the constant temperature 5° C (41° F). Table 11 shows the optimum stratification temperatures,[12] the effective range, and the length of time required for complete after-ripening of various seeds that have been studied at this Institute, with the addition of three studied earlier at the University of Chicago. The three additional ones are: *Crataegus mollis*, *Acer saccharum*, and *Juniperus* species. This table includes seeds with both dormant and non-dormant embryos that respond to low temperatures. This list could be considerably extended by drawing upon literature from other sources. It will be noticed that a number of seeds have an optimum stratification temperature of about 1° C (34° F), though most of them have 5° C (41° F) as the optimum. A number have 10° C (50° F) as the optimum, and some after-ripen equally well over a rather wide range, 1° to 5° C (34° to 41° F) or 1° to 10° C (34° to 50° F).

The degree of sluggishness in the early growth of excised dormant em-

Table 11. Effective Moist Pretreatment for Seeds Benefiting by Period of Low Temperature Before Greenhouse Planting.

Species	Best temp. (° C)	Effective range (° C)	Days at best temp.
Abies arizonica	1	1–5	30
Acer saccharum	5	5	35
Alisma Plantago-aquatica	5	1–10	30–60
Amelanchier canadensis	5	1–5	90
Aralia hispida	5	1–10	90–120
Arbutus menziesii	5	1–5	42
Asimina triloba	10	1–10	100
Belamcanda chinensis	5	5–10	120
Benzoin aestivale	5	5–10	120
Betula lenta *	5	1–10	60–75
" *lutea*,* *B. papyrifera**	5	1–10	30–60
Butomus umbellatus	5	3–10	30–120
Carya ovata	5	1–10	90
Celastrus scandens	5	1–10	60–90
Celtis occidentalis	5	1–10	60–90
Cornus florida	5	1–10	120
" *Kousa*	5	5	135
Crataegus coccinea	5	5	180
" *mollis*	5	5	75–90
" *mollis* †	5	5	135
" *Phaenopyrum*	1	1–10	60
Cupressus macrocarpa	5	5–10	60
Dictamnus albus	10	5–10	60
Diospyros virginiana	5	1–10	30–75
Gaultheria procumbens	1	1–5	60–90
Gentiana acaulis	5	1–10	60–90
" *Andrewsii*	1	1–5	30–60
" *crinita*	5	1–5	90–120
Hamamelis virginiana	5	1–5	60–90
Impatiens biflora	5	1–10	75
Iris versicolor	3	1–10	60–120
Juglans cinerea, *J. nigra*	5	5	100
Juniperus communis, *J. depressa*, " *prostrata*, *J. virginiana*	1–5	1–10	30–60
Libocedrus decurrens	1–10	1–10	30–60
Liquidambar Styraciflua	1–10	1–10	70
Liriodendron Tulipifera	5	1–5	150–180
Mitchella repens	5	1–10	90
Myrica carolinensis	10	5–10	60–90
Nyssa sylvatica	5	1–10	30–60
Physocarpus opulifolius	1	1–5	30–60
Picea canadensis, *P. excelsa*	5	1–10	30
" *Omorika*	5	1–10	42–60
" *pungens*	1	1–10	30–60
" *sitchensis*	5	1–10	30–60
Pinus austriacus	1	1–5	30–60
" *Banksiana*	5	1–15	30–60
" *caribaea*			

Table 11. — (*Continued*)

Species	Best temp. (° C)	Effective range (° C)	Days at best temp.
Pinus contorta	5	1–10	30–60
" *Coulteri*	5	1–10	30
" *densiflora*	10	1–10	30
" *echinata*	5	1–15	30–60
" *flexilis, P. insignis*	5	1–10	30–60
" *koraiensis*	10	1–10	30–60
" *Lambertiana*	5	1–10	90
" *Laricio*	1–5	1–10	30–60
" *monticola*	1	1–10	60–90
" *ponderosa*	1–5	1–10	30–60
" *resinosa*	1–10	1–10	30
" *rigida*	5	1–10	30
" *Strobus*	10	1–10	60
" *Taeda*	5	1–15	30–60
" *Thunbergii*	5	1–10	30
Polygonum acre	1–10	1–10	30–60
" *arifolium*	5	1–5	90–120
" *lapathifolium*	10	1–10	30
" *pennsylvanicum, P. virginianum*	10	1–10	60–90
Prunus americana	5	1–5	150
" *persica*	5	5–10	60–90
Ptelea isophylla	1	1–5	60–120
" *serrata*	1	1–5	150
" *trifoliata*	5	5	60–90
" *trifoliata* var. *mollis*	5	5	30–90
Pyrus arbutifolia	1	1–5	90
" *arbutifolia* var. *atropurpurea*	10	1–10	60
" (French pear)	5	1–5	60–90
" *Malus* var. *niedwetzkyana*	5	1–5	60
Ribes Grossularia	5	1–10	90–120
Rosa multiflora	5	5–8	50
Scirpus campestris var. *paludosus*	5	1–10	90–120
Sequoia gigantea	5	1–5	30–60
Smilacina trifolia	5	1–10	90–120
Sorbus aucuparia	1	1–5	60–120
Taxodium distichum	5	1–10	30
Thuja gigantea	5	1–10	30–60
" *occidentalis*	5	1–10	30
" *orientalis*	5	1–10	60
Typha latifolia	5	1–10	30
Vitis aestivalis	5	1–10	90
" *bicolor*	5	5	90–120
" (Concord grape)	5	5–10	90
" (Delaware grape)	5	5	90
Zizania aquatica	5	1–10	30

* Transferred to higher temperature ovens instead of greenhouse.
† Pericarps removed.

bryos varies considerably with the variety and species. Apple, pear, most *Crataegus* species, *Prunus americana*, and others behave much like the embryo of *Sorbus* shown in Fig. 26, in which only the cotyledon in contact with water grows; also in some of these the epicotyl elongates without any growth of the radical and hypocotyl (Fig. 30) when the seeds are placed on moist filter paper. The dormant peach and *Rhodotypos* embryos

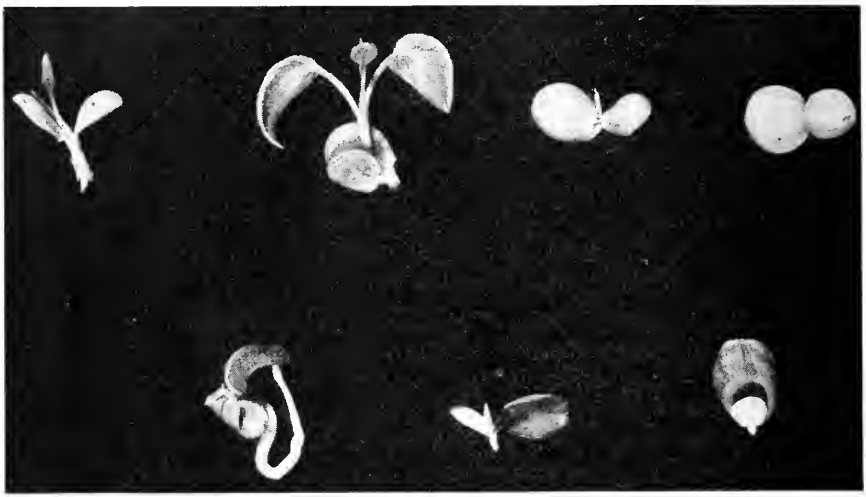

FIGURE 30. Excised non-after-ripened apple embryos grown in Petri dishes 14 days at room temperature.

are less sluggish than the ones mentioned above. The latter in the main show growth of both cotyledons and the hypocotyl and radical when placed on moist filter paper, but even in these the growth of the non-after-ripened embryo is very much slower than that of the after-ripened embryo.

Sorbus embryos attain sufficient after-ripening after six weeks' stratification at 1° C (34° F) in intact seeds to give vigorous early growth, as shown by their development when excised; but 12 weeks' stratification is needed for the intact seed to give full germination. Very recent unpublished work by Flemion indicates that the dwarfishness in the later growth of the embryo is not entirely overcome until the intact seeds have been stratified long enough to enable them to germinate completely. Seeds with dormant embryos will finally germinate at the optimum stratification temperatures, but they will do so more quickly if they are transferred to a somewhat higher temperature after complete after-ripening. Transferring partially after-ripened seeds to germinators at 20° C (68° F) is likely to throw them into secondary dormancy, and even fully after-ripened seeds do better if planted at relatively low temperatures such as are met in early spring planting. Indeed, Pack [89] found that various species of after-ripened *Juniperus* seeds germinated faster at the best after-ripening tem-

perature, 5° C (41° F), than they did at 10° C (50° F), and that 15° C (59° F) or higher temperatures were unfavorable for germination.

Dwarfish plants from dormant embryos. While dormant embryos grow very slowly and show unusual correlations in the growth of their several organs, Flemion [36, 38] was able to get seedlings from most of them by use of proper methods; she finally obtained plants by transferring the seedlings to soil. If placed in aerated water at room temperature so that water is in contact with the whole surface of the embryo, excised *Sorbus* embryos slowly form a seedling with the hypocotyl-radical elongated and both

FIGURE 31. *Rhodotypos kerrioides* seedlings. *Left:* grown from non-after-ripened embryos. *Right:* grown from after-ripened embryos.

cotyledons enlarged. Excised peach, apple, and hawthorn embryos can be germinated by placing them in moist peat at 25° C (77° F). This treatment will not give seedlings with dormant *Sorbus* embryos. The seedlings grown from dormant embryos at higher temperatures form dwarfish tops, although the root systems grow vigorously. Fig. 31 shows, at left, seedlings of *Rhodotypos* grown from dormant embryos and, at right, seedlings grown from low-temperature after-ripened embryos. The dwarfed seedlings have very short internodes and thick, deep green leaves in contrast to the long internodes and thin, lighter green leaves of the seedlings from the after-ripened embryos. Fig. 32 shows, at left, a seedling grown from a dormant peach embryo and, at right, a seedling from a low-temperature after-ripened embryo. In the dwarfed peach seedlings the internodes are very short, the leaves thicker, deeper green, and shorter, and often much deformed. To date, Flemion has been able to produce dwarfish seedlings from all species and varieties of rosaceous seeds with dormant embryos that she has tested, as well as with witch-hazel seeds; but her studies have not been extended to seeds of other families of plants with dormant embryos.

This dwarfishness in the seedling may persist for weeks, months, or even years or until some growth condition after-ripens a bud (generally a lateral bud) so that it acquires vigor of growth. The most effective condition now

FIGURE 32. Peach seedlings. *Left:* grown from non-after-ripened embryo. *Right:* grown from after-ripened embryo.

known for after-ripening such a bud is a period at low temperature. If the dwarfed seedling is placed at 5° C (41° F) for six weeks and then placed at a good growing temperature, one of the buds begins vigorous growth similar to that shown by the epicotyl of an after-ripened embryo. Fig. 33 shows a picture of a dwarfed seedling of *Rhodotypos* at two stages of dwarfish growth and the later vigorous growth of the terminal bud. Lammerts [74] has found that high temperatures will throw buds of the dwarfish peach seedling out of dormancy and that the rate of elongation of such buds is favored by a long day, especially by continuous illumination.

From what has been said above, it appears that low-temperature stratification does two things to dormant embryos: it overcomes the sluggishness in their early growth and it after-ripens the epicotyl so that in its later growth it forms a vigorous rather than a dwarfish plant above ground. The epicotyl of the seed is, of course, a bud and the second phase of low-temperature after-ripening described above is a phenomenon that occurs

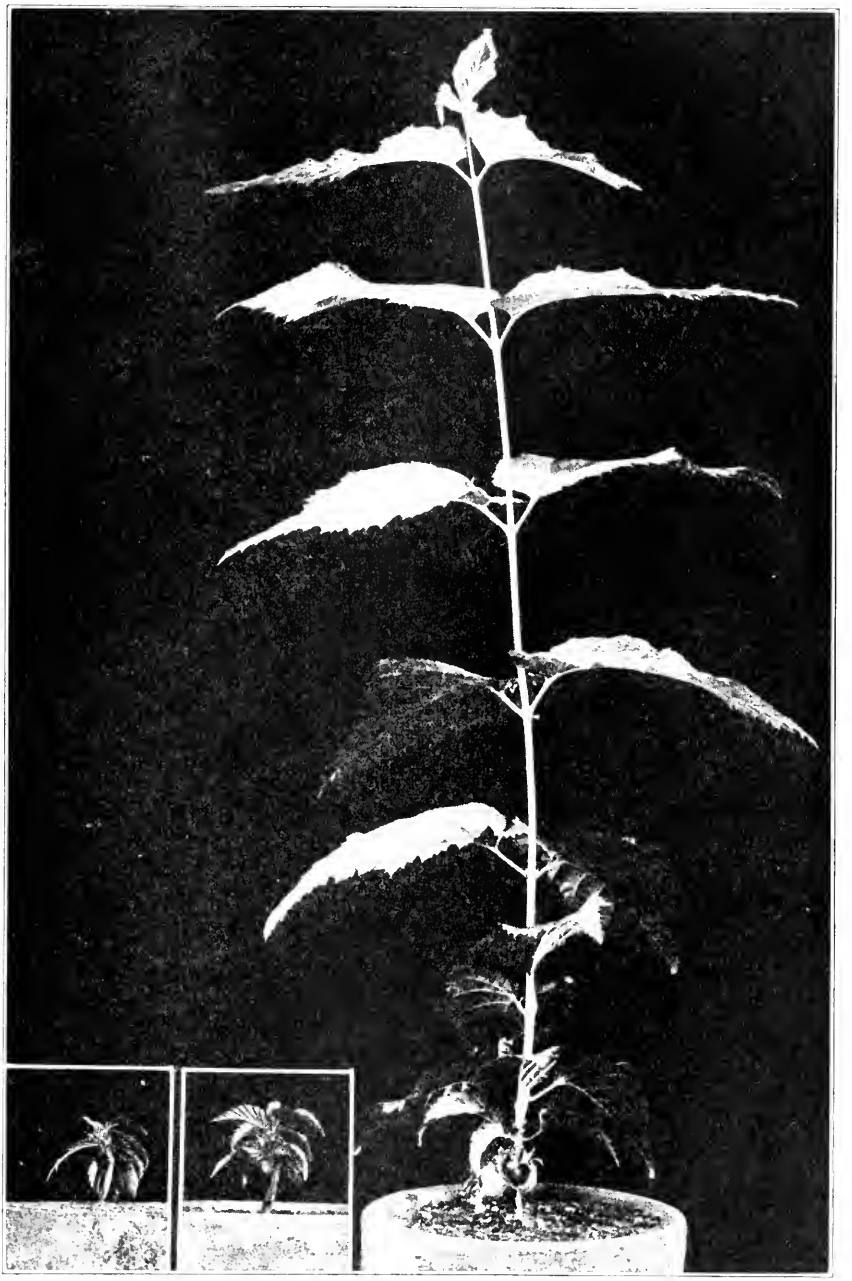

FIGURE 33. A plant from a non-after-ripened embryo of *Rhodotypos kerrioides* photographed 122, 161, and 226 days after planting. This illustrates the normal growth of the terminal bud which took place during the interval between 161 and 226 days.

generally in buds of trees and shrubs of the temperate zone. The buds go into the winter in the dormant condition and are after-ripened by the cold weather so they grow with vigor in the spring. Consider one example, the peach tree. Its leaf buds are after-ripened by the cold weather of winter and consequently take on vigorous growth in the spring. If one goes into Georgia he will note that the peach trees are smaller because of insufficient after-ripening of the buds during the short winters. If one goes far enough south, the peach no longer even persists, probably because there is not sufficient winter to after-ripen the buds even partially. The buds of trees of low-altitude tropical plants do not require low temperatures to after-ripen the buds and give them growth vigor. Perhaps one could select strains of peaches that had this need to a less degree and extend the culture farther south.

It is evident that the epicotyls of many seeds are not dormant in the sense we have just described and consequently do not need low-temperature after-ripening. However, we shall later discuss classes of seeds having dormant epicotyls that need low-temperature after-ripening but that do not have a sluggish growth in other parts of the embryo. In classes of seeds to be described later we shall frequently meet dormant embryos that require low-temperature stratification for after-ripening.

Davis and Rose [27] believed that the radical is the dormant organ of the *Crataegus* embryo, but Flemion [36] has shown that the epicotyl is the dormant organ in this embryo. The radical is sluggish in the early growth of the embryo, but once started, it grows with vigor. On the other hand, the eipcotyl shows a persistent dwarfishness in growth until it is after-ripened by a period of low-temperature exposure. This could be discovered only by forcing dormant embryos to form seedlings without low temperatures and by continuing the growth of these seedlings over long periods.

Very recently Flemion and Waterbury [44a] have thrown some additional light on the persistence and nature of the dwarfishness in seedlings grown from dormant embryos. After-ripened peach embryos were deprived of all or part of their storage material. Death resulted when both cotyledons were removed at the time of planting; but when they were removed ten days later, small but normal seedlings were obtained. When various parts of the cotyledons were removed at the time of planting, normal plants resulted, except that the plants were smaller when only one-third of one cotyledon remained. However, none of these small plants had the telescoping of internodes or other dwarfing characteristics so typical of seedlings obtained from non-after-ripened embryos. Dwarfish seedlings have apparently adequate root systems, for in dry weight determinations the ratio of root to top was always greater in the dwarfs. When the growing tip of a normal seedling was grafted on the stem of a dwarfish seedling, the result was a normal seedling, showing that the root system of the dwarf was capable of sustaining normal growth and that apparently there was no substance in the root which inhibited shoot growth. Interposing by

grafting a portion of the normal stem on the dwarfs or a portion of the dwarf stem on the normal seedlings had no effect whatsoever on the subsequent growth of the normal seedling or in overcoming dwarfishness. When the growing tip of a dwarfish seedling was grafted on a normal seedling, the subsequent growth of this tip remained dwarfish. Thus the seat of this dwarfishness is in the growing tip and not in the root or stem. The breaking of seed dormancy in the peach by low temperature is in a sense a treatment for overcoming bud dormancy, for normal development is obtained by subjecting either the seed or the dwarfish seedling to the required period at low temperature.

Chemical and physiological changes occurring in dormant embryos during low-temperature after-ripening. There have been a number of investigations of the chemical and physiological changes occurring in seeds with dormant embryos during after-ripening at low temperatures. Eckerson [30] studied several species of *Crataegus* seeds using microscopic, chemical, and physiological methods. The storage substances in these are in the form of proteins and fats with little soluble sugar and no other assimilable carbohydrates. Also the foods are stored almost entirely in the cotyledons. The initial change is increased acidity. Correlated with this is an increased water-holding power; increased catalase and peroxidase activity occurred as after-ripening progressed. Soaking the seeds in dilute acids hastened the after-ripening and the changes mentioned above. Eckerson does not know whether the increased acidity is a correlative change with after-ripening or whether it holds a causal relation to after-ripening. There is some evidence for the latter. There was also a great increase in water absorption by the embryo as after-ripening progressed.

Jones [69] studied sugar-maple seeds which have proteins and fats as storage material but also contain more than 6 per cent cane sugar. The best after-ripening temperature is 5° C (41° F) and several weeks are required to complete the process. During after-ripening there is a great increase in catalase, a considerable increase in reducing sugar, and a slight increase in peroxidase. There is no increase in water-absorbing power and the embryo is alkaline in both mature and after-ripened seeds. Pack [89, 90, 91] studied the after-ripening of seeds of several species of *Juniperus*. These seeds have their stored foods in the form of proteins and fats with little sugar and no starch. The optimum temperature for after-ripening is 5° C (41° F) and the time required is about 100 days. Recently Webster and Ratliffe [127] have shortened the stratification period of *Juniperus virginiana* seeds to less than two months by treating the seeds with a weak solution of lye before stratifying. Pack [89, p.58] lists the following changes that occur in the seeds of *Juniperus* during low-temperature after-ripening: "(1) rather rapid and complete imbibition, followed by a steady slow decrease in water content during after-ripening or until near germination; (2) increased H^+ ion concentration, especially of the embryo; (3) an increment of titratable acid; (4) a steady and enormous increase in the degree

of dispersion of the stored fat; (5) decrease in the amount of stored fat and protein, with an increase of sugar content and the first appearance of starch; (6) the translocation of food in the form of fat or fatty acids from endosperm to embryo; (7) a seven-fold increase in the amino acid content, and a complete disappearance of histidine from the endosperm;

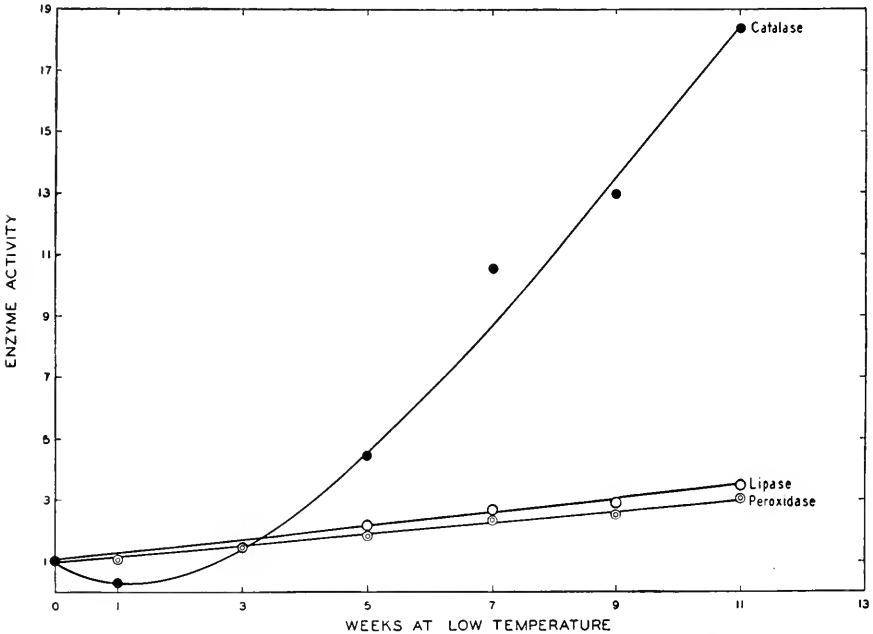

FIGURE 34. Catalase, peroxidase, and lipase activity of embryos of *Rhodotypos kerrioides* after various weeks in moist peat moss at 5° and 10° C (41° and 50° F) alternated weekly. The activities are calculated on the basis of the activity of the control expressed as one.

(8) an increase of soluble proteins with a marked hydrolysis of the stored proteins; (9) slight growth of embryo; (10) very slight increase of the respiration intensity; (11) increased respiratory quotient; (12) decreased intramolecular respiration; (13) a doubling of the catalase activity; and (14) the rise in vigor of seeds as shown by their resistance to fungal attack."

A later study of *J. scopulorum* seeds by Afanasiev and Cress [2] confirms many of the results found by Pack and shows that removal of the fruit and seed coats hastens the after-ripening of the seed in low-temperature stratification.

Flemion [35, p.158] summarizes the chemical and physiological changes occurring in *Rhodotypos* embryos during after-ripening as follows: "Analyses of the seeds at intervals of two weeks during the after-ripening period show that the seeds increase in catalase, peroxidase, and lipase activity and also increase in water absorption power, nitrogen-soluble in 80 per cent alcohol, titrable acid, and sucrose. The ether-soluble fraction de-

creases as after-ripening progresses." Fig. 34 shows the changes in catalase, lipase, and peroxidase as after-ripening proceeds. Lipase has the function in hydrolyzing the important storage substances, fats. Fig. 35 shows change in catalase, peroxidase, and lack of change in emulsin as *Sorbus* embryos after-ripen.[34] In this seed also there is no appreciable increase in amylase with after-ripening. This is to be expected, since there is no starch in the embryo until germination occurs.

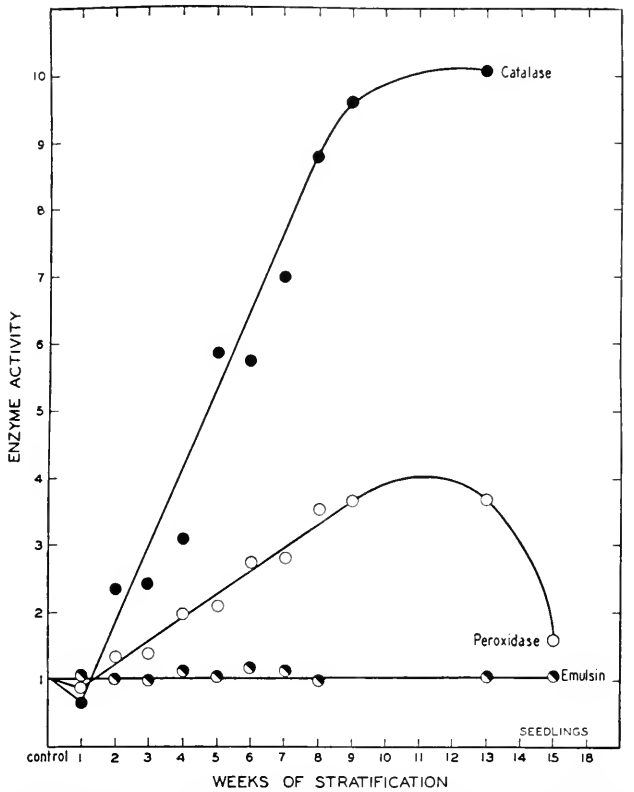

FIGURE 35. Changes in the catalase, peroxidase, and emulsin activities of *Sorbus aucuparia* seeds while after-ripening at 1° C (34° F).

In every case reported above, catalase has shown a great increase with degree of after-ripening of the seed or embryo. This enzyme decomposes hydrogen peroxide, and also organic peroxides; it is found in all living matter, increasing in general proportion but not in strict proportion to the physiological and metabolic activity. We know little about its function in organisms; it may aid in protecting them against over-accumulation of organic peroxides. The change in catalase content has received a great deal of attention in connection with after-ripening, germination, and storage of seeds. In connection with after-ripening, Fig. 36 is of interest. It shows the modification in the catalase content of *Sorbus* embryos main-

tained in germinators at various temperatures. At the optimum temperature for after-ripening (1° C, 34° F), except for a slight initial drop, there is a continuous rise until at the completion of the after-ripening, after

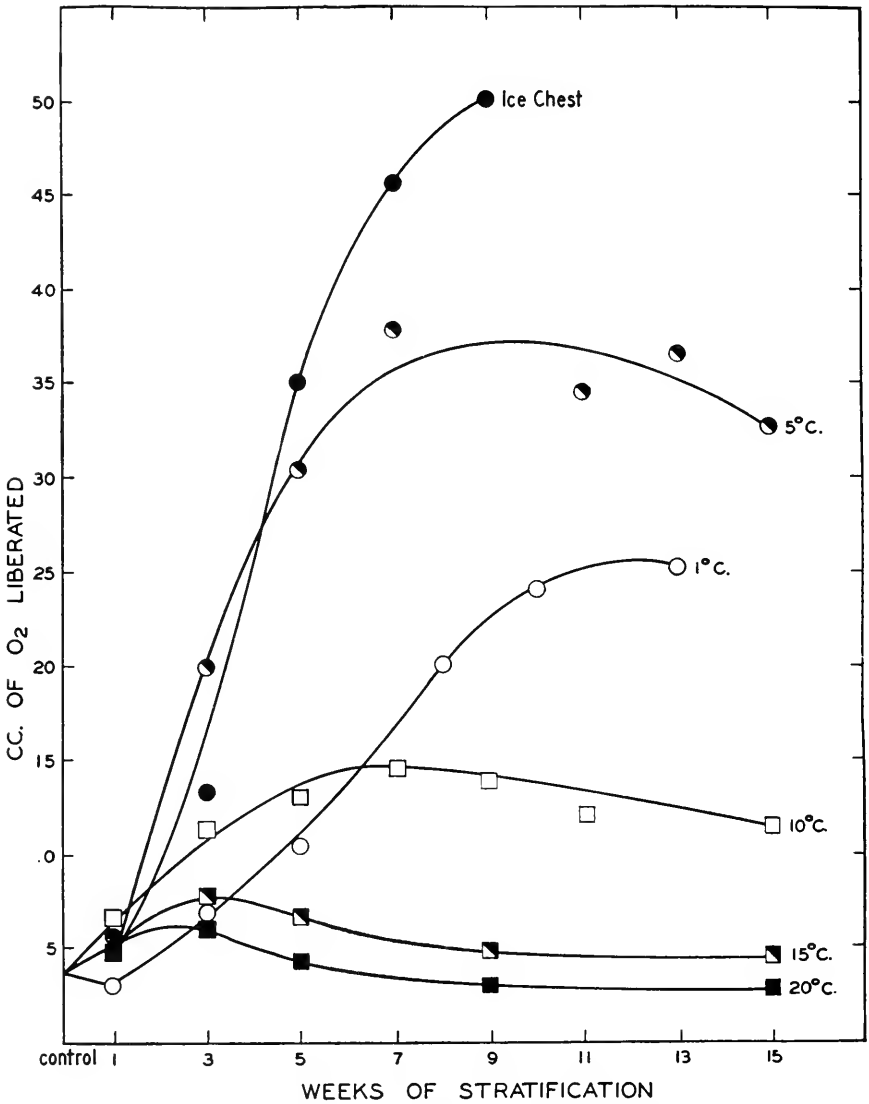

FIGURE 36. The catalase activities of *Sorbus aucuparia* seeds stratified for periods of 1 to 15 weeks at various temperatures.

12 weeks, the catalase has increased 5-fold. At the somewhat higher and variable temperature of the icebox, which is also a poorer temperature for after-ripening, it rose 12-fold in nine weeks. The catalase also rose faster at 5° C (41° F) than it did at the optimum after-ripening temperature. At

the still higher and still less favorable temperatures for after-ripening, the catalase content rose much less or actually fell after a small initial rise. While the catalase rises greatly with after-ripening of dormant embryos, the amount of rise is not a strict measure of the progress of after-ripening.

What is the nature of the changes brought about in seeds with dormant embryos during low-temperature stratification? First, there is an increase in enzymes, not only hydrolytic and oxidative — lipase, peroxidases, oxidases, catalase — but, judging from Pack's finding of a great increase in amino acid, probably proteases also; secondly, there is an accumulation of simple organic materials that can be readily used in building new tissues, sugars, amino acids, etc.; and finally, there is a transformation of insoluble, osmotically inactive substances to soluble, osmotically active ones, *i.e.*, fats to sugars and insoluble proteins to soluble proteins, amino acids, and other nitrogenous organic compounds. The formation of osmotically active substances may account for the free movement of water in after-ripened, rosaceous embryos in contrast to the difficulty of movement in dormant ones. The hypothesis that inhibiting substances may hold embryos in dormancy should not be forgotten, especially since the long stratification in moist medium gives good conditions for the outward diffusion of such substances. Opposed to this hypothesis, however, is the fact that there is a definite optimum temperature for after-ripening of any given dormant embryo and that the effective range of temperature in many cases is very narrow — also that the optimum is very low, 1° C (34° F) in *Sorbus*. If after-ripening were a matter of the leaching of inhibiting substances, one should expect a wide range of effective stratification temperatures in which high temperatures are more effective than low. As a matter of fact, high stratification temperatures make many dormant embryos still more dormant rather than after-ripening them. It is still harder to see how the continual dwarfishness in the epicotyl portion of the plant can be explained on the basis of inhibiting substances. The dormant slow-growing embryos are long in contact with moist peat or actually in aerated water before they are planted in soil; moreover, after they are grown in soil there is opportunity for any soluble inhibiting substance to move from the buds back into the stem and finally to the roots. Perhaps the dormancy in embryos is brought about by organization characteristics of the protoplasm involving insoluble substances.

Seeds with Non-dormant Embryos. Many seeds that do not have dormant embryos, as shown by the fact that they will germinate immediately and produce vigorous seedlings if the coats are broken, respond to low-temperature stratification. *Alisma Plantago* [21] seeds germinate readily and with vigor if the coats are broken. They also respond to low-temperature stratification in water, as shown in Table 11. Barton [12] and in unpublished work has shown the same to be true for *Butomus umbellatus*, *Scirpus americanus*, *S. campestris* var. *paludosus*, and *Zizania aquatica*, all the aquatic seeds she studied in this respect. The same is probably true of many other seeds

of water plants. The freshly harvested dormant cereals are forced to germinate by a few days of prechilling,[61, 105] or by use of low temperatures; also, breaking the coats forces these seeds to vigorous growth. Other dormant grass seeds have been found to respond to coat breaking and to stratification, or to prechilling in germinators. This is true of *Setaria macrostachya* [124] and other grass seeds.[125] Most dormant grass seeds germinate with vigor when the coats are broken or greatly weakened by treatment with sulphuric acid of various concentrations, and many respond to prechilling or stratification. A careful study of dormant seeds in this family will no doubt give a big list of seeds with non-dormant embryos that respond to stratification.

Barton [6] found that low-temperature stratification markedly increased the percentage germination and speed of germination of three of the southern pines: loblolly (*Pinus Taeda*), shortleaf (*P. echinata*), and slash (*P. caribaea*). Longleaf pine (*P. palustris*) seeds germinated much more promptly than seeds of the other three species but were benefited somewhat by stratification. Later, Barton [7] extended the studies to other pines and to several other conifers and found that seeds of many of these were benefited by low-temperature stratification. By examination of Table 11, it will be seen that *Abies arizonica*, *Taxodium distichum*, *Sequoia gigantea*, three species of *Thuja*, several species of *Picea*, and many species of *Pinus* are benefited by low-temperature stratification. Johnstone and Clare [68] confirm Barton's findings for Coulter's pine and show that seeds of the Torrey, Digger, knob-cone, and piñon pine are benefited by stratification.

Fig. 37 shows that one, two, or three months' stratification of *Pinus rigida* seeds at 5° C (41° F) gives full germination of practically all good seeds 15 days after planting, whereas the non-stratified seeds string along in their germination, giving only 30 per cent after 50 days.

Fig. 38 shows the effect of stratification on the germination of loblolly pine (*P. Taeda*) seeds. One to four months' stratification at 5° C (41° F) gave almost complete germination of all good seeds within 20 days. The germination of untreated seeds had scarcely started after 20 days and attained 40 per cent after 100 days.

In the spring sowing of coniferous seeds in nurseries, it is important to have seeds come up promptly and completely so the seedlings will have attained some size before the dry, hot days of summer. A month or so of low-temperature stratification just previous to spring sowing will accomplish this. This discovery is of great importance to nursery practice. Stratification is probably superior to fall planting, which is practiced in some nurseries. Stratified spring-sown seeds avoid the hazards of a winter in the soil incurring the danger of germination in mid-winter with later freezing and killing of the seedlings. Also the ravages of rodents are avoided. Stratification is also much simpler to apply and much more effective than light, which has been mentioned as a factor in the germination of certain coniferous seeds.

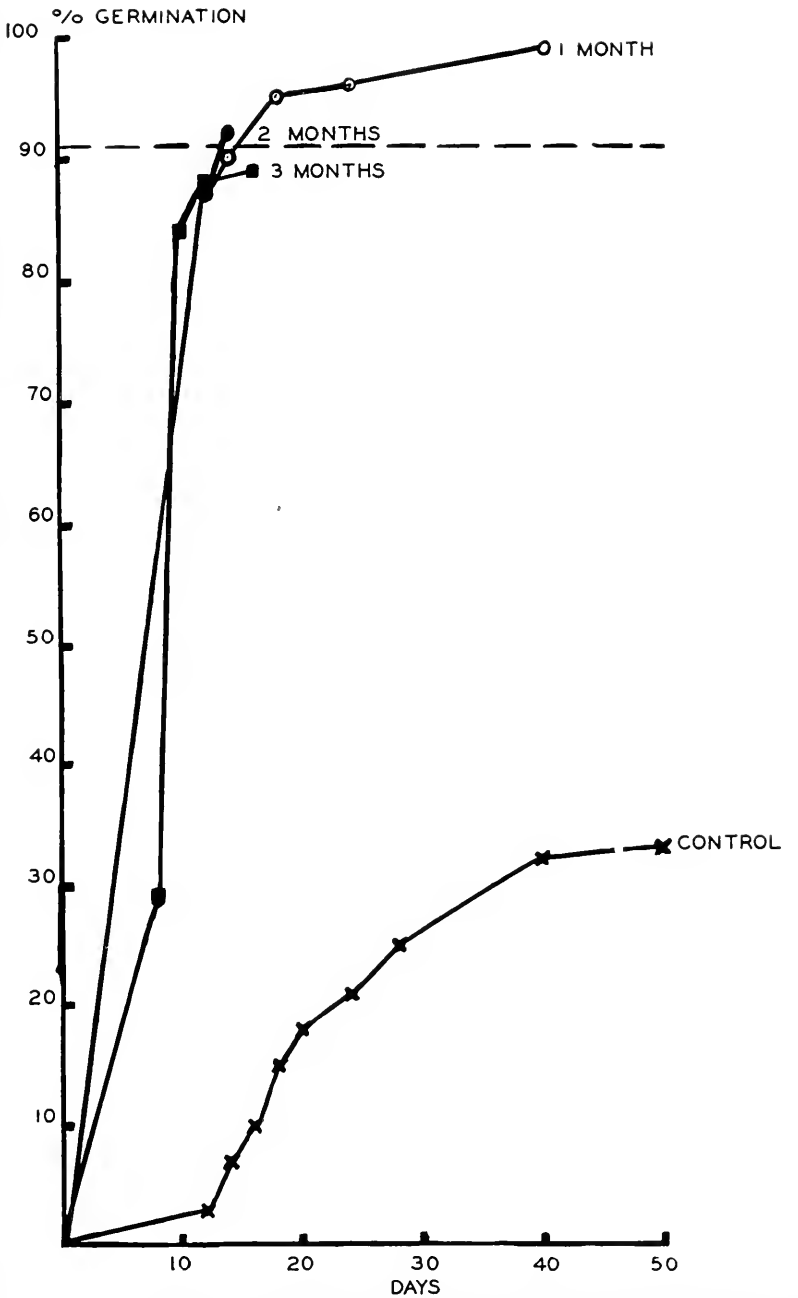

FIGURE 37. The effect of stratification at 5° C (41° F) for one, two, and three months on germination of the seeds of *Pinus rigida*. Broken line shows the percentage of good seeds as revealed by embryo tests.

We have classified coniferous seeds as seeds with non-dormant embryos that are benefited by low-temperature stratification. The main evidence that the embryos are non-dormant is the fact that conifer seeds in general will germinate and produce normal seedlings at 20° C (68° F) or above if

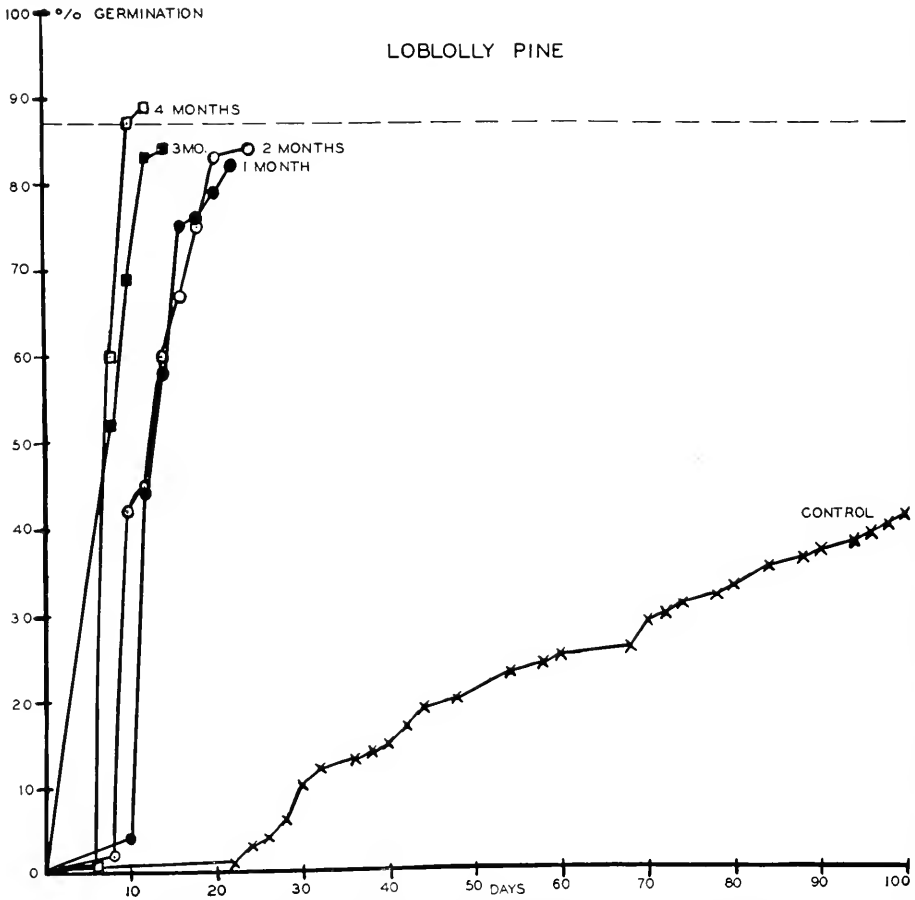

FIGURE 38. The effect of stratification at 5° C (41° F) for one, two, three, and four months on germination of loblolly pine seeds. Broken line shows the percentage of good seeds as revealed by embryo tests.

given sufficient time. One will see from the two sets of curves just mentioned that the time is likely to be rather long — more than 50 days for some species. Flemion in unpublished work has attempted to determine whether conifer embryos show any of the dormancy characteristics such as those already described for rosaceous seeds. Fig. 39 shows, at left, the typical growth of an Austrian pine embryo isolated from a seed that had been held in granulated peat three days at room temperature before it was excised and put into soil. At the right is shown a similar embryo taken from a seed after it had been in moist granulated peat at 5° C (41° F)

for one month before it was planted in soil. This is the desirable stratification time and condition for these seeds. In conifer seeds much of the stored food is in the endosperm. The difference in the growth of the embryo from the low-temperature stratification and that from the non-stratified seed may be explained by the movement of nutrients and accessory foods from the endosperm during the month of stratification. Flemion is planning experiments that will distinguish between nutritional effects of the endosperm and possible dormancy in the embryo.

FIGURE 39. Austrian pine seedlings. *Left:* seeds soaked for 3 days at 20° C (68° F) before excising the embryo for planting. *Right:* seeds stratified in moist peat for one month at 5° C (41° F) before excising the embryo for planting.

The question naturally arises, What are the effective changes that occur in dormant seeds with non-dormant embryos when they after-ripen in low-temperature stratification? This question cannot be answered at present. We have seen that both hydrolytic and oxidizing enzymes are formed or activated in seeds with dormant embryos, and that sugars, amino acids, and other soluble organic compounds are formed from more complex and less soluble compounds during low-temperature stratification. Perhaps similar changes occur in seeds with non-dormant embryos that give the embryos greater growing pressure. Low temperatures in plants in general lead to the formation of soluble sugars and other soluble substances. Likewise it is possible that essential changes occur in the seed coats. We must not forget also that moist stratification gives favorable conditions for the leaching of inhibiting substances. In these seeds, however, like those with dormant embryos, there are definite optimum temperatures for stratification and the effective range of temperature is rather narrow and low. Leaching ought to progress faster at high rather than at low temperatures; also one might expect it to proceed over a wide range of temperatures.

Two-Year Seeds.

Nurserymen often speak of two-year seeds. These are seeds that in nursery practice as well as in nature do not produce seedlings until the second spring after the seeds ripen. Work at the Institute has led to the grouping of these into three physiological categories.

(1) Seeds that need a period in the soil at good growing temperatures to permit microörganisms or other factors in the soil to disintegrate the coats, followed by a period at a low temperature to after-ripen the dormant embryos. These will be discussed under the heading "Seeds with Resistant Coats and Dormant Embryos."

(2) Seeds that need a warm-temperature period in the soil to produce a root system, followed by a low-temperature period to after-ripen the dormant epicotyl. These will be described under the heading "Dormant Epicotyls."

(3) Seeds that require a period of low-temperature stratification to induce root growth, followed by a high-temperature period permitting the root to grow, followed in turn by a low-temperature period to after-ripen the epicotyl, which later requires a higher temperature for good growth. These will be discussed under "Seeds That Require Two Low-temperature Exposures."

Seeds with Resistant Coats and Dormant Embryos. Seeds of this class differ from those of the group just discussed in that the coats (pericarps or other structures) must be removed or partially disintegrated before the embryos are in a condition to after-ripen in low-temperature moist stratification. In nature the resistant coats are partially disintegrated by agents in the soil, especially microörganisms. The latter require good growing temperatures for greatest activity. Hence in nature such seeds require a few months in soil at high temperature for removing the coat resistance, followed by a few months at a low temperature for after-ripening of the embryos, after which they will germinate. The coat resistance will be overcome during the first summer in the soil and embryos will after-ripen during the second winter so that the seeds are ready to grow the second spring. In nursery practice, if such seeds are to be grown out-of-doors without special treatment they should be sown in the spring. The coat resistance may be overcome by removing the coats mechanically or by corroding them with such agents as concentrated sulfuric acid. By using one of these methods of removing the coat resistance, seeds can be stratified during the first winter and made to grow immediately upon planting the first spring. As to seed or fruit coat, there are two classes of seeds in this group: one in which there is no suture line in the coats, and the whole surface of the coat is decomposed when the coat resistance (*Symphoricarpos*) is removed, and the other in which there is a dehiscent line at which the coat resistance is removed by decomposition. In the latter, the coats open into equal valves (*Crataegus*) or a valve comes off with dehiscence (*Cotoneaster*).

Flemion [37] reports that one investigator was unable to obtain any germination of snowberry (*Symphoricarpos racemosus*) seeds, while another investigator obtained only 50 per cent after two years in the soil. Flemion was successful in getting nearly complete germination of these seeds within a year. She summarizes her results as follows: [37, p.101]

"In order to induce germination in seeds of *Symphoricarpos racemosus* it is necessary that the seed coat be disintegrated. This can be accomplished by placing the seeds for a period of three or four months in moist acid peat moss at 25° C, or by soaking the seeds in concentrated H_2SO_4 for 75 minutes, or by H_2SO_4 treatment and several weeks at 25° C. After the required changes in the seed coat have occurred it is still necessary to after-ripen the embryo, which can be brought about by a period of six months at 5° C. Of these three methods which modify the seed coats, the combination of H_2SO_4 treatment and a short period at 25° C is the best, and germination percentages of 60 to 90 may be obtained in this way.

"For the production of seedlings on a large scale the best method is to plant the seeds in flats in spring and place out-of-doors in a cold frame which is covered with a board cover during the winter months. Germination occurs the following spring. In nature, as in our laboratory experiments, the seeds respond to a high temperature followed by low temperature. During the period at high temperature (summer months) conditions are favorable for the modification of the seed coats; the embryos are then after-ripened during the subsequent cold winter months.

"That the seed coats undergo changes during dry storage at room temperature is shown by the fact that a suboptimal treatment of sulphuric acid followed by low temperature produced a maximum germination from seeds which had been stored about three months. Practically no seeds stored nine months or longer germinated although they were shown to be still viable when subjected to a more effective treatment.

"The seeds increased several-fold in catalase and peroxidase activity during the period of after-ripening at 5° C. When at 25° C the activity of these enzymes does not increase, but there is instead a slight decrease."

Pfeiffer [92] made a study of the building up of the seed coat during the development and maturing of the seed, as well as of decomposition of the coat in the soil. Fig. 40 shows the general structure of the seed and coats. The outer longitudinal, many-celled layer of fibers, as well as the circumferential inner and somewhat thinner layer of fibers are derived from the ovary wall. Both consist of long, thick-walled cells with small lumina and give the coat its marked leathery toughness. Pfeiffer summarizes her work in part as follows: [92, p.121]

"The components of the fiber walls and the integument epidermis include cellulose, pentosans, and lignin. Deposition of the substances is in this sequence; the greater deposit of lignin is in the integument epidermis.

"These wall substances become subject to decomposition by fungi from

the medium when seeds are kept in moist peat moss or soil at favorable temperatures. The coats soften and readily disintegrate, thus removing a mechanical barrier in seed germination.

"The coats of seeds kept free from fungi, but under similar conditions of moisture and temperature, do not undergo these changes.

"In seed coats exposed to sulphuric acid for different periods of time, the fiber tissue is reduced in amount in proportion to the length of exposure. The longer exposures favor the development of fungi in subsequent holding in peat moss at 30° C. Too long exposure is disadvantageous for seed germination, probably because of excessive development of fungi, possibly because of change in the inner cuticle rendering it non-resistant to fungus entrance.

"The fibers of the coat and the thin-walled tissue in the placenta region are permeable to water, as indicated by entrance of salts and methylene blue. The inner cuticle is apparently impermeable. Both inner and outer cuticles seem to be barriers to the progress of fungi under normal conditions.

"The embryo is small with a short suspensor radical, short hypocotyl and cotyledons and an undeveloped stem tip. There seems to be a tendency toward increase in size and differentiation with keeping in moist peat moss at 5° C, which is more marked if this is subsequent to exposure to sulphuric acid and an interval at 30° C."

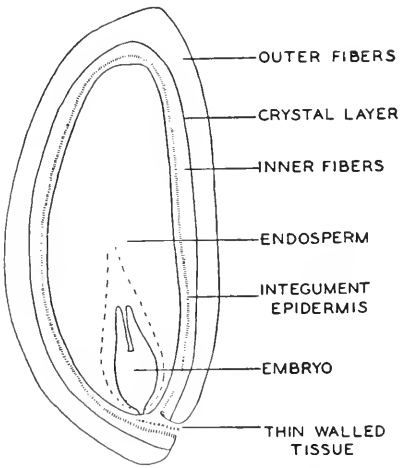

FIGURE 40. Diagram of median longitudinal section of *Symphoricarpos racemosus* seed (16.5 ×).

Later, Flemion and Parker [44] showed that the germination behavior of *Symphoricarpos orbiculatus* is similar to that of *S. racemosus*, except that the former responds to a higher stratification temperature. Flemion [43] found that addition of nitrates or other nitrogen compounds to the peat during the warm temperature period in the peat hastened the decomposition of the tough seed coats. It is a well-established fact that when microorganisms decompose cellulosic materials in the soil, they consume much

available nitrogen and are often limited in the decomposition by lack of available nitrogen. We have already learned that nitrates and other nitrogen compounds substitute for light in light-favored seeds. Gassner [52] found that nitrates substitute for light in forcing the germination of *Chloris ciliata* seeds, although the enveloping tissues are not permeable to nitrates. This raises the question as to what extent nitrates favor the germination of light-favored seeds in darkness by furthering the decomposition of the coats by organisms.

Table 12. Percentage Germination of *Symphoricarpos orbiculatus* Seeds When Mixed in Moist Peat Moss and Kept at Various Temperatures.*

Temp. (° C)	Percentage germination					
	0.5 year	1 year	2 years	3 years	4 years	5 years
1	0	0	0	1	2	3
5	1	2	3	3	5	10
10	5	43	62	66	70	74
15	0	0	0	1	2	5
20	0	0	0	0	0	0

* Duplicate lots of 200 seeds of 1934 crop; experiment started March 6, 1935.

We have seen that the coats of *Symphoricarpos* seeds are decomposed by organisms most rapidly in the soil at relatively high temperatures, and that the embryos after-ripen best at about 5° C (41° F). Can an intermediate constant temperature be selected that will permit both processes to occur in succession, followed by germination? Table 12 shows the germination of seeds of *S. orbiculatus* at constant temperatures, 1°, 5°, 10°, 15°, and 20° C (34°, 41°, 50°, 59°, and 68° F). Only 10° C (50° F) gave any considerable germination, amounting to 43 per cent after one year and finally rising to 74 per cent after five years. At 5° C (41° F) there was only 10 per cent germination after five years and at 15° C (59° F) only 5 per cent after five years. Temperatures much below 10° C (50° F) permit only very slow disintegration of the coats, and temperatures much above 10° C (50° F) do not lead to after-ripening of the embryos.

Flemion [41] has found that seeds of various species of *Crataegus* differ markedly in their requirements for germination. *C. cordata* [*Phaenopyrum*] and *C. coccinea* belong to the class previously discussed. They need two and one-half to five months' low-temperature stratification to after-ripen. If planted outside in the fall, they after-ripen during the winter and produce seedlings the following spring. They are not two-year seeds. The seeds of *C. flava*, *C. punctata*, *C. Crus-galli*, and *C. rotundifolia* are two-year seeds. Fall planting will not produce seedlings the first spring, but seedlings are produced the second spring after fall planting. This gives a summer in the soil for overcoming coat resistance. Spring planting of these produces seedlings the next spring. This gives a summer in the soil

for coat changes and a winter for embryo after-ripening. Fig. 41 illustrates this behavior. Seeds of *C. rotundifolia* were planted in flats in the fall of 1932 and placed in three sorts of cold frame at Yonkers. Row A shows photographs of these flats in June of 1933 after one winter in soil. None

FIGURE 41. Per cent germination of various *Crataegus* seeds when planted in the fall of 1932 and placed in various cold frames. A and B, *C. rotundifolia* photographed June, 1933 and July, 1934 respectively. C, *C. flava* photographed July, 1934.

has germinated. Row B shows photographs of the same flats in July of 1934. Row C shows the seedling production for *C. flava* the second spring after fall planting in flats in cold frames. No seedlings of either species were produced when the flats were kept continuously in the greenhouse.

Fig. 42 shows that seeds of three of these more resistant species of *Crataegus* will not germinate after five or even nine months in soil at 5° C (41° F) followed by six weeks at good growing temperature. They all give abundant seedling production if the flats are kept four months at 21° C (70° F), then at 5° C (41° F) for five months followed by six weeks at a higher temperature. In these the four-months' period of high temperature can be shortened to two or three weeks by decomposing the coats partially with concentrated sulfuric acid before the high-temperature period. By such treatment the total high- and low-temperature after-ripening periods

can be reduced to less than six months. In this way the more resistant *Crataegus* seeds can be made to germinate the first spring after maturity. In the case of less resistant seeds Flemion says: [41, p.422] "Although seeds of *C. arnoldiana, C. carrierei, C. mollis, C. sanguinea,* and *C. tomentosa* germinate after a period at low temperature, more seedlings are obtained when the seeds have been treated in a moist medium for several weeks at 21° or 25° C prior to the low-temperature treatment."

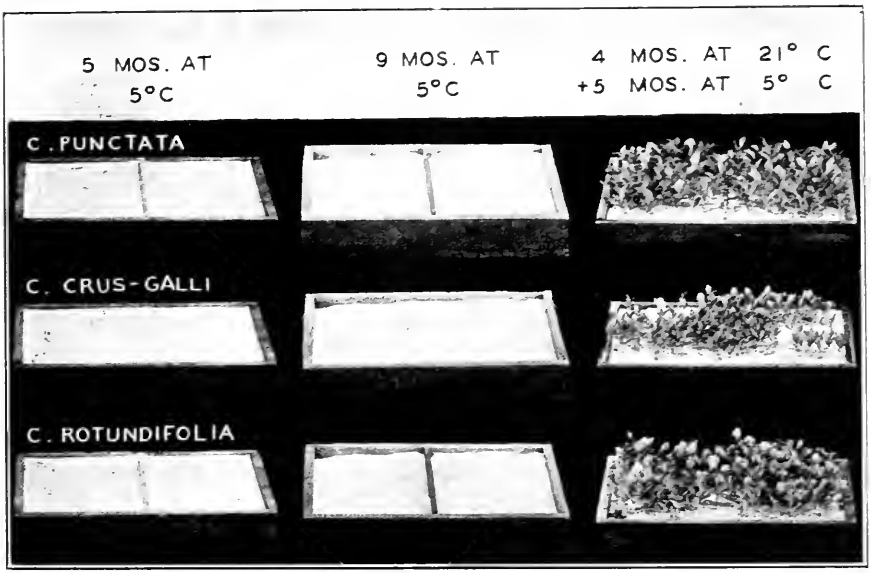

FIGURE 42. Seedling production of various *Crataegus* species. Lots of 500 seeds each were planted on Nov. 26, 1932, in flats and kept for various periods at 5° C (41° F) with and without a previous four months at 21° C (70° F). Photographed six weeks after being transferred to a warm greenhouse.

Cotoneaster seeds [53] behave much as do *Crataegus; C. Dielsiana* and *C. Zabelii* show 100 per cent germination within four months when kept in a germinator at 10° C (50° F), while *C. acutifolia, C. apiculata, C. horizontalis, C. lucida,* and *C. divaricata* show very little germination in this condition even after ten months. These five all respond, as do the more resistant species of *Crataegus* seeds, to a period in soil at high temperatures for overcoming coat resistance, followed by a period at low temperature to after-ripen the embryos. Likewise the high temperature can be partially or entirely eliminated by proper treatment with concentrated sulfuric acid.

Table 13 shows the data on the seeds described above and a number of other seeds [9, 54, 56] belonging to this class that have been studied at the Institute. It gives the best temperatures for the high-temperature period, as well as the low-temperature period that follows; the range of effective temperatures for each treatment; the number of days required for each

period; and the length of time for concentrated sulfuric acid treatment if it is used to overcome the coat resistance.

Table 13. Effective Pretreatment for Seeds Requiring Periods at Both High and Low Temperatures before Planting in the Greenhouse.

Species	Best temp. (° C)		Effective range of temp. (° C)		Days at best temp.		Effective H_2SO_4 time treatment
	High	Low	High	Low	High	Low	
Aralia racemosa *	25	5	14–25	1–10	30–60	90–120	10 min.
Arctostaphylos Uva-ursi †	25	10	20–25	5–10	60–90	120–240	2–4 hr.‡
Cornus canadensis *	25	1	25	1–5	30–60	120–150	10–30 min.
Cotoneaster divaricata	14–25	5	14–25	1–5	90–120	90–120	2.5 hr.
" *horizontalis* *	14–25	5	14–25	1–5	90–120	90–120	1.5 hr.
Crataegus Crus-galli	25	5	25	5	120	180	2 hr.
" *flava*	25	5	25	5	120	180	2 hr.
" *Oxyacantha*	25	5	25	5	90	180	2 hr.
" *punctata*	25	5	25	5	120	180	2 hr.
" *rotundifolia*	25	5	25	5	120	180	2.5 hr.
Halesia carolina *	20	5	14–27	1–5	30–90	60–90	—
Rhodotypos kerrioides *	25	5	25–30	1–10	30	90	—
Symphoricarpos orbiculatus	25	10	25	10	90–120	150	30–40 min.
" *racemosus*	25	5	25–30	5	90–120	180	75 min.
Taxus cuspidata	20	5	20–25	1–5	90	120	—
Tilia americana *	20	5	14–20	1–5	120	90–150	20 min.

* Give some germination with pretreatment at low temperature only but much better with high plus low.

† Neither high temperature nor H_2SO_4 alone sufficient to overcome coat effect. Best results when these two treatments were used together.

‡ Effective length of treatment depends on whether entire nutlet stones, stone pieces, or single seeds are used.

One should realize that there is no hard-and-fast line between seeds of this class and those of the previous class. Seeds with only moderately resistant coats may respond fairly well to simple low-temperature stratification; yet they give a higher percentage of seedlings if first exposed for a period to a high temperature in the soil, followed by low-temperature stratification. This is true of *Rhodotypos kerrioides* seeds.

Pfeiffer has shown beyond doubt that organisms in the soil decompose the non-dehiscent coats of *Symphoricarpos* seeds. We have assumed that organisms decompose the coats with dehiscent lines at these lines. This, however, is questioned by some workers. Müller [85] claims that seed coats with dehiscent lines are weakened at these lines only by water absorption and not by soil organisms, acids, and other agents unless these lines (planes) are composed in part or wholly of cellulosic materials. In this group of seeds the fact that the coat resistance is overcome only at higher temperatures that permit action of microörganisms is evidence against Müller's conclusion.

Dormant Epicotyls. At the Institute we have found a number of seeds in which the epicotyl has to be after-ripened by a period of low-temperature exposure after the radical and hypocotyl have grown. Table 14 shows the seeds studied to date that belong to this group. In all of these the germination and formation of the root system — a process that takes place at

Table 14. Effective Treatment for Producing Plants from Seeds with Dormant Epicotyls.

Species	Requirement for root production		Pretreatment for shoot production	
	Temp. (° C)	Time (mos.)	Temp. (° C)	Time (mos.)
Asarum canadense	15–30 * or 10–30 *	3	5	3
Lilium auratum	20	3–6	1–10	2–3
" *canadense*	"	3–6	1–10	2–3
" *japonicum*	"	3–6	5–10	3–4
" *rubellum*	"	3–6	1–10	3
" *superbum*	"	3–6	5–10	2–3
" *szovitsianum*	"	3–6	10	3–4
Paeonia (herbaceous)	15–30 *	2–3	5–10	3
" (tree)	"	2–4	5–10	2–3
Viburnum acerifolium	20 or 20–30 *	6–17	5	2–3
" *dentatum*	"	6–17	5–10	½–2
" *dilatatum*	"	7–9	5–10	3–4
" *opulus*	"	2–3	3–15	1–2
" *prunifolium*	"	7–9	3–15	1–2

* Daily alternation.

higher temperatures — is rather slow, requiring from two to three months in the herbaceous peony and *Viburnum Opulus*, to 6 to 17 months in *V. acerifolium* and *V. dentatum*. One might expect low-temperature stratification of the seeds to hasten the growth of the root system, but such has not proved to be the case. It is evident that there ought to be some way of getting more prompt root production in this type of seed, but it has not been discovered to date. In some of these seeds the percentage of germination is also low. All these seeds will produce some seedlings the second spring if the seeds are properly planted early in the spring. The tree and herbaceous peonies and *V. Opulus* will give a good stand of seedlings the second spring if planted in late spring or early summer. Some of the seeds of forms like *V. acerifolium*, *V. dentatum*, *V. dilatatum*, and *V. prunifolium* are likely to carry over to the third spring or later for complete seedling production. With the conditions met in nature, which are generally far from the optimum, seedling production in all these may extend over several years.

Fig. 43 shows the behavior of tree peony seedlings [8] with hypocotyls 1 to 3 cm planted in pots and placed at 1°, 5°, 10°, 15° C (34°, 41°, 50°,

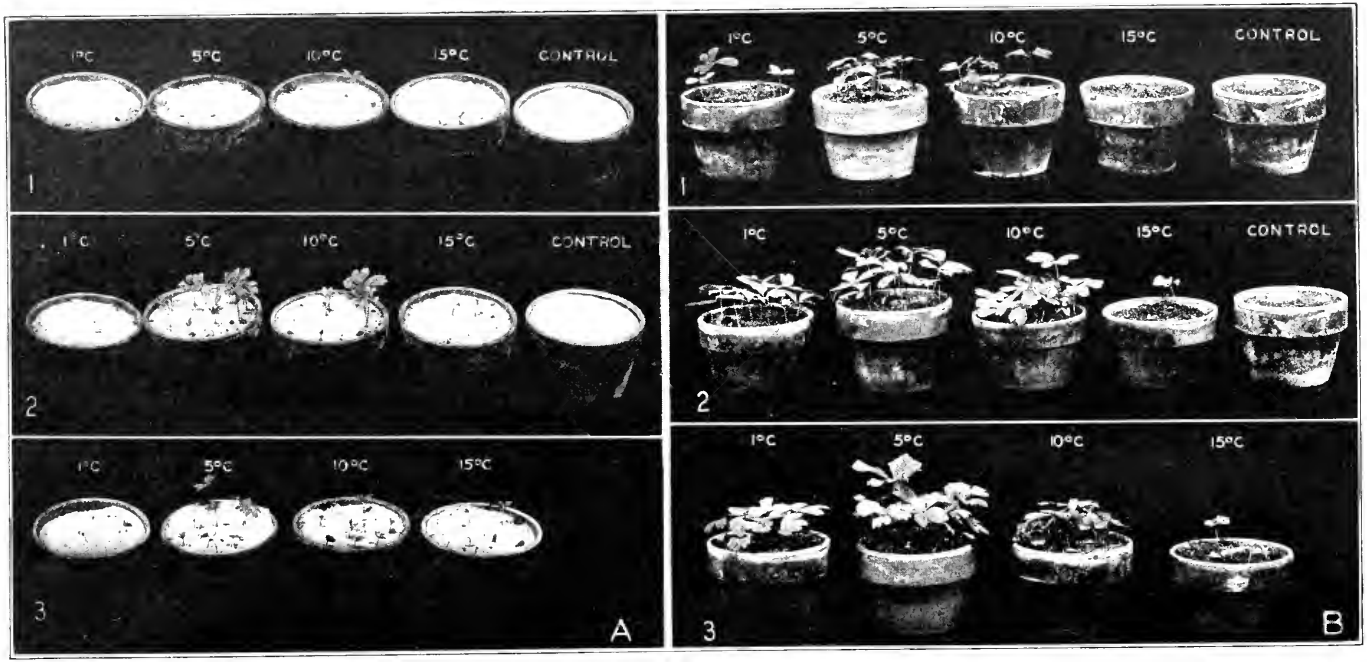

FIGURE 43. The effect of low temperature treatment for (1) two months, (2) two and one-half months, and (3) three months, on production of tree peony shoots in a greenhouse at 13° C (55° F). Twenty seedlings (with hypocotyls 1 to 3 cm long) planted in each pot. A, after three weeks in greenhouse; B, after seven weeks in greenhouse.

59° F), and greenhouse temperatures for 2, 2½, and 3 months, and then transferred back to greenhouse temperatures and photographed after three weeks and seven weeks. Examination of this figure shows that 5° C (41° F)

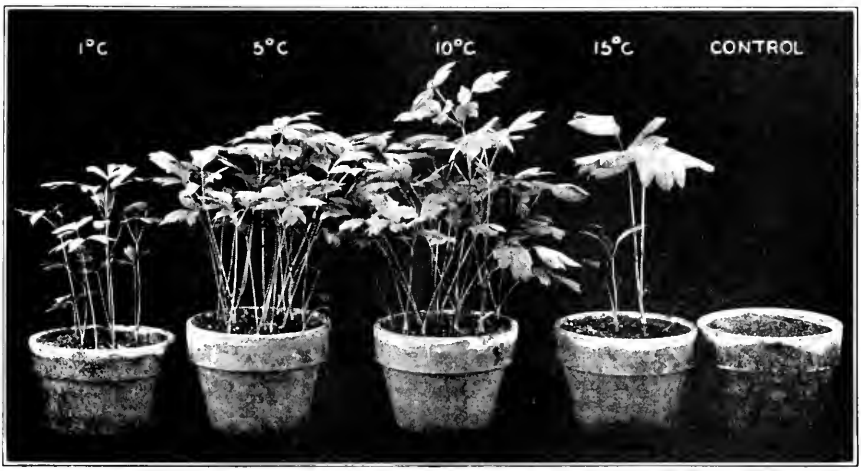

FIGURE 44. Tree peony seedlings, one year and seven weeks after transfer to greenhouse at 13° C (55° F) following a pretreatment of two and one-half months. Kept in board-covered cold frame over winter.

is the best temperature used for after-ripening the epicotyls. This temperature gave a good epicotyl growth after 2 months of low temperature, followed by greenhouse temperature. There was somewhat less growth from the 10° C (50° F) exposure and still less at 1° C (34° F) exposure for 2 months. With 2½ and 3 months' exposure 1° C (34° F) and 10° C (50° F) were only slightly less effective than 5° C (41° F). The exposure at 15° C (59° F) gave little after-ripening of the epicotyl and the control at 20° C (68° F) gave none. Fig. 44 shows some of the seedlings in Fig. 43B after an additional year's growth. Fig. 45 shows the seedling production by

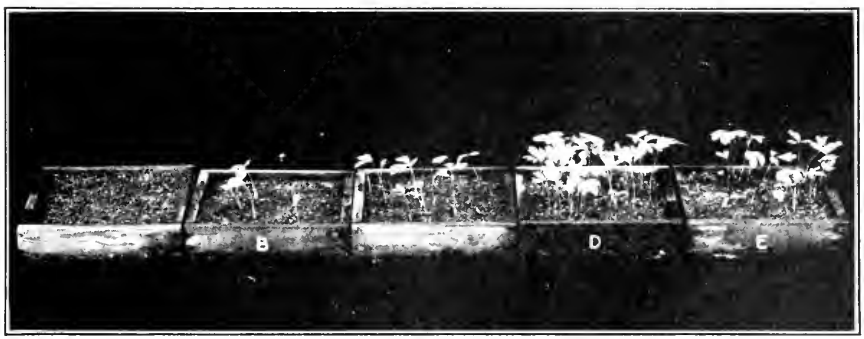

FIGURE 45. Seedling production of tree peony in May 1933 from seeds planted in flats which were placed in a board-covered cold frame. Seeds planted: A, Dec. 1931; B, Feb. 1932; C, March 1932; D, May 1932; and E, July 1932.

tree peony seeds when the seeds are planted in flats at different times of the year and kept in cold frames, with board covers. Those planted during the spring and summer of 1932 gave no seedlings until the spring of 1933. During the summer of 1932 the roots grew and during the winter of 1932–1933 the epicotyls after-ripened and seedlings came up in the spring of 1933.

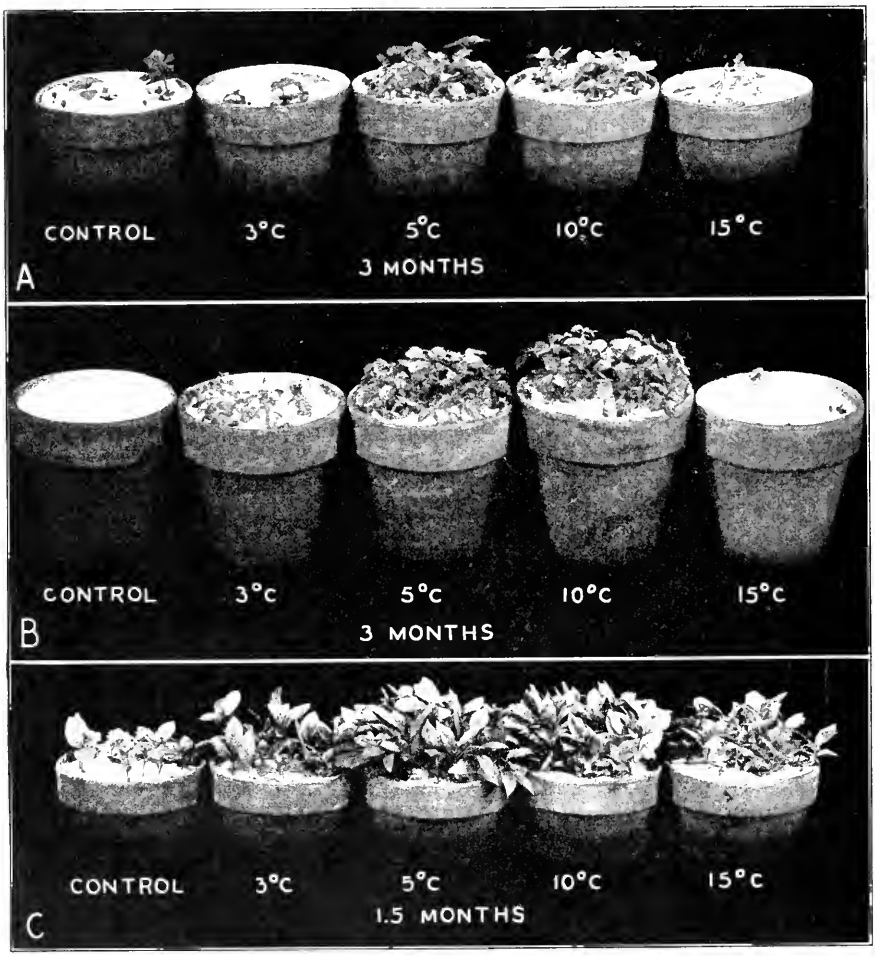

FIGURE 46. The effect of low temperature on shoot development of *Viburnum* species. A, *V. acerifolium*. B, *V. dilatatum*. C, *V. prunifolium*.

The May planting gave the best results. This gave the roots adequate time to grow without growing so long that foods of the seeds were exhausted before the epicotyl developed ready to manufacture foods. The earlier plantings may have led to exhaustion of the foods of the seeds before the epicotyls functioned, but the early decay of some of the seeds at the low temperatures was also a factor. The July planting did not give sufficient time for root development before the cold weather set in. The May plant-

ing gave 25 per cent seedling production, which is a good yield for commercial production but below that attained with good seeds by more fully controlled inside culture. Fig. 46 shows the effect of various low temperatures for three months on epicotyl after-ripening or shoot development of three species of *Viburnum*.[55] For *V. acerifolium* and *V. dilatatum* 5° and 10° C (41° and 50° F) are both effective temperatures for epicotyl after-ripening. *Viburnum prunifolium* has a less limited range of temperature, 3° to 15° C (37° to 59° F) being effective. In this as in *V. acerifolium* there is a small percentage of epicotyl development without cold exposure. Also it will be noted that the cold exposure period required by *V. prunifolium* is only $1\frac{1}{2}$ months contrasted with 3 months for the other two.

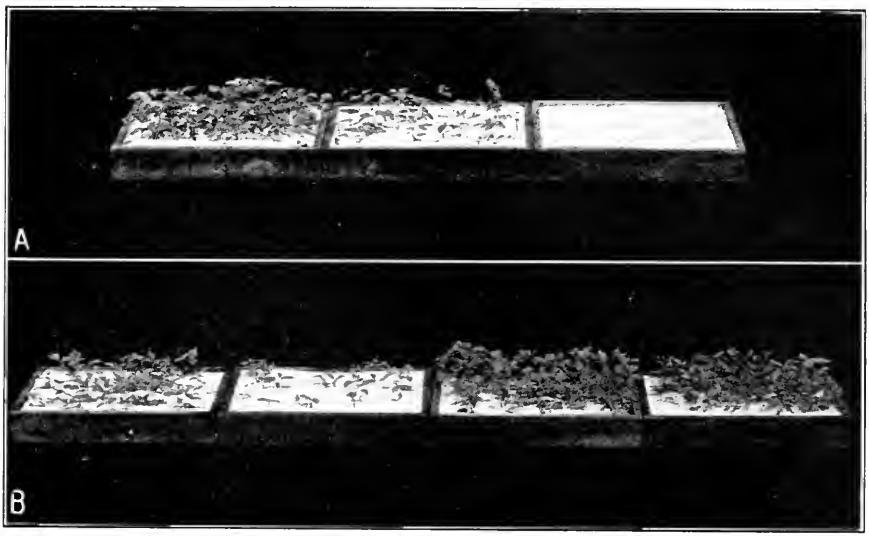

FIGURE 47. A, *Viburnum acerifolium*. The effect of various planting times during summer on seedling production the following spring. *Left to right:* Planted April 1st, June 1st, September 15th, 1936. B, The effect of storage on seedling production of *V. acerifolium* planted May 1, 1936. *Left to right:* Room temperature cleaned, in pulp; 5° C (41° F) cleaned, in pulp.

Seedling production of the most stubborn Viburnums can be accomplished out-of-doors by early spring planting. Of course, the seedlings do not come until the second spring. Fig. 47A shows the effect of planting *V. acerifolium* seeds at different times during the summer upon seedling growth the next year. April 1 was better than June 1, and September 1 gave no seedlings. The earliest planting gave the slow-growing roots 6 to 8 months to grow and get established before winter set in. Evidently 4 to 6 months, as shown by the June 1 planting, was not sufficient for many of the slower growing seeds to form roots. To avoid the hazard of two winters in the soil, these seeds should be spring planted. Fig. 47B shows that it is better to store the seeds of this species during the winter at 5° C (41° F) than at room temperature, whether they are dried in the pulp or

cleaned. Giersbach found that *V. nudum* and *V. scabrellum* did not need epicotyl after-ripening and offered no problem in germination. These are more southerly forms. It must not be forgotten that the temperature relations described in this and previous sections, as well as those to be described later, probably apply to colder temperate zone plants and probably not to torrid zone plants unless they are high-altitude forms. The species of *Lilium* needing low-temperature after-ripening of the epicotyl after roots are formed were studied by Barton.[11]

In unpublished work, Flemion has found that *Chionanthus virginiana* and *Symplocos paniculata* seeds have dormant epicotyls that require low-temperature after-ripening after the roots start to grow. In the first of these seeds the roots grow much more promptly than in most seeds of this class. In fact, many of the roots start soon after the seeds fall to the ground and before winter sets in. In nature, the epicotyl will after-ripen the first winter and the seedling will come up the first spring. These are not two-year seeds.

In seeds that need a high-temperature period in a germinator to dispose of coat resistance and a low temperature to after-ripen the embryo, we noted that a constant intermediate temperature could be used that permitted both changes to occur. The time required for both processes and complete germination, however, was greatly lengthened by using this compromise, intermediate constant temperature instead of the optimum temperatures for each individual process. No doubt in the type of seeds being discussed in this section such an intermediate constant temperature could be selected that would permit development of the roots followed by epicotyl growth. In all these seeds temperatures as high as 10° C (50° F) permit epicotyl after-ripening, and in two Viburnums even 15° C (59° F) is effective. No doubt constant temperatures of 10° C (50° F) or higher would permit both processes to go on. This, however, is of no importance in practical horticulture, for it would greatly lengthen seedling production time and it has no significance in nature, because long-maintained constant temperatures do not occur in the temperate zone.

One might question the wisdom of having seeds that produced a root one year and had to wait until the next year for the epicotyl to develop foliage for feeding the root. These seeds ripen in the fall and go through the hazards of one winter before even a root forms. If the roots start early in the spring they draw on the stored foods all summer and no doubt exhaust them before winter. The tardiness of the root formation in many of these seeds probably delays root growth until late summer or fall, which lowers the draft on stored foods the first year. It is probable that a relatively small percentage of the seeds of this group ever produce seedlings in nature. This may also be true of most of the seeds of wild plants that have such a complex system of after-ripening and germination. The situation is quite different in horticultural practice. Once one knows the tricks of a given seed he can put it under the optimum conditions for each phase

of after-ripening and germination and come out with a high percentage of seedling yield in minimum time, although in some cases the time is not so short at that.

Seeds Requiring Two Low-temperature Exposures. Barton [13] has made an extensive study of the germination of *Trillium grandiflorum* seeds. The following method of treating the seeds gave the highest percentage of seedlings in the shortest time: the seeds were planted in pots in moist soil and kept for three months at 5° C (41° F) for after-ripening; then they had three months in a greenhouse, which produced a root system; then five months at 5° C (41° F) to after-ripen the dormant epicotyls; and finally a period in the greenhouse to grow the epicotyl and develop a complete seedling with top and root. This means two low-temperature periods to after-ripen the seed and the epicotyl respectively, each followed by a high-temperature period for growing the root and finally the epicotyl. This method requires 12 to 14 months to produce a high percentage of seedlings. In nature the seeds would after-ripen the first winter, the root system would grow the next summer, the epicotyl would after-ripen the second winter, and the plant appear above ground the second summer. This is a two-year seed in the sense the nurserymen use this term. In nature, the four different periods would not be at the optimum conditions a large percentage of the time either as to temperature or as to duration of exposure. Consequently there must be a high wastage of seeds in nature.

Table 15. *Trillium grandiflorum*, 1940 Crop. Root and Shoot Production after Various Temperature Treatments from Duplicate Lots of 100 Seeds Each. Planted in Soil in Pots.

Treatment					Percentage seedling production			
First low temperature period		Months in greenhouse	Second low temperature period		Percentage roots		Percentage epicotyl growth	
° C	Months		° C	Months				
None	None	17	None	None	7	6	1	0
None	None	6	10	3	35	32	0	0
5	3	3	5	3	83	84	54	38
5	3	3	5	5	90	86	80	80
10	3	3	10	3	86	78	63	46
10	3	3	10	5	69	81	63	72

Table 15 shows the seedling production from various periods of temperature exposure. Continuous greenhouse temperatures for 17 months gave 7 and 6 per cent of root growth and 1 and 0 per cent of epicotyl growth. Six months of greenhouse exposure followed by three months at 10° C

(50° F) gave 35 and 32 per cent of root growth and no epicotyl growth, because there was not a low-temperature period to after-ripen the epicotyls after the roots had grown. Judging from the later figures in this table, the six months' initial period in the greenhouse interfered with the after-ripening for root growth. Compare 35 and 32 per cent with the percentages below in the same columns (Table 15). Three months at 5° C (41° F), followed by three months in the greenhouse and then three months at 5° C (41° F), gave 83 and 84 per cent of root production and 54 and 38 per cent of shoot production. When the second cold period was lengthened to five months, there was about the same root production but much higher shoot production — 80 and 80 per cent against 54 and 38 per cent. As Table 15 shows, 10° C (50° F) is almost as effective as 5° C (41° F) for after-ripening for both root and shoot growth.

Table 16 lists several other seeds that belong to this class — *Caulophyllum thalictroides*, *Smilacina racemosa*, and *Trillium erectum* strictly so because there is very little root production without a prechilling period. *Polygonatum commutatum*, *Sanguinaria canadensis*, and *Convallaria majalis* belong in part to this class of seeds and in part to the previous class, for there is considerable root production without a prechilling period; but such a period increases considerably both the percentage and speed of root production. For instance, *Convallaria* seeds give 46 per cent root production after several months in the soil without prechilling, whereas seeds that have been stratified for three months at 5° C (41° F) give 92 per cent root production rather promptly. If one examines the length of time each of the low-temperature periods (and also the high-temperature periods) requires according to the optimum conditions Barton has found for getting the epicotyl ready to grow into a plant, one will realize not only the complexity of the process but its time-consuming nature. This adds up to 8.5 months for *Caulophyllum* seeds, 7 to 18 months for *Convallaria*, 11 months for *Polygonatum*, 12 months for *Sanguinaria*, 14 to 19 months for *Smilacina*, 9 to 14 months for *Trillium erectum*, and 9 to 12 months for *T. grandiflorum*. So far as persistence of the species is concerned, one wonders whether nature has not overdone dormancy in these seeds, certainly as to complexity and perhaps as to time required. Yet if nature wanted to become even more complex and difficult she could do so right in line with the things we have noticed in the several classes of dormancy mentioned above. Seeds that required initially a high-temperature period in the soil to overcome coat resistance, followed in succession by a low-temperature period to after-ripen for root growth, a high-temperature period for root growth, a low-temperature period for epicotyl after-ripening, and a high-temperature period for growth of the plant would add one more step to the after-ripening process. If such a temperate zone seed existed and matured in the late fall, it would not germinate until the third spring after maturing: it would be a three-year seed. Has nature gone to this extreme with any seed? If so, the folly has not been discovered.

Table 16. Effective Treatment for Seeds with Dormant Epicotyls Requiring or Benefited by Low-Temperature Pretreatment for Root Production.

Species	Pretreatment for root production			Months in greenhouse about 21° C for root growth	Pretreatment for shoot production		
	Best temp. (° C)	Effective range of temp. (° C)	Months at best temp.		Best temp. (° C)	Effective range of temp. (° C)	Months at best temp.
Caulophyllum thalictroides *	5	3–5	5	½	5	5–10	3
Convallaria majalis †	5	5–10	2–3	2	5	5–10	3–5
Polygonatum commutatum †	5	—	4	3	—	3–5	4
Sanguinaria canadensis †	5	5–10	3	6	5	5–10	3
Smilacina racemosa *	5	1–5	6–10	3	10	5–15	5–6
Trillium erectum *	5	—	6–9	2	5	—	1–3
Trillium grandiflorum *	5	—	3–4	3–4	5	—	3–4

* Require low-temperature pretreatment of seeds for root production.
† Increased root production after low-temperature pretreatment of seeds.

There is one other peculiarity that Barton and Schroeder [14] found in certain seeds mentioned in Table 16: the epicotyl could not be after-ripened by a low-temperature exposure until it had lengthened sufficiently to break the epicotyl sheath. This stands in contrast to the situation with the peony and other seeds described in the previous section of this chapter;

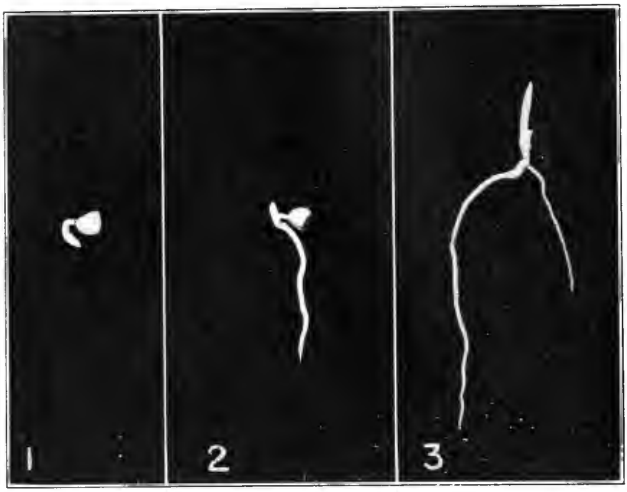

FIGURE 48. Stages in the development of the seedlings of *Convallaria majalis*. *Left to right:* Stage 1, the protrusion of the radicle and hypocotyl; Stage 2, the first evidence of shoot development; Stage 3, further growth of the shoot to break through the cotyledonary sheath.

in these cases the epicotyl could be after-ripened as soon as the root started, without the necessity of previous elongation. This has been worked out in detail for *Convallaria majalis* and *Smilacina racemosa* seeds. Fig. 48 shows three stages in the early development of the seedlings of *Convallaria*.[14] It must reach the stage shown in "3" of this figure before a cold period for after-ripening the epicotyl is effective. The epicotyl goes into dormancy at this stage of growth and not at earlier stages. This is very similar to the situation with *Botrychium lanceolatum* and with many of our early spring flowers that grow up every spring from bulbs or tubers. The bud that forms the spring shoot is formed the year before and elongates considerably before it goes into dormancy, no doubt to be after-ripened by the cold of winter ready for early spring growth.

We have seen for *Symphoricarpos orbiculatus* seeds that an intermediate constant temperature, 10° C (50° F), can be chosen that will permit all the after-ripening processes to proceed, including overcoming coat resistance, after-ripening of the dormant embryo, and finally growth of the seedling. This temperature is not optimum for any of the processes, and consequently lengthens the time for seedling production, in contrast to using the optimum temperatures for each individual process. No doubt in all the cases described above, where temperature is an important factor

in the various phases of after-ripening and growth of seeds, such a constant intermediate temperature could be used to consummate the complete process; but nature in the temperate zone does not deal in constant temperatures and the practical grower cannot easily maintain such a constant temperature with other necessary growth conditions, nor can he wait so long for the plants.

The facts of seed dormancy stated in this and in previous sections show the inadequacy of ending germination studies with the mere growth of the root. Studies thus terminated would entirely miss epicotyl dormancy and the dwarfishness in seedlings due to the growth of non-after-ripened epicotyls. We always follow the growth of the seedling for many months or even years to catch any peculiarities in the later development of the seedlings that might result from seed dormancy.

A Period of Dry Storage After-Ripens Many Seeds

A period of dry storage may be quite as important a factor in after-ripening dormant seeds as low-temperature stratification, especially if one considers the number of species that respond to each treatment. Many light-sensitive seeds [20] lose their light-sensitiveness partially or completely if kept for several months in dry storage. *Viscum album* and *Arceuthobium oxycedri* seeds are notable exceptions, for the first is killed by continuous darkness, and neither germinates under any conditions without light. There are probably other exceptions among light-sensitive seeds. Most seeds of cultivated grains and other grasses after-ripen in dry storage. In the cereals the period of after-ripening varies with species, varieties, and races from a few days to several months. According to Pietruszczyński,[93] it is longest in oats, shorter in barley and wheat, and shortest in rye. The period is shorter in winter than in spring cereals, in early than in late ripening varieties, and in dry than in wet seasons. In wild grasses, several months of dry storage are generally required for complete after-ripening. Most weed seeds studied in this respect after-ripen in dry storage. Even seeds with dormant embryos [26, 34] can be at least partially after-ripened by dry storage, although this treatment is not nearly as effective as low-temperature stratification.

Kroeger [72] has recently studied the progressive after-ripening of *Impatiens balsamina* seeds with increasing periods of dry storage. An examination of lot 1 in Fig. 49 shows that, as the dry storage period increased from 0 weeks to 43 weeks, the speed and total percentage of germination increased. The fresh seeds gave 32 per cent germination after 20 weeks in the germinator, while the seeds that had been dry-stored for 43 weeks gave 100 per cent germination in one week. Even four weeks of dry storage raised both the speed and final germination, which reached 70 per cent after 20 weeks. The speed and final percentage of germination rose as the dry storage period was lengthened progressively to 9, 16, and 25 weeks.

Evidently, after-ripening was complete after 43 weeks of dry storage. Lot 10 in Fig. 49 shows a similar relation between the period of dry storage and degree of after-ripening. In this case, the later collection of seeds

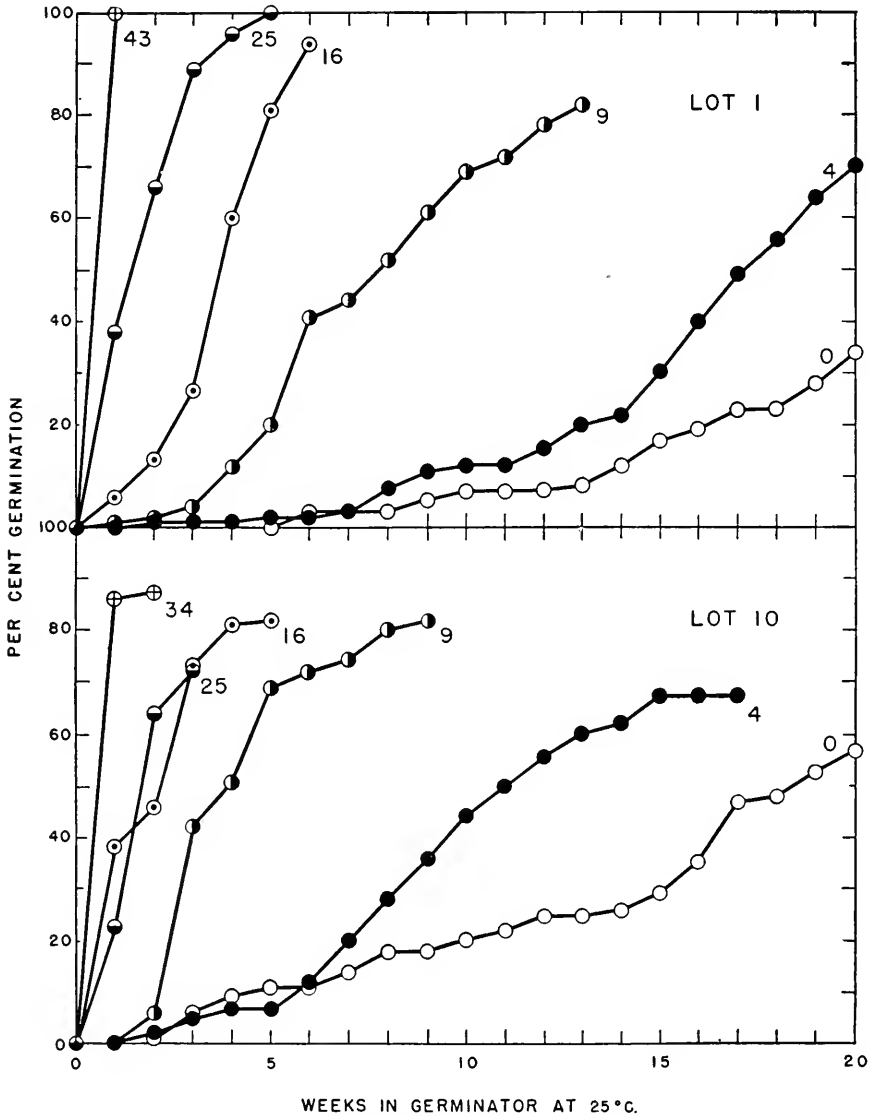

FIGURE 49. Effect of weeks of dry storage at room temperature and date of harvest on after-ripening of seeds of *Impatiens balsamina*. Lot 1 was collected August 14–19 and Lot 10, October 14–16.

shows higher germination of the fresh seeds, perhaps due to partial after-ripening on the plants; also it appears that after-ripening was not complete after 34 weeks of dry storage. Kroeger also found that for fresh seeds or

for partially after-ripened seeds the percentage and speed of germination was increased by two weeks' moist stratification at 5° C (41° F). Various grass seeds respond similarly to both dry storage and low-temperature stratification, or prechilling.

Obviously, we would like to know the essential changes that occur in dormant seeds in dry storage that enable them to germinate. We asked the same question about low-temperature stratification and found the answers only in small part satisfactory. Answers to this question are also only partially satisfactory. There are, however, a number of hypotheses and facts that throw some light on the question. On the basis of culture of excised embryos from dormant and after-ripened barley grains on nutrient gelatin and on the basis of transplanting embryos from dormant and after-ripened grains on endosperm from dormant and after-ripened grains in all possible combinations, Windisch [129] concluded that the embryos from dormant grains themselves show dormancy, and that during dry storage of the grains the epithelium of the scutellum is essentially modified. There is much evidence that dormancy in many kinds of seeds of the grass family is determined by intactness of the coats, and that breaking the coats causes prompt, vigorous germination giving no evidence of embryo dormancy (Crocker and Harrington [22] for Johnson grass; Harrington [61] for wheats, oats, and barley; and Atwood [5] for wild oats). Many investigators have found that dormant cereals will germinate promptly if placed in a germinator at lower temperatures, such as 10° to 12° C (50° to 54° F) or 14° to 15° C (57° to 59° F). Flemion, in preliminary unpublished work at the Institute, has found that wheat and oats grown from dormant grains, by opening the coats, show the same vigor in the later growth of the seedlings as those grown from after-ripened grains. As we have seen from Flemion's work cited above, dwarfishness or lack of dwarfishness in the seedling is the best criterion for embryo dormancy.

There is the possibility that freshly harvested seeds contain inhibiting substances that volatilize or decompose during dry storage. Shuck [107, 108] finds that fresh lettuce seeds form inhibiting substances when put into a germinator at a high temperature, 25° C (77° F), in darkness. Repeated growth of batches of seeds on the same filter paper leads to the accumulation of inhibiting substances on the paper that inhibit later sowings. Water and soil are more favorable germination media for these seeds than filter paper. He believes this is the case because they have greater power to absorb the inhibitors. Even soaking in cold water seems to remove the inhibitors. He also finds that light and low-temperature germinators overcome the inhibitors. We have already discussed other cases where inhibiting substances prevent germination. This hypothesis deserves serious examination in all seeds that after-ripen in dry storage.

Cereal grains are hastened in after-ripening by heating at 35° to 40° C (95° to 104° F) [4] for 2 to 4 days; the heating is effective without drying, although dry heat adds to the effectiveness. Of course, heat will drive off

volatile inhibitors if they are present. Gadd [51] thinks that at first seed coverings are alive and thus deprive the embryos of oxygen, and that when the coats die oxygen gets to the embryo. We have found that freshly harvested lettuce seeds that will not germinate at 30° C (86° F) in air also will not germinate at this temperature under one or two atmospheres of pure oxygen. They will, however, germinate promptly at this temperature if the coats are broken. It might seem that a 10-fold increase in oxygen pressure would be sufficient to allow some to reach the embryos. Death of living cells in the coats might also favor the outward diffusion of inhibitors.

Table 17. Percentage of Water Held by Seeds of *Impatiens balsamina* after Different Periods of Soaking; Freshly Harvested Seeds; Seeds Dry-Stored 8 Weeks. Dry Weight Basis. Temperature of Soaking 20° C. Weighings Made on Duplicate Lots for Each Soaking Period.

Storage period, weeks	Percentage water in seeds after hours soaking						
	0	3	6	12	24	30	48
0	12.1	20.9	34.6	46.1	50.4	50.8	50.9 *
8	6.2	23.0	38.9	51.9	56.7	56.8 *	

* Maximum water absorption.

Barton, in unpublished work at the Institute, has found that certain seeds that after-ripen in dry storage increase greatly in the initial rate of water absorption and somewhat in the final total amount held by them when fully imbibed as the dry storage period increases. Table 17 shows this relation for *Impatiens* seeds freshly harvested and dry-stored in the laboratory for eight weeks. It will be noted that freshly harvested seeds bore 12.1 per cent water while the dry-stored seeds bore 6.2 per cent. In spite of this initial difference, after three hours' soaking, the dry-stored seeds contained 23.0 per cent water against 20.9 per cent for the freshly harvested; in three hours of soaking the dry-stored seeds had absorbed 16.8 per cent of their dry weight while the fresh ones absorbed 8.8 per cent. She got similar results with *Rumex, Amaranthus,* and lettuce seeds. The initial rate of water absorption and the total amount absorbed increase with the period of dry storage as after-ripening progresses, and is above that which occurs from drying without after-ripening.

While the statements above throw some light on the changes involved in after-ripening of seeds in dry storage, the great number and range of species and varieties of seeds in this category demand that many of these seeds, including several representatives from every family of plants involved, be examined in the light of all the hypotheses and facts mentioned above, and in the light of new hypotheses that will be suggested by such investigations.

In examining all the foregoing discussion on dormancy of seeds, it will be seen that nature secures delayed germination in seeds by a great variety of methods and not by a single method. It is not improbable that dry storage likewise leads to the after-ripening of seeds by several different essential changes in the many kinds of seeds involved.

Temperature a Factor in Overcoming Dormancy

We have already spoken of the importance of daily alternating temperatures in substituting for light in light-sensitive seeds. There are many other records in the literature of seeds that germinate much better at alternating temperatures than at optimum constant temperatures. This is so important that alternating temperatures are used as a regular procedure in the commercial testing of many seeds. Seeds in nature in the soil experience such a daily alternation of temperatures — warm in the daytime and cool at night. There are many seeds that germinate at temperatures at or near the freezing point, and some of them germinate only at such low temperatures. There are other seeds that require high temperatures for germination.

Schroeder and Barton [104] found that seeds of some high-altitude alpines (*Calochortus macrocarpus, Camassia Leichtlinii, Lewisia rediviva*) germinate only at low temperatures. The first germinate best at 5° C (41° F) and will not germinate fully at temperatures much above this. Annual delphinium seeds do not germinate well at temperatures above 15° C (59° F). These low-temperature seeds can be successfully grown at temperatures above the low maximum for germination by pregerminating [10] at favorable low temperatures before planting at higher temperatures. We have already mentioned the fact that many seeds that need low-temperature stratification will germinate when they are after-ripened right at the optimum stratification temperature. As will be seen in Table 11, this temperature is 1° C (34° F) for several seeds studied. We have also emphasized the fact that seeds after-ripened at low temperatures may go back into secondary dormancy if put in a germinator at too high a temperature. While one generally thinks of physiological processes in plants increasing in intensity as the temperature rises above the freezing point, the after-ripening of seeds goes on fastest near the freezing point and falls off as the temperature rises; and in some seeds the germination proceeds fastest just a little above the freezing point.

Just as some kinds of seeds are attuned to very low temperatures for after-ripening and germination, others require relatively high temperatures for germination. At the Institute we have tested a number of crops of *Amaranthus retroflexus* seeds for their temperature requirements for germination immediately after harvest and after various periods of dry storage. Immediately after harvest these seeds require a temperature of 35° to 40° C (95° to 104° F) for germination, and they germinate promptly at this temperature. As they remain longer and longer in dry storage they

will germinate at lower and lower temperatures, until after several months of dry storage they germinate, although slowly, at 10° C (50° F). We have already mentioned the fact that the intact upper seed of the cocklebur requires about 33° C (91° F) for prompt and complete germination, and that an excised embryo has a minimum germination temperature of 18° C (64° F). It is probable that high-temperature requirements for germination determine the late appearance of crab grass, *Panicum sanguinale*,[122] *Portulaca oleracea*, and other weeds [81] rather late in the growing season in this latitude. From what has just been said it is evident that many seeds remain dormant in a germinator because the temperature is too low or too high or lacks variation.

Quick Vitality Tests for Dormant Seeds

Seeds of farm and garden plants are tested for viability before they are put on the market. This is relatively easy to do by germination, for some of these seeds will germinate fully within four or five days under the proper conditions; and even the slower ones, like the blue grasses, will germinate within 28 days under the good conditions provided in the seed-testing laboratories. The testing of farm and garden seeds for viability and purity has returned many-fold the expense of maintaining thoroughly equipped state, government, and private laboratories. No such adequate methods and equipment have been available for comparable tests of forest and horticultural seeds that show great delays. For many of them, ordinary germination tests are not available, because it takes so long to after-ripen and germinate the sample that there is not enough time left to after-ripen the main part of the seeds for early spring planting. In two ways it is more important to be sure of high viability in these seeds than it is for farm and garden seeds; considerable effort and expense must be put on stratification, and failure of a crop due to poor seeds cannot be recouped to any degree the same year by replanting. On the other hand, the total crop value from farm and garden seeds is many times that from dormant forest and horticultural seeds.

Probably due to its work on dormant seeds, the Institute in the early thirties began to receive samples of dormant seeds from nurseries for testing. If the samples came in as late as January it was impossible to stratify the seeds and later germinate them so that the viability tests could be furnished to the grower in time for him to stratify the seeds and have them ready for early spring planting. Flemion had been excising embryos from dormant seeds and growing them on moist filter papers to determine whether the embryos were the seat of dormancy and how the dormancy of the embryo expressed itself in the later growth of the seedling, as described in a previous section. This led to her method of quick vitality tests for dormant seeds, which consists of growing the excised or partially excised embryo at room temperature on moist filter paper.

For a long time botanists have been trying to get reliable methods for determining quickly the viability of seeds without bothering to germinate them. Flemion [40] gives a review of this literature to which one may turn for citations. As early as 1876 Dimitriewicz found that sections of good grain seeds turned a deep rose color in sulfuric acid in five minutes, whereas sections of poor seeds required 15 minutes. Lesage used dilute potassium hydrate for determining viability in garden cress seeds. Heat of respiration, electrical response ("blaze current"), and electrical conductivity have been suggested as means of quick vitality tests. Catalase content has been used. This offers difficulties because many dead seeds contain catalase, and dead *Amaranthus* seeds [22] are as rich in catalase as viable seeds. Davis [25] found that soaking seeds in warm (32° C, 89° F) water overnight lowered the catalase in dead seeds and increased it in live seeds, and on this basis developed a viability method. This method, however, has been questioned as to general usefulness. Some dyes penetrate dead embryos readily, but living embryos much less readily. Indigo carmine 1:2000 is the best dye for this purpose. Several chemicals enter the seeds and are reduced by the respiratory activity of seeds producing color reduction products. Soaking seeds in solutions of para- or ortho-dinitrobenzene for 20 hours, followed by treatment with ammonia for one hour, gives an orange coloration in live seeds. Tellurites, tellurates, selenites, and selenates enter seeds and are reduced to the elements tellurium and selenium, giving purple color with the former and yellow with the latter. Tetrazolium salts [73] have been very recently recommended as substitutes for selenium salts because they are less toxic. They are reduced to red formazanes.

In three papers [39, 40, 42] Flemion describes in detail the methods of excising embryos and of running the viability tests on 38 different species of plants representing 10 different families. In later unpublished work she has extended this method to 15 other species of dormant seeds representing 3 additional families. In some of the seeds, excising of the embryo without injury is a fairly difficult process and requires considerable technical skill. This is especially true of *Symphoricarpos*, which has a tough, leathery coat and a rudimentary embryo embedded in the endosperm. A quick vitality test on this seed not only requires much work but 6 weeks to get results. This seed represents an extreme case and is of no practical significance. In most seeds of practical interest the excising of the embryo is relatively simple; and the period for running the viability tests ranges from 3 to 14 days, about the time required for germination tests of farm and garden seeds. In all cases, Flemion finds that viability tests determined with excised embryos check closely with germination obtained by after-ripening the dormant seeds and later germinating them. Even the relative vigor of the several embryos is clearly shown, as it is with the speed of germination of the after-ripened seeds.

Let us examine the application of the Flemion method to several different dormant seeds shown in Figure 50. In A are embryos of *Rhodotypos kerrioides*. Beginning at the left is an embryo just removed from a swollen seed of 1937 crop; the next 5 in succession are sample embryos from the seed crops of 1932, 1934, 1935, 1936, and 1937, all removed and kept in the germinator five days in 1937. Note that the embryo of the 1932 crop is disintegrating and note the increased growth from the 1934 crop to the 1937. In excised "dormant" *Rhodotypos* embryos both the cotyledons and the roots grow rather promptly, though not nearly as promptly as the low-temperature after-ripened embryos, as we have already seen; and the rate of growth increases with vigor of the embryos and decreases with the age of the seed. The behavior of embryos from the 1932 and 1934 embryos kept on moist filter paper 5 additional days, or 10 days in all, are shown in B. The 1932 embryo is dead and decaying, while the 1934 embryo has such low vigor that it has not enlarged, although it has developed more chlorophyll. It is evident that this test shows not only the embryos that are alive but also the relative vigor of the living embryos of the several crops.

Apple embryos are shown in C: the one at the left, a freshly excised embryo, followed by 2 dead embryos and 2 live ones after 6 days on moist filter paper. The apple embryo is more sluggish in its early growth than the *Rhodotypos* embryo. There is merely an enlargement and the greening of the cotyledon in contact with moist filter paper in some embryos; in others both the cotyledons and the roots grow in the 6-day test.

In D *Crataegus Crus-galli* embryos are shown in a 10-day test run started February 1, 1938. Beginning at the left, first, second, and third are embryos from 1928, 1930, and 1933 crops respectively, all non-viable. The fourth embryo is of the 1935 crop which has not yet had time to enlarge. The fifth embryo is of the 1936 crop in which the intact carpel had been stratified one year at 5° C (41° F); but the embryo had not after-ripened under this condition, due to the resistant coat, as shown by the growth of only the cotyledon in contact with the moist filter paper. *Crataegus* embryos are very sluggish in their early growth, and manifest their viability in these tests by the enlargement and greening of the cotyledon in contact with the moist filter paper and their lack of viability by decay. The test period for these should run for several weeks.

Sorbus aucuparia embryos are shown in E: beginning at the left is a freshly excised embryo of *Sorbus aucuparia;* embryo of the 1930 crop, decaying; and embryo from the 1937 crop with cotyledons enlarged and green. The second and third have been on moist filter paper for 6 days beginning November 18, 1937. The dormant *Sorbus* embryos are more sluggish in their growth than dormant *Rhodotypos* embryos and less sluggish than apple and *Crataegus* embryos.

Witch-hazel embryos appear in F after 5 days on moist filter papers (November 18, 1937), 1934, 1935, and 1936 crops. The one-year-old embryos gave good growth, the two-year-old embryos little growth, and the three-year-old none. In G is shown the reaction of the 1934 embryos (dead) and the 1936 embryos, high viability, to para-dinitrobenzene-ammonia treatment; in H is the reaction of the same to 1 per cent potassium tellurite treatment; and J shows the development of these embryos after 5 days on moist filter paper. The color difference from the reduction of the two chemical compounds is not very conclusive as to degree of vitality.

Prunus americana embryos are shown in K, all from viable seeds after several weeks on moist filter paper. These embryos are extremely sluggish in their early growth and show viability mainly by slow growth and greening of the cotyledon in contact with the moist filter paper and by the occasional growth of a root. The dead embryos soon disintegrate.

The fringe-tree embryos appear in L. In order, beginning at the left, are: dead embryo and live embryos after 4, 6, and 20 days. In M are Douglas fir embryos: beginning at left, freshly excised, viable, and non-viable embryos after 4 days. *Pinus rigida* embryos are shown in N. From left to right, freshly excised, dead embryo, embryo with low vitality, and one with high vitality after 8 days on moist filter paper. In selecting the embryos for this plate (Fig. 50), the embryo that most nearly represented the average growth or behavior in duplicate cultures of at least 50 embryos was used.

FIGURE 50. Development of excised embryos on moist filter paper in Petri dishes at room temperature: K, natural size; E, M, and N 4 ×; all others 2 ×. (*See explanation on opposite page.*)

What are the relative merits of viability tests based on color reactions and those using excised embryos? The color reaction requires only 24 to 48 hours, whereas the excised method requires 4 to 14 days. In both cases the embryos must be at least partially removed, but for the color tests less care needs to be taken against injuring the embryo. Finally, Flemion claims that the main advantage of the excised embryo method is its reliability. Often worthless embryos show considerable color with the color tests. In the excised embryo method the embryo shows its viability by a growth reaction of some sort. It is a direct viability test.

Let us select two of many cases where the excised embryo method was applied to practice. A seedsman reported that he had a chance to purchase 2000 bushels of Douglas fir cones that had been found in a squirrel cache. The seeds looked good, but a test for viability was desired; also the offer had to be accepted in a few days, whereas an ordinary germination test required about 60 days. Within 5 days by the use of this quick viability test Flemion was able to advise that 95 per cent of the seeds were dead and the other 5 per cent showed very low vitality. The largest distiller of witch hazel has always used wild growth as the source of wood for distillation. This source became scarce and had to be gathered at greater and greater distances from the still. A few years ago the distiller decided to start a grove near the still as a wood source. A quick vitality test showed that the peck of seeds they had for this purpose was entirely dead. The collection of seeds the next autumn proved viable and was after-ripened with three months of stratification at 5° C (41° F). Several hundred thousand seedlings are now ready to transplant into the proposed grove.

The excised embryo test for viability of dormant horticultural and forest seeds is used extensively at Boyce Thompson Institute, and is gradually being adopted by seed-testing laboratories.

Chemicals as Forcing Agents for Dormant Seeds

As we shall see in a later chapter, Denny and co-workers have been very successful in forcing dormant buds with chemicals. Among the chemicals tested to date, few have proved successful in forcing dormant seeds. Nitrogen compounds for light-favored seeds and concentrated sulfuric acid for hard seeds and seeds with resistant coats are notable exceptions. The latter, however, is a corrosive action. A few kinds of dormant seeds are forced by one or more of the following substances: carbon dioxide, mercury salts, hydrogen peroxide, anesthetics, etc., but none of these is generally effective as a forcing agent. We have tried bud-forcing chemicals, hormones, and other compounds, on both dormant seeds and embryos, without promising results either in forcing the dormant seeds to germinate or dormant embryos to grow with vigor. It is possible, however, that later researches will find chemicals that will parallel temperature manipulations in eliminating seed and embryo dormancy.

Summary

We have seen the advantages to plants of delayed and time-distributed germination of seeds in furthering the persistence of species, and the advantages to man of at least a temporary dormant period in seeds in furnishing him seeds for food and for propagation of plants. We have seen how delayed germination presents difficulties in propagation and in fighting weeds on the farm and in the garden. We have seen that delayed germination of seeds in nature is secured by a variety of means: by hard coats that allow no water to enter until decay or other factors corrode the coats; by requiring light or darkness for germination so that the first seeds are prevented from germinating if they are covered with soil and the latter if they are not; by coats, very thin in the upper seed of the cocklebur, that reduce the oxygen supply below that needed for germination; by dormancy in embryos that gives them sluggishness in early growth and manifests itself by dwarfishness in the later growth of the seedling, unless previously given a low-temperature period in the soil which after-ripens the embryos and perhaps aids in coat destruction; by dormancy of the epicotyl that is after-ripened by a period of low-temperature exposure only after the root has started; by demanding a high-temperature period in the soil for decomposition of coats by soil agents, especially organisms, followed by a low-temperature period for after-ripening the embryos; by demanding a low-temperature period to after-ripen the seed so that the root can grow, followed by a high-temperature period for root growth, and in some seeds for epicotyl elongation and rupture of cotyledonary sheath, followed again by a low-temperature period for epicotyl after-ripening, and a high-temperature period for growth of the epicotyl.

At every phase of this study we have seen the great significance of seed and fruit coats, non-living or in the main non-living, in securing this delay. We have seen that two big factors in after-ripening temperate zone seeds and preparing them for germination are periods of low-temperature moist exposure and periods of dry storage; and hardly less important is the coat-disintegrating action of soil agents, especially organisms which act best at growing temperatures. We have discussed various methods for quick vitality tests of seeds that require long periods for germination, the best of which seems to be isolating or partially isolating the embryos and placing them on moist filter paper. Finally, the main problems in delayed germination and dormancy of seeds are yet to be solved. The work to date has thrown much light on the way dormant seeds of various types behave in nature; it has put into the hands of commercial and amateur growers methods of growing many kinds of seeds that could not be grown before; and it has done much to define the big physiological and biochemical problems still to be solved. No doubt further research will shorten the time needed to get complete germination of the seeds that are the most difficult to handle.

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CHAPTER 4

Physiological Effects of Ethylene and Other Unsaturated Carbon-containing Gases

Early Experiments

Beginning at the University of Chicago and continuing later at Boyce Thompson Institute in a much more detailed way, a group of investigators have made extensive studies of the effects of gases upon plants. In the later phases, the researches were extended to the effect of some of the gases upon animals. In regard to the physiological effect of the gases upon plants we may speak of two groups: (1) gases low in lethal action and high in anesthetic and growth-modifying effects, among which are certain unsaturated C-containing gases such as ethylene, acetylene, propylene, and carbon monoxide; (2) gases mainly lethal that have only minor physiological action otherwise, including hydrocyanic acid, mercury vapor, sulfur dioxide, ammonia, chlorine, and hydrogen sulfide. The physiologically active gases will be discussed in this chapter and the lethal gases in the following chapter.

This work was initiated by a question from a Polish greenhouse operator, a good grower but painfully short on English, "What is the effect of illuminating gas on carnations?" Answering his question raised others; as a result, much of the work reported in this and the two chapters that follow was an outgrowth of his question. Since the hormone work at the Institute originated in the anesthetic and formative effects of ethylene upon plants, all the work reported in the hormone chapter is a continuation of the answer to the Polish greenhouse operator's query. The author has been assigned two botany problems in his time. One was the reason for the difference in behavior of the two seeds in the cocklebur, assigned by Professor Chas. F. Hottes of the University of Illinois (1902), and the other was the illuminating gas problem (1908) here discussed. The previous two chapters show what has come of the first assignment, and this and the next two chapters set forth the results of the second. Each, of course, has involved the cooperation of a number of investigators, not only in the laboratories with which the author has been associated, but in laboratories throughout the world. The literature on the remarkable physiological effects of the unsaturated C-gases has become almost voluminous since 1908, though most of it has appeared since 1930; only a portion can be cited in this popular discussion. Which assigned the better problem, the learned university professor or the practical greenhouse operator, quite innocent of higher learning?

The greenhouse operator had lost the carnation crop in several of his greenhouses each fall for two years after a cold spell had frozen crust on the ground. Near the greenhouses affected was a honey-combed illuminating gas pipe. When there was no crust on the ground the gas escaped upward into the air. With a frozen crust on the ground the gas moved horizontally and escaped into the greenhouse. With the ventilators closed during the cold spells the gas was held in the houses.

Crocker and Knight [7] tested the effect of the Chicago illuminating gas (a water gas) on carnations. The buds and flowers proved most sensitive. One part of illuminating gas to 40,000 of air stopped the growth of young and medium-aged buds, and 1:80,000 caused freshly opened flowers to close, "go to sleep," within 12 hours, and they would not open again even in absence of the gas. As manufactured illuminating gas is a mixture of many gases, the question naturally arose, Which of the constituents are effective? Neljubow [56] had previously investigated the reason for the peculiar growth of certain plants in "laboratory air" and found it due to a trace of illuminating gas. He found that ethylene produced the "horizontal nutation" of the garden pea epicotyl in concentrations as low as 1 ppm of air. The ethylene in the air changed the geotropic equilibrium position of the garden pea epicotyl gradually from the vertical to a more and more declined position as the concentration of ethylene increased, until 1 ppm of ethylene brought the tip of the seedling into a horizontal position or made it diageotropic. Fig. 51 shows the results of repeating Neljubow's experiments 43 years later upon another variety of garden pea. The controls show the great amount of elongation in the epicotyls in three days, and also that they are negatively geotropic, *i.e.*, grow vertically. One ppm of ethylene reduced the rate of elongation markedly, caused the portion of the pea that grew while in the gas to take a declined position; 1.33 ppm reduced the elongation much more and caused the part growing while in the gas to become diageotropic and the growing part to increase in diameter; and 4 ppm reduced elongation still more, with a consequent shorter swollen and diageotropic region. It is interesting that these results check so closely with Neljubow's, although a different variety of pea was used under a different set of conditions. He got horizontal nutation in 1 ppm of ethylene; Crocker and Knight got it with 1.33 ppm of ethylene. They found further that 1 ppm of ethylene prevented the growth of young carnation buds and 0.5 ppm of ethylene caused open flowers to close.

From this work Knight and Crocker concluded that ethylene was the effective constituent of illuminating gas in bringing about the injury to the carnation buds and flowers. The crude methods of analysis available at that time indicated that the gas bore about 3 per cent ethylene, whereas it should have borne 4 per cent according to the flower and bud response. The discrepancy can be explained by errors in analysis and variation in the sensitivity of the buds and flowers, as well as variations in the ethylene concentration of the gas. Later work has also shown that, while certain

plant organs respond to very low concentrations of ethylene, the concentration may vary many per cent without a noticeable difference in response. The authors also spoke of the toxicity of ethylene. Later work has shown that ethylene is not a highly lethal chemical, that is, tissue-killing, but

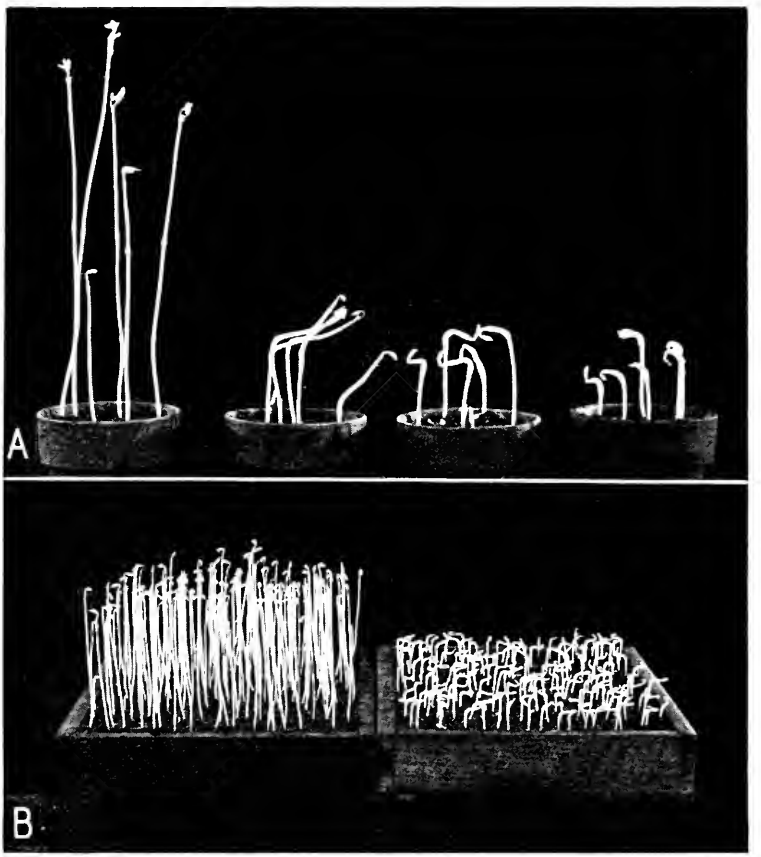

FIGURE 51. Response of the etiolated epicotyl of garden pea to ethylene. Seedlings 3 to 4 cm high exposed to ethylene for 3 days room temperature. A (*left to right*): control, 1 ppm ethylene, 1.33 ppm ethylene, 4 ppm ethylene. B: (*left*) control; (*right*) 1.33 ppm ethylene.

rather an anesthetic and a growth modifier. The failure of the buds to grow was due partly to anesthetic action, and the closing of the flowers was an irreversible growth response called hyponasty.

As ethylene is hard to detect chemically in the low concentrations that produce plant responses, Knight and Crocker [44] attempted to find plant responses that would serve as reliable and delicate tests for traces of ethylene. They tried various sorts of peas as test plants and found certain varieties of sweet peas, Gladys Unwin, for example, even more sensitive than garden peas. One-tenth ppm of ethylene inhibited the elongation of the

epicotyl of this pea measurably; 0.2 ppm caused slight declination, together with much greater inhibition of growth; and 0.4 ppm caused still greater reduction in elongation, a greater declination from the vertical, and a swelling of the declined portion. This constituted the "triple response" of Knight and Crocker. They proposed the use of either the declination or triple response of the sweet pea seedling as a means of detecting the presence of ethylene.

Later, several workers [4, 8, 17, 35] have shown that ethylene causes a modification in the relative rate of growth on the upper and lower sides of the petioles of leaves of many kinds of plants so that the leaves curve downward, giving the epinastic response. Fig. 52 shows this response in the tomato plant.

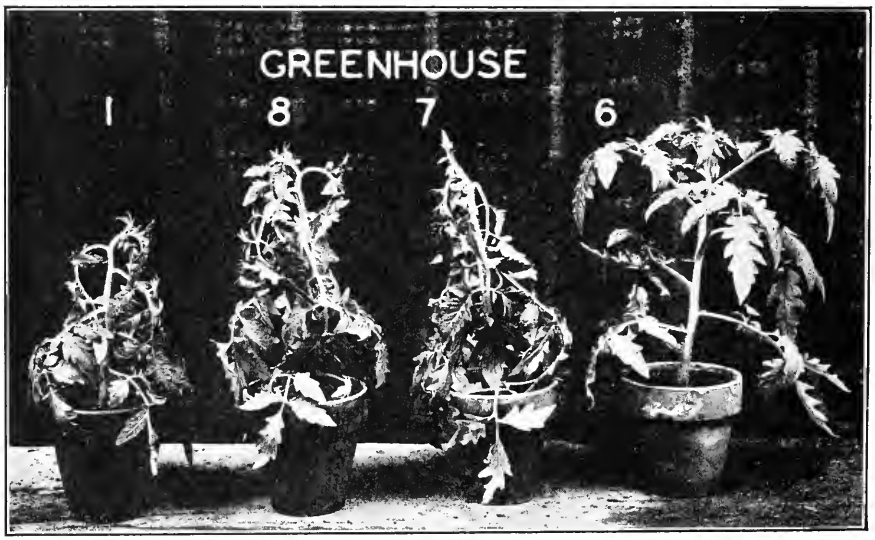

FIGURE 52. Tomato plants after exposure for 28 hours in various houses of a commercial range suspected of gas injury. No. 1, a house in which Acacias showed almost complete bud and leaf fall; No. 8, a house in which newly forced roses were showing leaf fall; No. 7, a house in which roses were showing similar injury to No. 8; No. 6, a house in which roses showed no injury.

Besides ethylene, acetylene, and propylene, carbon monoxide (CO) and perhaps butylene will induce the declination or the triple response in the sweet pea seedling and the epinasty of leaves, but these three gases must be used in much higher concentrations than ethylene to be effective. Table 18 shows the minimum effective concentrations of the several unsaturated C-gases for inducing declination of the sweet pea seedling and epinasty in the tomato leaf.[8] The gases of the olefin series (ethylene, $CH_2{=}CH_2$, propylene, $CH_3CH{=}CH_2$, and butylene, $CH_3CH{=}CH{\cdot}CH_3$*) fall off rapidly with increase in length of the chain. Acetylene, $CH{\equiv}CH$, with a

* Formula given is one of three isomers.

Table 18. Comparative Effectiveness of Gases in Producing Declination in Sweet Pea Seedlings and Epinasty in Tomato Petioles.

Gas	Minimum parts per million needed to produce	
	Declination in sweet pea seedlings, according to Knight and Crocker *	Epinasty in tomato petiole †
Ethylene	0.2	0.1
Acetylene	250	50
Propylene	1000	50
Carbon monoxide	5000	500
Butylene	—	50,000

* 3 days' exposure used. † 2 days' exposure used.

triple bond and chemically more active than ethylene, is much less effective in inducing either response; more than 1200 times the concentration is needed to induce the pea response and 500 times the concentration to induce the tomato response. Other unsaturated short chain compounds [8] such as allyl alcohol, $CH_2{=}CH{-}CH_2OH$, acrolein, $CH_2{=}CH{-}CHO$, and isoprene, $CH_2{=}CH{-}C(CH_3){=}CH_2$, did not induce these responses. Allyl alcohol killed the tomato in 125 ppm of air and acrolein in 30 ppm of air. Denny [15] has found three out of 77 other volatile chemicals tested that produce leaf epinasty: ethyl bromide, ethyl iodide, and propyl chloride. Later we shall see that most so-called plant hormones induce this reaction. The latter in general have low volatility and neither the three halides Denny mentions nor the hormones are likely to be present at all, or at least in a way that will interfere with the tests we describe below for ethylene and other effective unsaturated C-gases.

Responses Induced by Gases Containing Unsaturated Carbon

The four effective gases mentioned above induce the following responses in plants: (1) epinasty of leaves; (2) proliferation of tissues; (3) abscission of leaves, flowers, and fruits; (4) anesthesia and inhibition of growth; (5) coloring and other ripening changes in fruits; (6) other metabolic changes in living plant tissues; (7) root and root-hair initiation; and (8) other physiological effects.

Epinasty of leaves. The effective gases cause epinasty in leaves by inducing a rapid elongation in the upper side of the leaf or petiole. This is shown in Fig. 53.[8] In this case only the older basal leaves of the tomato plants in which the base of the petioles had ceased to elongate were used. The gas starts growth again in the tissue on the upper side of the base of the mature petiole. In young leaves in which the petiole is still growing even at the base, the gas causes growth on the upper side of the petiole in excess of that on the lower side, and thus turns the leaf downward, the curvature extending over a great part of the length of the petiole. After

leaves have responded in gas and the plants are put in gas-free air, the young leaves recover their former position completely, and the older leaves partially, by growth on the lower side, or hyponastic growth. Leaves of plants rotated on a horizontal clinostat show much less epinastic response to ethylene than do leaves of plants in a stationary, upright position; and leaves of inverted plants show little or no epinastic response to ethylene. With the plant upright in air, the position of the leaf is determined mainly by its geotropic equilibrium position, but in part also by autonomic epi-

FIGURE 53. Tomato plants showing change in length of upper faces of petioles when sealed in Wardian cases for 24 hours: *left*, in air; *right*, in 1 part of Yonkers gas to 10,000 of air.

nasty; [8] in some leaves light plays a part. With a little ethylene in the air, either the geotropic equilibrium position is changed or the geotropic effect is so weakened that autonomic epinasty plays the main role in leaf position. In some leaves no doubt geotropism is still a factor in determining the ethylene position of the leaf, while in others autonomic epinasty is the main determinant. We shall see later that petioles of some leaves in the presence of ethylene continue to grow on the upper side until they actually form coils. In such cases it would seem that autonomic epinasty and not geotropism is the determining factor.

Out of 202 species and varieties of plants tested,[8] 89 gave leaf epinasty in the presence of ethylene and 113 did not. Fig. 54 shows the type of response in four different plants. In *Fuchsia* the petioles decline somewhat, but the blade shows the greatest epinastic curvature. In buckwheat and sunflower the growth on the upper side of the petiole is so extreme that it

FIGURE 54. Ethylene-induced epinasty of leaves. All plants sealed in Wardian cases for the periods and in the concentrations of gases mentioned below and photographed immediately after removal from the cases: 1. Fuchsia, check; 2. Fuchsia in 500 ppm of ethylene for 24 hours; 3. Buckwheat, 1 part to 10,000 of Yonkers gas, equivalent to 3 ppm of ethylene, for 72 hours; 4. Sunflower, 1 part to 10,000 of Yonkers gas, 48 hours; 5. Paper White narcissus, 1 part to 10,000 of Yonkers gas, 96 hours.

produces coils in some of the petioles. In narcissus only the young leaves respond, and the inequality of growth on the two faces of the leaves is sufficient to produce coils at the tips of the leaves. The epinastic response of tomato leaves has already been shown in Figs. 52 and 53, and it will be shown for other plants in later figures.

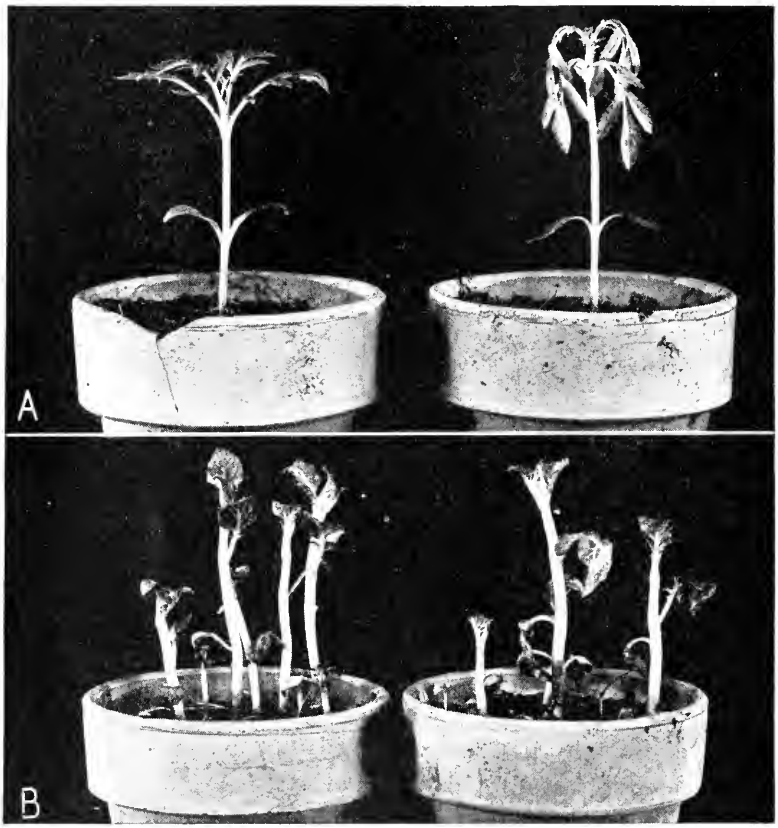

FIGURE 55. A, Marigold plants. *Left:* control. *Right:* in 1 part of ethylene to 1 billion parts of air for 20 hours. B, Potato plants. *Left:* control. *Right:* in 1 part of ethylene to 300 million parts of air for 24 hours.

Since leaf epinasty is a growth response, it is most easily induced in thrifty plants growing under optimum conditions; but there is a great difference in the minimum concentration of ethylene or other effective gases needed in the air to induce leaf epinasty in various kinds of plants. The tomato is moderately sensitive. The leaves of thrifty plants in good growth condition will respond to 1 part of ethylene to 10 to 20 million of air. In old leaves under long exposure epinasty will occur in 1 to 25 million or less of ethylene (Fig. 59). As is shown in Fig. 55, leaves of the young potato plant respond to 1 part of ethylene to 300 million of air, and the African marigold leaves to the extreme dilution of 1 part of ethylene to 1 billion of

air. A medium-sized thimble holds about 2.5 cubic centimeters. One such thimbleful of ethylene placed in a room 80 feet long, 32 feet wide, and 32 feet high gives 1 part of ethylene to 1 billion parts of air, or just enough, after it is equally diffused throughout the room, to induce epinastic response in the leaves of the African marigold. Air is a rather rare medium compared with the density of water or plant tissue, also ethylene has low solubility in water and probably in plant tissues, especially at 25° C (77° F), a good growing temperature. The percentage by weight of ethylene in the plant tissue in the atmosphere described above must be almost fancifully small. The epinastic response of the marigold to ethylene must rank high in the extreme sensitiveness of an organism to a chemical. Vitamins and hormones are noted for producing physiological effects in extreme dilutions and, as we shall see later, there are good reasons for considering ethylene a plant hormone.

Destructive or dry distillation or incomplete combustion of carbon compounds produces ethylene, carbon monoxide, and other carbon gases. As a result, there are several sources of ethylene and carbon monoxide in the air: artificial illuminating gas; automobile exhaust and exhaust from other internal combustion engines; furnaces when the oxygen supply is inadequate; improperly trimmed or adjusted oil or gas stoves or torches; [43] pipe, cigarette, or cigar smoke; and burning brush or rubbish piles. Finally, burning a sheet of paper [44] in the air produces some ethylene and carbon monoxide because the heat is not great enough to burn all distillation gases. There are three known natural sources of ethylene: it is given off by coal in the mine or in storage; one natural gas [8] is known to contain a trace of ethylene; and respiring living plant tissues produce ethylene. In traces of the gas mixtures mentioned above, it is the ethylene that induces the responses and not the other three. This is because ethylene is so much more effective than the others; also propylene and acetylene when present are generally in lower concentrations than ethylene in these mixtures. In some, CO is more concentrated than ethylene; in water gas there are about 24 per cent CO and about 3 per cent of ethylene. But in the lowest concentration of this gas in air that will induce epinasty, because of the ethylene present, CO exists in about $\frac{1}{625}$ sufficient concentration to induce the response. Epinasty does, however, serve as an indirect test for CO for it generally accompanies ethylene in the gases mentioned above.

Epinastic response of leaves has proved a very useful and delicate test for traces of unsaturated C-gases both in practice and in research.[12, 15] This test has been accepted in courts as evidence of illuminating gas in greenhouses.[4] Fifteen or twenty years ago there were frequent and some very extensive injuries to plants in commercial greenhouses by artificial illuminating gas that escaped from leaking pipes, seeped along under the frozen crust, and came up into greenhouses. Numerous researches on the effect of illuminating gas and its constituents, mainly ethylene, upon greenhouse plants have reduced this loss tremendously. The epinastic response

has given a very sensitive and exact means of detecting the presence or absence of the gas in the greenhouse. This and various other detrimental changes caused in plants by traces of ethylene have been impressed upon the minds of both the greenhouse owners and the gas companies. As a result, the greenhouse owners now recognize gas injuries in their incipiency and, what is more important, the gas companies take great care in testing for leaks and repairing them in the neighborhood of greenhouses.

There is one precaution that has not received enough attention, namely, the trapping of water drains from greenhouses. We have observed cases where gas leaks several blocks from a greenhouse caused injury. The gas escaped into the storm sewer, traveled through it, and into the greenhouse through the untrapped drainage tile. Water drainage lines from greenhouses should have cemented joints so as to be air-tight. They should also have a trap outside the greenhouse with an upright vent outside the trap. While this would prevent injury in some cases, in many others it would not. Gas will seep many rods through the ground under a frozen crust and escape up into the greenhouse through the unfrozen ground. The real way to prevent injury is to see that there are no gas leaks even in the general neighborhood of a greenhouse. The epinastic response has been used in submarines [5] to determine whether any exhaust gases are escaping into the hull.

This test even has its place in home and social adjustment. The local gas company had trouble in convincing two lady school teachers that there was not a gas leak in their apartment. The ladies were shown that the tomato could detect about $\frac{1}{1000}$ the least concentration the human nose could detect. The tomato plant indicated no gas in the apartment. The teachers agreed that they were smelling something else. The author gave a judge's wife a beautiful *Crassula arborescens* plant. After it had been in the house two weeks many of the leaves had fallen. The tomato plants indicated leaks in the 20-year-old gas stove, but a much bigger leak in the gas meter under the front room. The judge's wife got a new stove and the gas company repaired the meter. The judge heard from his neighbors for his penuriousness in failing to buy a new stove until the evidence was overwhelmingly against him. Oortwijn Botjes [61] used the epinasty of tomato leaves to show that ripening apples produce ethylene. Other investigators [16, 25, 26, 55] have used epinasty in tomato, potato, and marigold leaves and horizontal nutation in the pea seedling to demonstrate ethylene emanations from respiring tissues of several kinds of plants. Many other uses of this response in research will be mentioned below.

Proliferation of tissues. Ethylene induces the proliferation of plant tissue, especially of cork cambium, as well as enlargement of cells. Harvey and Rose [37] showed that when illuminating gas flowed slowly through soil in which plants grew it caused the development of massive soft white tissue at the base of the stem and on the larger roots of *Hibiscus*, also on roots and lower part of the stem of *Catalpa* seedlings. This tissue resulted both

from a multiplication of cells (hyperplasia) and from an increase in size of some of the cells (hypertrophy). Fig. 56 shows the effect of ethylene in inducing proliferation of tissue in the lenticels of young *Hibiscus* stems. In

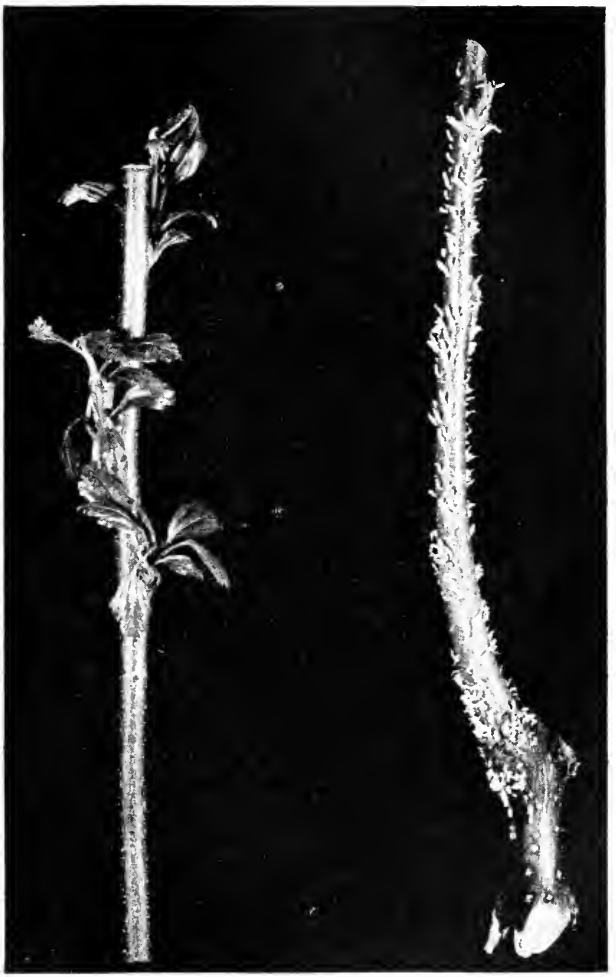

FIGURE 56. Twigs of *Hibiscus* cut from tree in late January and placed in moist chambers for 10 days. *Left:* control in air. *Right:* treated in ethylene 10 ppm. Note that ethylene induced undifferentiated outgrowth from the lenticels and stopped bud growth.

the picture the outgrowths look like roots. Instead they are undifferentiated tissue. It will be noted also that ethylene hindered the growth of buds. The enlargement in the tip of the sweet pea seedling (Fig. 51) induced by ethylene probably results mainly from cell enlargement.

When the illuminating gas flowed through the soil more rapidly, this response did not occur, but the roots and base of the stem were killed. As

we shall see later, illuminating gas contains cyanides which are soluble; in heavier flow of gas these accumulate in the soil in sufficient concentration to kill the plants, so the ethylene response can not occur. Wallace [68] found that intumescences in the apple stem in response to stimulation by ethylene gas arise through three fundamental changes in the tissues affected, namely, solution of cell walls already showing marked secondary thickening, enlargement of cells, and proliferation of cells. Ethylene induced intumescence in apple twigs in the extreme dilution of 1 to 100 million of air. This approaches the sensitiveness of the potato and African marigold leaves which require over 1 to 300 million and 1 to 1 billion of ethylene in the air, respectively, to induce epinasty. We shall see later that many of the plant hormone types of chemicals induce proliferation of tissues.

Abscission of leaves, flowers, and fruits. One of the more general effects of ethylene and other effective gases is to cause leaf, petal, flower, and fruit fall by inducing growth in cells of the abscission layer. This is in part because the flat cells of the abscission layer enlarge and become spherical (hypertrophy), although proliferation of cells may occur in some cases. No doubt abscission is also furthered by the tendency of ethylene to induce the solution of insoluble pectins of the middle lamella of the cell walls. We shall discuss this effect of ethylene later in this chapter. Fig. 57 shows leaf fall in *Crassula arborescens* and petal fall in *Salvia* induced by ethylene in cigarette smoke in the first, and by ethylene in illuminating gas in the second.

Ethylene has been used in Oregon to defoliate roses at time of digging. For this purpose a moderately tight chamber having high humidity and a temperature of 70° to 75° F (21° to 24° C), not exceeding 85° F (30° C), is used. Tank ethylene may be used at the optimum rate of 1 cu. ft. to 100,000 cu. ft. of space. Apples stored in the chamber give the optimum ethylene concentration when there is a bushel to every 400 to 500 cu. ft. of space. Kerosene stoves and rose hips proved a less desirable source of ethylene because the ethylene concentration was not sufficiently high. The defoliation required four days under optimum conditions. Ethylene has been used to loosen the shucks of English walnuts [67] and pecans,[2, 21] with improvement in the color of the product in the former. In both cases the nuts could be harvested earlier, as soon as the meats were ripe, without waiting for the shucks to loosen on the tree. This prevented later injury in the dry, hot air of California and Arizona. The optimum conditions in the shucking chambers were 1 to 1000 of ethylene and a temperature somewhat above 70° F (21° C) with a period of two to four days. Emanations from pecan shucks induced epinasty in potato plants; the pecan tissue produces ethylene, but evidently not enough to induce quick shucking. Ethylene probably induces earlier growth in the abscission layer between the shuck and shell. The cytological changes, however, have not been studied.

Anesthesia and inhibition of growth. Doubt [17] found that the proper concentration of ethylene produced complete rigor in *Coleus*, from which the

plant recovered without injury when the gas was removed. In proper concentrations of ethylene, *Mimosa pudica* was unable to respond to the contact or heat stimulus; it recovered after removal from the gas, but showed some later injury. Neljubow [56] and Knight and Crocker [44] showed still

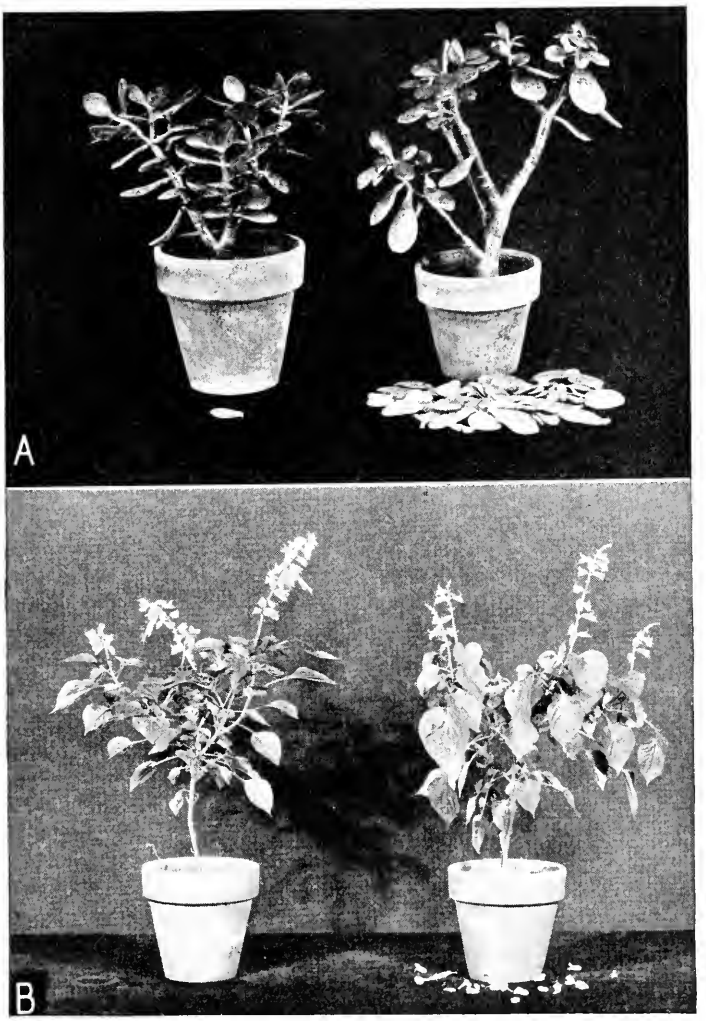

FIGURE 57. A, *Crassula arborescens* showing leaf fall. *Left:* control. *Right:* sealed in a 10-liter bell jar with 3 puffs of cigarette smoke for 5 days. B, *Salvia* showing petal fall. *Left:* control. *Right:* treated 24 hours with 1:10,000 illuminating gas.

earlier that the rate of elongation of etiolated pea seedlings was reduced by very low concentrations of ethylene in the air, and that as the concentration of ethylene increased, the rate of elongation fell until in relatively low concentrations the elongation ceased, that is, growth rigor was produced. Anesthetized pea seedlings showed no killing of tissue and resumed

a rapid rate of elongation when removed from the gas. There is an increase in diameter in the growing region near the tip of the seedling, even when it is in a concentration of ethylene that stops all elongation. This forms a knob near the tip, as is shown in Fig. 51, with the high concentration of ethylene.

A number of later studies [6, 8, 69] have been made on the anesthetic action of ethylene, acetylene, propylene, and carbon monoxide upon plants and lower animals. In plants the order of effectiveness of the several gases, that is, the minimum concentration inducing rigor, is the same as for epinasty and declination of the pea seedling. It took very high concentrations of these gases to induce rigor in insects and centipedes and the order of effectiveness of the several gases showed no relation to that found for plants. Butylene, which is almost without effect on plants, is the most effective in anesthetizing insects and centipedes, requiring from 5 to 40 per cent for the various species. It also partially paralyzes the organisms. Ethylene, which is the strongest anesthetic for plants, along with acetylene, was the least effective on insects and centipedes, requiring a complete atmosphere for anesthesia; 30 to 75 per cent of propylene and 80 to 90 per cent of carbon monoxide were required to anesthetize these organisms.

It is evident from the facts stated above — and will be borne out by those to be stated later — that besides anesthesia these gases have many other effects on plants, namely, epinasty, proliferation of tissue, abscission, etc. Probably most of these secondary effects can be avoided by using gases in concentrations that produce complete rigor. Epinasty of leaves, however, is induced in concentrations of ethylene that result in complete rigor in other parts of the plant, as shown by time-lapse motion pictures [8] of tomato plants in air and in air containing 2 ppm of ethylene. The photographing was continued 24 hours with the treated plant in the gas, followed by 24 hours with the treated plant in ethylene-free air, in order to get both the response and the recovery. The exposures were made 96 times per hour to give a speed of movement on the screen 600 times that occurring in the plants themselves. The top of the check plant was in continuous movement due to unequal rate of growth on the several flanks of the stem; also the leaves on the control plant showed movement during the course of the experiment. Especially conspicuous was the sleep movement during the first night of the experiment. This movement was less conspicuous during the second night due to bad growth conditions furnished by the experiment.

The movements of the gassed plant must be considered under two heads: the response movements and the recovery movements. Soon after the plant was placed in the anesthetic, the tip of the plant ceased to move and showed no movement during the rest of the exposure. It was in rigor. Moreover, the sleep movement of the leaves did not occur. Instead, noticeable epinasty of the petioles began within two to three hours after exposure. The first leaf to show this was the third from the top, followed successively by

the leaves below on the growing part of the stem and then by the two tip leaves. Several hours later the older leaves on the non-elongating part of the stem showed epinastic movement. The leaves on the growing part of the stem had completed their epinastic movements within 8 or 10 hours and the leaves on the more mature part of the stem within 15 or 20 hours. Younger leaves curved throughout the length of the petioles, while in the older leaves the curvature was limited to the base of the petioles. After the leaves had come into equilibrium by epinastic response, all movement in them ceased for the duration of the exposure. The whole plant was in rigor. Two or three hours after the gassed plant was put into ethylene-free air, the recovery from the epinasty began and continued in about the same order and, for the younger leaves, with nearly the same speed as the response. The leaves on the growing part of the stem showed complete recovery to the original position, while the older leaves showed only partial recovery. Also within two or three hours after removal of the plant from the anesthetic, vigorous movement started in the tip and continued throughout the period. The tip of the plant recovered completely from the rigor.

The time-lapse pictures of the sunflower showed similar behavior. Comparable time-lapse pictures were made of the sensitive plant, using 0.5 per cent of CO as the anesthetic. Complete anesthesia was induced in this plant, and complete recovery occurred after removal from the gas, as shown by loss of power to respond to contact stimuli while in the gas and recovery of this power after removal from the gas. When this plant is anesthetized, however, there is a slow, non-correlated movement of the individual leaves and leaflets that gives the plant a disorganized appearance (Fig. 58). The movements of the leaves and leaflets of this plant are brought about by changes in osmotic pressure in cells of the pulvini at the base of leaves and leaflets, and not by growth. The slow, non-correlated movements in the leaves and leaflets in the anesthetized plant would have been entirely overlooked if it had not been for the time-lapse pictures.

When plants are exposed to ethylene in concentrations below the rigor-producing dosage, the rate of growth is retarded, with or without other secondary effects, depending upon the kind of plants treated and the concentration of the gas used. A study was made of the effect of low concentrations of ethylene (1 part to 10 million and 1 part to 25 million of air) on the growth rate of seeds and seedlings of wheat, buckwheat, tomato, clover, and corn growing in soil in pots. These experiments were carried out in two continuous and regulated air-flow Wardian cases in a special greenhouse. This apparatus will be illustrated and described in the next chapter. The rate of air flow through each chamber was 200 cubic feet per minute. By means of a calibrated capillary flowmeter a measured amount of ethylene was added to the air passing through one flow-chamber to give the concentration of ethylene desired. Six pots each of seeds and seedlings of each kind of plant were used in the control and treated chamber.

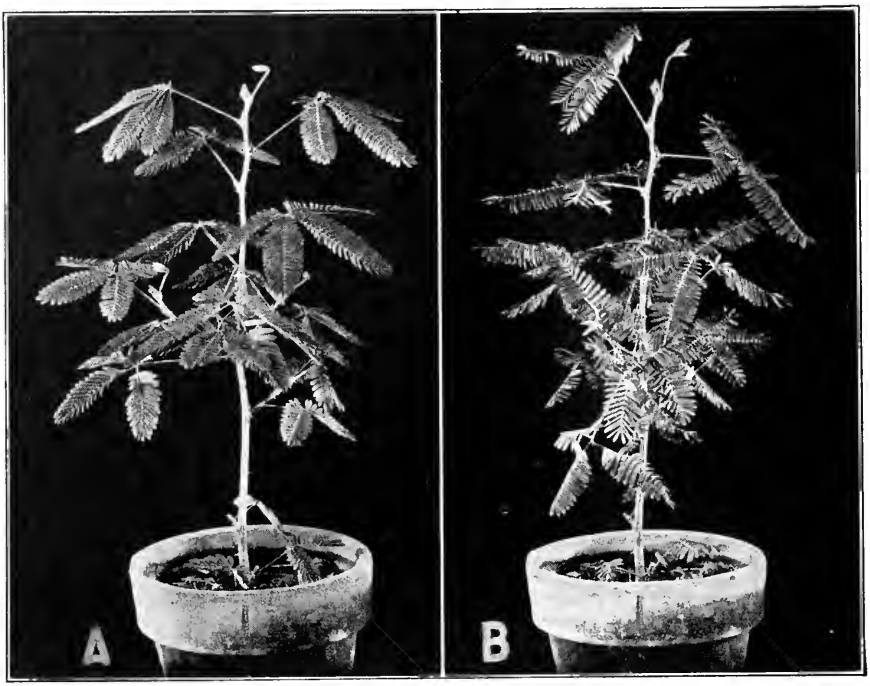

FIGURE 58. The anesthetic effect of carbon monoxide on plants. A, *Mimosa pudica* control. B, The same plant as "A" after exposure to one per cent carbon monoxide for 18 hours. Note the change in the normal equilibrium position of the leaves and the disturbance of normal correlation.

Fig. 59 and Table 19 show the effect of these low concentrations of ethylene upon the growth rate of seeds and seedlings. For the illustration a typical pot was selected in each case and photographed, and for the table all seedlings of each kind and lot were measured at the beginning and end of the experiments and the average elongation during the experiment calculated. Examination of the figure shows that 1 part of ethylene to 10 million of air causes a marked reduction in growth rate and 1 part to 25 million an evident reduction. There is no killing of tissue; the growth inhibition is an incipient anesthetic effect. The table shows that 1 part of ethylene to 10 million of air reduced the rate of elongation by the following percentages: clover, 50; tomatoes and buckwheat, 40; and wheat, 25.

Table 19. Per Cent Inhibition of Growth in Length by Ethylene.

1 part of ethylene to 10 million of air. Continuous fumigation for 4 weeks			
Clover	50	Buckwheat	40
Tomatoes	40	Wheat	25

Measured by volume or wet or dry weight increase, the reduction in percentage growth would be much greater. It will be noted that the lower concentration induced epinasty in the older leaves of the tomato plant. Even so, the question arises whether epinasty of tomato leaves is sensitive enough to detect the lowest concentration of artificial illuminating gas in greenhouses that will retard growth. If not, epinasty in the leaves of potato or marigold will do so. Greenhouse operators will do well to keep young plants of all of these growing in their greenhouses continuously if there is any danger of injury from leaking gas pipes.

From observing the work on the effect of ethylene on plants done in the botany department at the University of Chicago, Dr. Luckhardt of the animal physiology department of the same institution became interested in the effects of ethylene upon animals. He and associates [49, 50] found it to be a rather remarkable anesthetic for higher animals and man, but it must be used in high concentration, 80 per cent or more, with oxygen. The authors cited describe its use in 800 operations at the Presbyterian Hospital at Chicago. They mention several advantages of ethylene as an anesthetic in surgery under the headings: (1) ease of induction and rapidity of recovery; (2) relaxation without cyanosis; (3) absence of sweating; (4) absence of respiratory irritation; and (5) narrow anesthetic margin. They list as disadvantages: (1) odor; (2) oozing from wound; and (3) danger of explosion. They state that it is no more explosive, however, than ether. In 1938 Luckhardt was awarded the Alpha Omega medal as discoverer, with J. Bailey Carter, of ethylene as an anesthetic with qualities superior to nitrous oxide.

Chipman,[3] an anesthetist of Washington, D. C., ranks ethylene-oxygen first among anesthetics known and in use up to 1931. Dr. H. M. Livingstone, Chief Anesthetist at the University of Chicago Clinics, says that ethylene-oxygen is enjoying its greatest popularity in the midwest and some in California. She describes [48] the use of ethylene-oxygen in 6590 cases, mostly without the accompaniment of other anesthetics. She also states that to date she has used ethylene-oxygen in about 50,000 cases without death or explosion. Luckhardt states that John S. Lundy [52] of the Mayo Clinic used ethylene-oxygen in an even greater number of cases without mishap, but that lately he is using mainly an intravenous anesthetic.

Dr. Poe [62] considers ethylene-oxygen the best anesthetic known to date and finds no danger of explosion if it is properly applied. Drs. Guthrie of Sayre, Pa., and Woodhouse of Cedar Rapids, Iowa, confirm this view and describe 35,500 operations conducted with ethylene-oxygen either as the sole anesthetic or in conjunction with ether. They say:[29, p.1850] "It is our opinion that ethylene is an excellent anesthetic agent for general surgical use and that an unjust prejudice exists in the minds of the surgical profession with respect to its widely heralded disadvantages." The anesthetic used by anesthetists seems to depend to a great degree upon the anesthetic in use where they were trained. On the whole, however, ethylene-oxygen

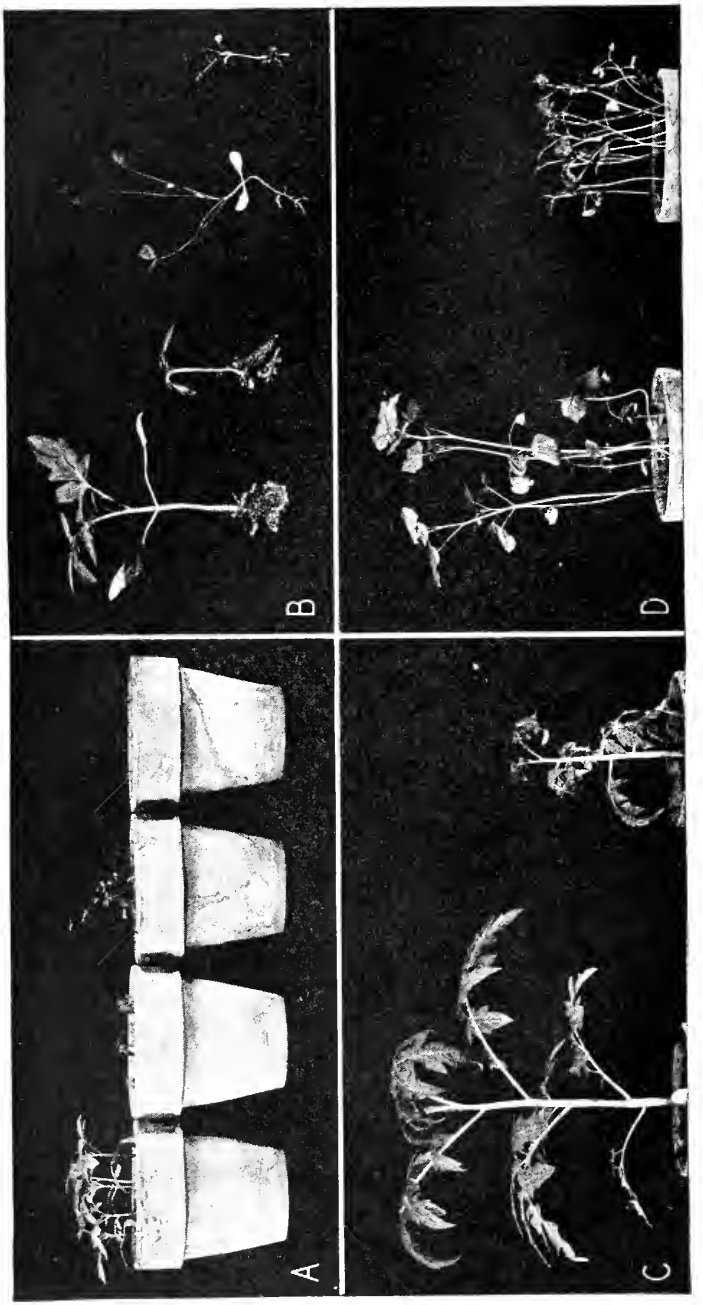

FIGURE 59. *(Please see legend on opposite page.)*

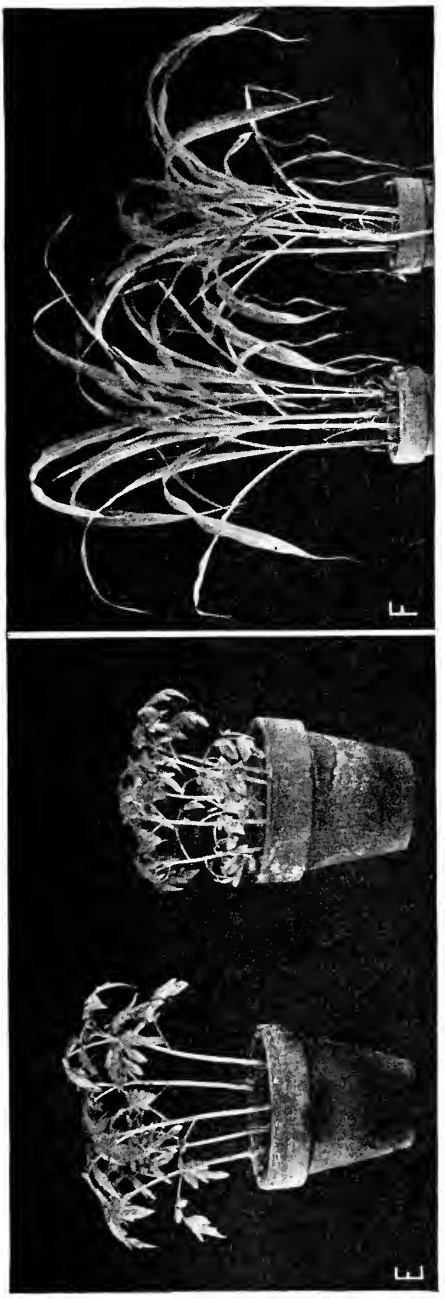

FIGURE 59. Effect of ethylene on growth of plants. A, Seeds of tomatoes and clover growing during 27 days in air and in 1 part of ethylene to 10 million of air. *Beginning at left:* control tomato, gassed tomato, control clover, and gassed clover. B, Typical plants from (A) photographed in same order. Note great reduction in growth rate caused by ethylene. C, Seedlings of tomatoes growing for 27 days. *Left:* in air; *right:* in ethylene 1 to 10 million. D, Buckwheat seedlings, same treatment and arrangement as (C). E, Seedlings of tomatoes growing for 21 days. *Left:* in air; *right:* in 1 part of ethylene to 25 million of air. F, Seedlings of corn, same treatment and arrangement as (E). Note evident reduction in growth caused by this trace of ethylene in both kinds of plants, also the epinasty shown especially by older leaves of the tomato.

has had rather extensive use and is in great favor with the anesthetists who do use it.

Coloring and ripening of fruits. Denny [9, 10, 11] was the first to note the effectiveness of ethylene in coloring citrus fruits. When commercially mature as determined by their size, color, or chemical composition, citrus fruits are often still green in color. For years the citrus growers had been burning kerosene stoves in their storehouses to hasten the proper coloring of the fruits. At first they thought the stoves did this by raising the temperature, but it was discovered that the stoves gave off some gas that increased the rate of chlorophyll decomposition. Denny found that low concentrations of ethylene in storage houses would hasten the coloring. Any concentration from 1:5000 to 1:5 million of the air had some effect, with 1:5000 to 1:1 million representing the most effective range of concentration. Very high concentrations, such as 80 per cent, slowed the process. The process was fastest at about 82° F (28° C). Besides accelerating the coloring of lemons, ethylene increased the rate of respiration 100 to 250 per cent, depending upon the concentration of the gas and other conditions. Acetylene and carbon monoxide were effective, but must be used in much higher concentrations; and butadiene showed some effect.

In practical application of this discovery the rooms were gassed two to four times a day and aired out at least once a day. Ethylene came to be very generally used instead of the kerosene stoves. The significance of this discovery was well put by the late Dr. Henry G. Knight, former Chief of the U. S. Bureau of Agricultural Chemistry and Engineering, on the occasion of his receipt of a medal from the American Institute of Chemists for his achievements in agricultural chemistry. *Science* [63] reports a portion of his speech as follows: "As a single, dramatic example of the returns obtainable on small outlay in research, Dr. Knight cited the case of the ethylene gas treatment of oranges, to bring a bright color to the skins of some types of fruit that persist in staying green after the oranges themselves are ripe. 'The treatment bleaches out the predominant green color and leaves the orange a beautiful natural yellow. The chemical investigations leading to the development of this treatment, which is now in rather general use, cost the taxpayers of the country about $4,000 and is estimated to be worth about $4,000,000 a year to the producers of citrus fruits in Florida alone and about the same amount to producers in California. And yet some people say that research doesn't pay. . . .'"

Ethylene induces the decomposition of chlorophyll in many different plants and plant organs. This is not a direct effect of ethylene upon the chlorophyll but an indirect effect through the protoplasm. In high concentrations, such as 80 per cent, ethylene inhibits the decomposition, as Denny showed for the lemon, because it produces partial rigor in the protoplasm and lowers the speed of many of its activities. Fig. 60 shows that illuminating gas (the ethylene portion) induces the decomposition of chlorophyll in rose leaves.[73]

R. B. Harvey [38] recommends the use of ethylene, 1000 ppm or more dilute, for blanching celery. Acetylene was less effective. Hibbard [40] confirmed Harvey's conclusions that ethylene hastens the blanching of celery, but found that it also reduces the growth. It hastens the yellowing of the green part of McIntosh apple skin but had no effect on the development of the red pigment. Other investigators have found that ethylene hastens several other ripening processes in fruits. These will now be described.

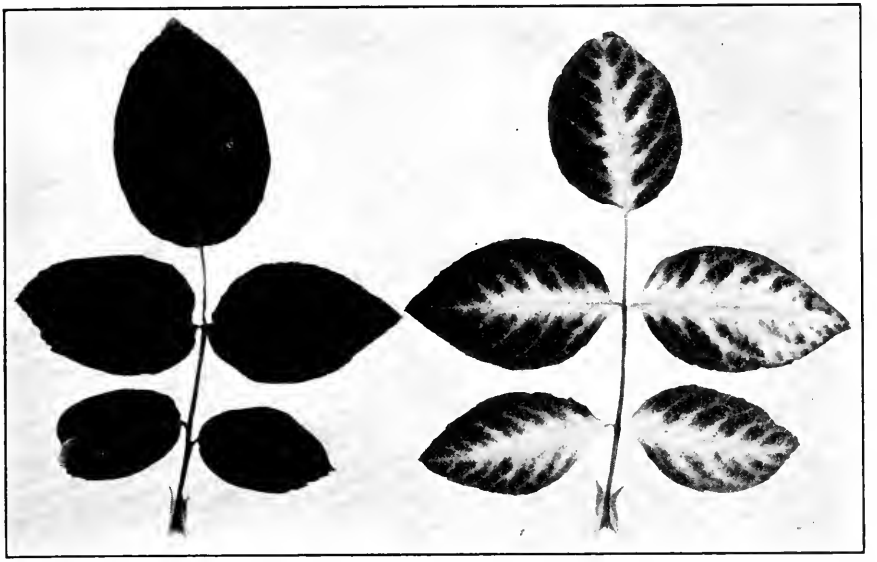

FIGURE 60. *At left:* control. *At right:* leaves of *Rosa* (hybrid tea) variety Madame Butterfly showing the yellowing of the veins of leaflets 3 days after removal from a 48-hour treatment in 1:5000 illuminating gas.

Other metabolic changes in living plant tissues. E. M. Harvey [36] was one of the early workers to make a detailed study of the effect of ethylene upon the metabolism of plant tissues. He also reviewed the earlier literature on the effects of anesthetics upon plant metabolism. He grew inch-long epicotyls of the sweet pea for 72 hours in air containing 1 ppm of ethylene and compared the chemical composition of these with epicotyls grown in air. Ethylene caused the simple soluble substances to increase at the expense of higher soluble and insoluble forms. Sugars, amino acids, amides, polypeptides, lipoids, etc., soluble in hot alcohol-ether, increased 8 to 9 per cent while the insoluble substances — proteins, starch, cellulose, lignocelluloses, etc. — showed a corresponding decrease. Reducing sugars increased about 11 per cent and non-reducing sugars decreased about 3 per cent. Amino acids plus amides increased and the polypeptides decreased. The fats were lower in the treated epicotyls and the cellulose and proteins were about 3 per cent lower. The acidity was not changed. Respiration was lowered, the author believed, because the ethylene was

used in too high a concentration. Denny [10] points out that Harvey got increased respiration in one of his determinations, namely, the one with shortest exposure, probably before the anesthetic effect became evident.

A number of investigators [40, 42] have determined the effect of ethylene upon the metabolism of celery and a number of fruits, citrus and pomaceous, generally using 1000 ppm of ethylene in the air. Ethylene caused an increase in reducing and simple sugars, at the expense of polysaccharides if the latter were present. If not, the simple sugars were decreased because ethylene increased respiration. In all the fruits ethylene hastened coloring and ripening. Ethylene decreased the tannin and catalase [42] and increased the peroxidase content. Ethylene, 1 ppm in air,[41] increased the respiration of potatoes in storage from the first and increased the soluble sugar content in later storage. There was one exception to this; in tubers rich in sugars caused by a period of storage at low temperature, ethylene did not increase respiration. If such tubers were de-sugared by a storage period at high temperature, ethylene again stimulated respiration.

Hansen [31, 32] has confirmed the findings mentioned above for the effects of ethylene upon fruits and added another very important metabolic change. Ethylene in 1000 ppm in air hastens the hydrolysis of insoluble protopectin of cell walls of the fruits into soluble pectin. This accounts for the more rapid softening of fruits when ripened with ethylene. Hansen also found that ethylene hastened the formation of pectin from protopectin in citrus rinds and English walnut shucks. This no doubt is a factor in shucking walnuts with ethylene. Hansen also found that little ethylene was produced by green fruits and that ethylene production increased as the fruit ripened. Ethylene did not induce ripening and softening of fruits after they had been in cold storage at 31° F (−0.5° C) for three weeks.

Lynch [53] suggests that ethylene acts as a respiratory coenzyme in the ripening fruits, and Nord [60] suggests that ethylene increases the enzymatic formation of plant hormones. Englis and Dykins [20] find that ethylene does not modify the rate at which salicin, a glucoside, is hydrolyzed by the enzyme emulsin. They conclude that ethylene, in modifying the metabolism and hastening the ripening of fruits, does not act directly upon enzymes but indirectly upon the living protoplasm of the fruit.

Root and root-hair initiation. The discovery that the unsaturated C-gases induce rooting was accidental. For some years Zimmerman and Hitchcock had been studying the rooting of cuttings, and during this time had been seeking without success chemicals that would induce rooting. On the side they were working with the author on the effect of illuminating gas and its constituents on greenhouse plants, a problem that would seem to have no relation to their main researches. In general the exposures of the plants to the gases were for a duration of three to four days.

On one occasion a tomato plant was exposed to 1 per cent CO in a bell jar, and because of a holiday making a long week-end was kept in the gas several days more than usual. The gassed plant showed many roots along

the stem and the control plant none. The results are illustrated in (A) control and (B) treated plant in Fig. 61. So far as the author knows, this was the first time an effective root-inducing chemical was reported. Carbon

FIGURE 61. Tomato plants treated with carbon monoxide gas. A, Control in Wardian case for 11 days. B, Plant exposed to one per cent carbon monoxide gas for 5 days and then held in Wardian case for 6 more days at the end of which time it was photographed. Note the slender root growth made after the plant was removed from gas. C, Control plant. D, Plant with a flask of carbon monoxide sealed over a leaf. Note the epinastic response of the leaves, indicating that the gas is taken in through the leaf and transmitted to all parts of the plant.

monoxide [70] showed root-inducing effects on many plants. The paper reporting these results was awarded the A. Cressy Morrison Prize in Experimental Biology in 1932 by the New York Academy of Sciences. Later,[71]

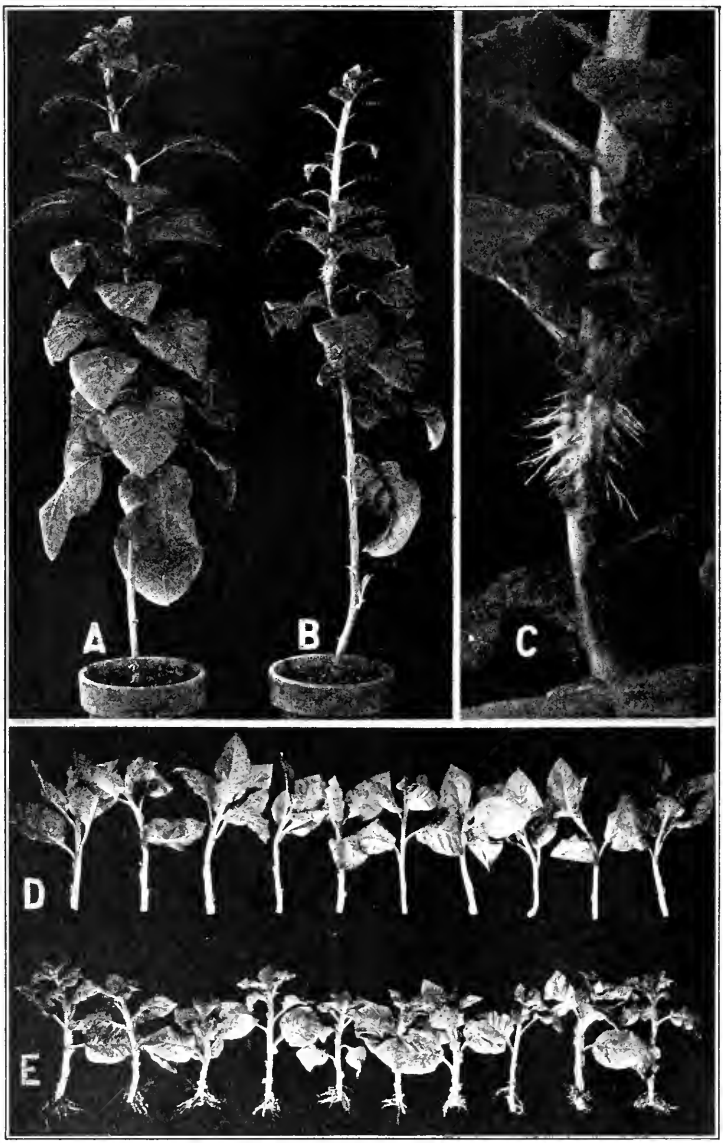

FIGURE 62. *Nicotiana tabacum* (Turkish variety) exposed to one per cent carbon monoxide gas. A, Control plant kept in Wardian case. B, Plant exposed to gas 15 days, then allowed to stand in air two days, after which time it was photographed. C, An enlargement of the rooting region of B. D, Tobacco cuttings from control plants in Wardian case for 10 days. Photographed 5 days after having been placed in rooting medium. E, Cuttings from plants treated with one per cent carbon monoxide for 10 days and then placed in rooting medium for 5 days, after which time they were photographed.

ethylene, propylene, and acetylene, like CO, were found to induce rooting. These gases were effective in lower concentrations than CO, as has been found for other plant responses, but the minimum root-inducing concentration was not determined for any of them. In some plants roots were induced along considerable stretches of the stem, as shown in Fig. 61 for

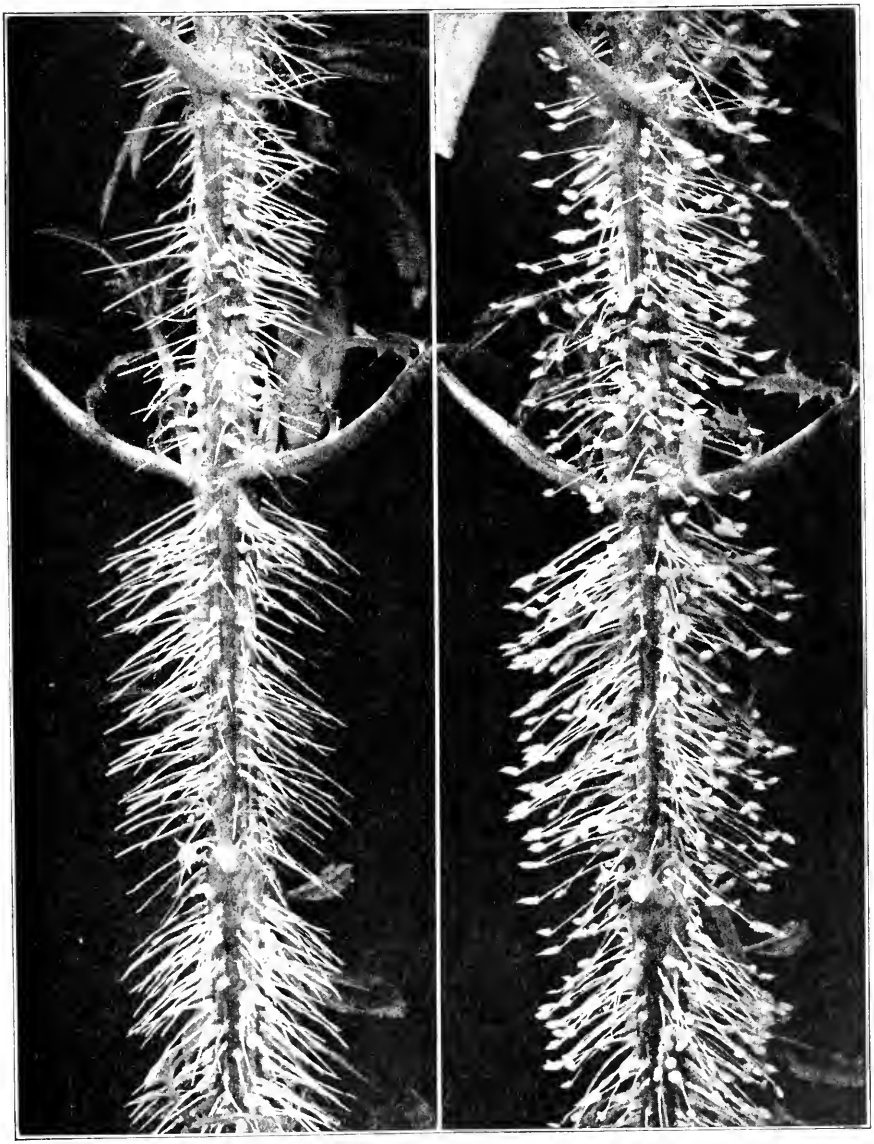

FIGURE 63. African marigold to show the effect of acetylene on orientation of roots to gravity and on formation of root hairs. *Left:* normal growth of roots six days after a three-day exposure to the gas. *Right:* the same roots after a 48-hour exposure to 0.25 per cent acetylene. Root hairs were induced and the roots changed their direction of growth.

tomato and in Fig. 63 for the African marigold. In other plants the roots grew only in the region of the stem that was elongating at the time it was subjected to the gas, as in tobacco (Fig. 62) and in a number of other plants. In still other plants the roots developed only at the nodes. The gases also induced development of roots on leaves and roots. The greatest growth of roots was obtained in intact plants if they were subjected to the gas for the induction period and then placed in gas-free moist air for further growth. While the gases induce rooting they also inhibit later elongation of the roots. These gases induce root-hair formation also, as is shown in Fig. 63.

While in one respect Zimmerman and Hitchcock had found the thing they had long been seeking, namely, chemicals that induce rooting, these chemicals could not be used in propagation. Since they are gases they move readily throughout the plant,[39, 72] probably traveling through the extensive intercellular system of plants. Because of this they induce roots at places where roots are not desired, all along the stem in some cases and at especially susceptible zones in others. Consequently Zimmerman and Hitchcock sought non-volatile or slightly volatile chemicals that would induce rooting only at the point of application. This led to the study of other plant hormones discussed in a later chapter.

Other physiological effects. Several investigators have found that the unsaturated C-gases induce the earlier formation of flowers in pineapple [47, 65] and flower and leaf buds in mango.[22] In the Hawaiian Islands acetylene has been used to induce earlier flowering of pineapples and to spread the harvest over a greater period of the year. Use of these gases on tobacco [1, 28] during fermentation improved the color and smoking quality and increased the rate of fermentation of the leaf. Ethylene, 100 ppm,[30] is said to improve the germination and baking performance of freshly harvested and high-moisture wheat in storage.

Respiring Plant Tissue Produces Ethylene

Elmer [18, 19] in 1932 observed that apples gave off a volatile substance that inhibited the growth of potato sprouts. A year later Oortwijn Botjes [61] reported that emanations from apples caused epinasty in tomato plants and horizontal nutation of the etiolated pea seedling. She found that ethylene absorbents would remove the effective gas from apple emanations. Gane [25] found that aerobically growing yeast (but not anaerobically growing yeast) produced a substance that prevented the growth of a pea seedling. The substance was absorbed by bromine water, an ethylene absorber. He showed [24] that ripe apples in air produce a substance that modifies the growth of several kinds of plants. In an atmosphere of nitrogen the apple did not produce emanations that modified the growth of the pea seedling, but seemed to hasten ripening of bananas. Apples killed by freezing no longer produced effective gases. The effective gases from apple were absorbed in bromine water [23] and ethylene dibromide was identified

in the absorbent. He found [27] that green Bon Chrétien pears stored at 0° C (32° F) produced vapors that ripened bananas. Denny and Miller [16] and Denny [12, 13, 14] found that most of the many kinds of living plant tissues tested emanated a chemical that induced leaf epinasty in the potato. Among the tissues giving the response were fruits (mature and green), seeds, flowers, leaves, leafy stems, young shoots, roots, tubers, anthers, pistils, and petals of a number of different kinds of plants. A few living tissues gave negative results: potato tubers (whole or cut into pieces); seedlings of wheat, corn, and oats; mycelium of *Rhizopus nigricans*, two mushrooms, and baker's yeast. By absorbing emanations from a greater number of seedlings of oats, wheat, corn, and several other seedlings in a mercuric nitrate-nitric acid reagent and later releasing the absorbed gases with HCl, a positive response was obtained. It may be that all living plant tissues produce ethylene, but some in very low amounts. Ripe apples produce relatively large amounts. The flesh of the squash and dandelion flowers are less active. Seedlings of Cruciferae tested produced both toxic (probably mustard oils) and leaf epinasty-inducing emanations. Denny found that tomato stems in the horizontal position, out of equilibrium with gravity, produced more of the effective emanations than similar stems in the vertical position.

Quantitative determinations have been made of the amount of ethylene produced by fruits and contained in fruits, as well as of the effect of stage of ripening and other conditions upon the amount of ethylene produced. Niederl, Brenner, and Kelley [59] converted the ethylene from ripening bananas to acetylene and determined the latter as silver acetylide. They estimate that 100 pounds of bananas produce 0.1 to 0.2 cc of ethylene during the ripening period. Nelson [57] finds that McIntosh apples after nine months of storage contain 0.12 mg of ethylene per kilogram of apples. He also found [58] that for six varieties of apples studied, the ones with longer storage lives showed less capacity to produce ethylene. There is a general correspondence between the ethylene content and respiratory activity of apples. Ethylene has a hydrolytic action on ripening fruits, but the effect is probably not directly upon the enzymes. Ethylene may be consumed as well as produced by the ripening bananas.

Hansen [33, p.556–557] summarizes his quantitative measurements of ethylene production by pears in part as follows: "In fruit in air at 20° C the rate of ethylene production increases during the climacteric rise in respiration, reaches a peak at the respiratory climax, then declines during the post-climacteric period. During the climacteric rise, ethylene production increases seven- to eighty-fold, while rate of respiration approximately doubles. Each variety was found to have a characteristic maximum rate of production. The maximum rate for Bartlett, a variety which maintains its capacity to ripen for only a short period of time when kept at a storage temperature of 0° C, is 3.25–4.48 ml per kg-day. The maximum rate for Anjou, a variety which maintains its capacity to ripen for a long period of

time when kept at cold storage temperatures, is 0.57–0.78 ml per kg-day. The maximum rate of respiration for Bartlett is approximately double that for Anjou. Under anaerobic conditions, the production of ethylene is either greatly retarded or entirely inhibited. In the fruits used for these experiments, there was found but little difference in the production of CO_2 under aerobic and anaerobic conditions. The maximum rate of ethylene production occurs at 20° C. At higher temperatures production decreases and is totally inhibited at 40° C. Respiratory activity as measured by either CO_2 production or O_2 consumption is greatly accelerated between 20° and 40° C."

The question naturally arises whether living plant tissues produce the other effective gases: acetylene, propylene, and carbon monoxide. As has been stated above, wherever the minimum effective concentrations of the several gases for producing a plant response have been determined, ethylene has proved effective in very much lower concentrations than the other gases. Hansen and Christensen [34] state that solubility tests indicate that ethylene is the active gas evolved by the fruits, with similar unsaturated hydrocarbon gases, such as acetylene, propylene, and butylene, not present in sufficient amounts to be detected by the bromination procedure. Langdon [45] found that the atmosphere in the float and air channel of giant kelp, *Nereocystis leutkeana*, is 1.1 to 12.2 per cent CO, varying widely from plant to plant and little with time of day. Langdon and Gailey [46] believe the CO is a product of aerobic respiration, since it occurs only when oxygen is present and equally abundant in light and darkness. Rigg and Henry [64] confirm the previous work on the origin of the CO in the kelp. As we have seen, the concentration of CO in the kelp float is sufficient to cause many formative changes in various plants. We have no evidence, however, that CO is produced by most plants and even if it is, probably not in sufficient concentration to have formative effects. The same is true of acetylene, propylene, and butylene.

Since plant products, especially fruits, produce ethylene which has marked physiological effects upon other living plant organs, some discrimination must be used in storing various plant products in proximity to each other. Cut carnations [51] in cold storage rooms with apples become sleepy. Ripe apples and high ethylene-producing apples [66] hasten the respiration and ripening of slow-maturing apples stored with them. A number of storage practices may be modified by these findings on the production of ethylene by plants and the effect of ethylene on plants. In the case of apples being used to defoliate roses,[54] the association is beneficial.

Summary

The researches described above and carried out mainly in the United States have shown that ethylene, acetylene, propylene, and carbon monoxide have many far-reaching and interesting effects upon plant development and plant metabolism. These researches were early stimulated by the

work of Crocker and Knight (1908) in answering the practical question on the effect of artificial illuminating gas upon carnations. Denny's discovery (1923) of the effectiveness of ethylene in coloring lemons was the second great stimulus to work in this field.

Qualitatively the four gases act similarly on plants; so far as tested, each will produce the responses that any other produces provided they are all used in the proper concentrations. Quantitatively they are very different; the minimum effective concentration of ethylene is very much lower than that of acetylene and propylene, and the minimum effective concentration of carbon monoxide is much higher than that of the last two. In general, the effectiveness can be expressed as ethylene > acetylene and propylene > carbon monoxide.

Ethylene modifies the geotropic equilibrium position of plant organs, causing such changes as declination or horizontal nutation of pea seedlings, leaf epinasty, and change in the orientation of roots. Leaf epinasty of the tomato seedling and potato shoot has been used rather extensively in practice in detecting the presence of ethylene in the air and through it the presence of traces of a number of gas mixtures, such as illuminating gas and exhaust gases from internal combustion engines. Leaf epinasty has been especially useful in scientific research for detecting the emanation of ethylene from respiring plant tissues. The declination or horizontal nutation of the pea is the least sensitive of the tests. With a very sensitive variety of sweet pea, using declination as the indicator, one might detect 0.2 ppm of ethylene in the air. With leaf epinasty of the tomato one can detect easily 0.1 ppm of ethylene in the air. If one observed only the mature leaves of the tomato and gave a long exposure, 0.04 ppm of ethylene could be detected. The leaf epinasty of the potato furnishes a considerably more delicate test than that of the tomato. Finally, leaf epinasty of the African marigold is the most sensitive test known for ethylene. It will detect 0.001 ppm of ethylene in air. On the basis of the small amount of the chemical needed to induce the response, this is one of the most delicate responses of an organism to a chemical known to date.

Ethylene causes cells that are dormant and would otherwise not grow, to enlarge and divide, forming soft, rather massive tissues. This is especially true for lenticular and cortical tissue. Traces of ethylene will even cause cells with thickened walls to dissolve the walls and to enlarge and multiply. The proliferation of tissue is induced by concentrations of ethylene as low as 0.01 ppm in the air.

Ethylene induces the abscission of leaves, flowers, petals, and fruits by inducing flat cells of the abscission layer to enlarge and become spherical, thus furthering organ drop. Hydrolysis of the insoluble protopectins of the middle lamella of cell walls is also a factor in abscission. Ethylene is used for defoliating plants when desirable in horticultural practice. This is probably the response that makes ethylene useful in shucking English walnuts and pecans.

Ethylene is an excellent anesthetic. Ethylene will anesthetize certain plant organs in concentrations as low as 1 ppm in air. It is interesting, however, that while it anesthetizes one part of the plant, it may start growth in another part. In concentrations far below the anesthetic dosage it still slows the growth, and the inhibition of growth falls very slowly as the concentration falls. Dilutions of 0.1 ppm of ethylene reduce the rate of elongation of plant organs 25 to 50 per cent, and 0.04 ppm gives easily measurable reduction in rate of elongation. It is likely that much lower concentrations will inhibit elongation in some of the more sensitive plants.

Butylene, which is almost inactive with plants, will anesthetize centipedes and various insects in concentrations 5 to 40 per cent of the air and partially paralyzes the organisms in higher concentrations; 30 to 75 per cent of propylene is required; and 80 to 90 per cent of carbon monoxide; ethylene and acetylene must be used as a full atmosphere. The most effective of these for insects and centipedes is least effective for plants and the most effective anesthetic for plants is the least effective for insects and centipedes.

Ethylene is an excellent anesthetic for mammals, lacking as it does some of the undesirable qualities of ether and nitrous oxide. It must be used in high concentration, 80 per cent or a higher concentration, with oxygen. It has been used extensively in surgery. Many anesthetists are very enthusiastic about its use and others are quite as antagonistic.

Ethylene induces the decomposition of chlorophyll in the living plant and has been used extensively to hasten the proper coloring of ripe citrus fruits, especially lemons, and to bleach celery. It also hastens many other ripening changes in fruits and has been used commercially for this purpose with tomatoes, bananas, and other fruits.

Ethylene induces or hastens many other metabolic changes in living plants. These changes are mainly hydrolytic, such as transformation of insoluble protopectins to soluble pectins, higher carbohydrates to soluble sugars, proteins to polypeptides and amino acids.

Ethylene and the physiologically similar gases induce root and root-hair formation.

Finally, many if not all respiring plant tissues produce ethylene. The amount of ethylene produced varies greatly with the kind of plant and with the organ of the plant, as well as with the age of the organ. Apples and pears produce much ethylene at the climacteric stage of the fruit; bananas produce less; and the potato tuber little, if any. Since ethylene is produced by plants, often in sufficient concentrations to modify development, it may be considered a phytohormone.

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CHAPTER 5

Effect of Certain Lethal Gases upon Plants and Animals

The study of the effect of physiologically active gases upon plants led a group of investigators at the University of Chicago and later a group of research workers at Boyce Thompson Institute to investigate the effect of other gases upon plants and animals.

Injury to Trees and Shrubs from Leaks of Artificial Illuminating Gas

When we discuss illuminating gas injury to trees and shrubs along streets or in parks, there are three factors that must be considered that are not involved in gas injuries in greenhouses: (1) soluble constituents of the gas will accumulate in the soil about the roots in the first case, whereas they will be absorbed by the soil and not reach the air of the greenhouse in the second; (2) the roots as well as, or even more than, the tops of the plants must be considered in tree and shrub injury; and finally, (3) there is a free movement of air outside, that will take the gases away from the tops of the plants instead of holding them about the plants, as is the case in the greenhouse.

Harvey and Rose [14] found that when root systems of plants were sealed in the soil and subjected to moderate concentrations of Chicago artificial illuminating gas various abnormal growths occurred. These responses were the same as those occurring when ethylene was added to the soil in amounts equal to that in the gas. The following growth changes occurred in one or another of the several kinds of plants treated: coiling or swollen growth near the tip of roots, proliferation of cortical cells on the upper part of roots and lower parts of stems, and fall of leaves. In the last case the ethylene was absorbed by the roots and passed up through the stem to induce leaf fall. In high concentrations of illuminating gas the roots were killed and the whole plant died. Harvey and Rose also flowed illuminating gas through soil bearing plants, very slowly in some cases, and very rapidly in others. In the former they observed ethylene-induced growth responses and in the latter death soon occurred, which they attributed in part to the lack of oxygen, though admitting that toxic substances in the gas may have played a part. They observed that with slow flow of gas, all the starch disappears from the cortex of the roots, and believe that this, together with the proliferation of tissue on roots and lower parts of the stems, may serve

as a point for diagnosing gas injury, if not on the trees killed, at least in adjoining shrubs and trees that received a lower dosage of the gas.

Hitchcock, Crocker, and Zimmerman [15] later reviewed the literature and carried out many experiments on injury to plants by illuminating gas seeping through the soil.

Exposure of Roots in Soil to Flowing Gases

The tomato plant proved to be very sensitive to illuminating gas flowing through the soil in which it grew, and was much used in this investigation. Fig. 64B shows that 2 cubic feet of Yonkers illuminating gas flowing through a pot bearing a small tomato plant leads to the final death of all the roots and the lower part of the stem, so that the plant later collapsed. Even 1 cubic foot of gas killed the root system. Table 20 shows the effect of flowing several different gases through the soil of pots bearing six different kinds of plants. The most toxic of these gases is unscrubbed Yonkers illuminating gas. Even 1 cubic foot of this killed all the roots, and 4 cubic feet killed all the underground parts of the tomato plant, which collapsed. The injury decreased in the following order for the other plants: willow, maple, cherry, silver bell, and privet. Scrubbing the gas through water before passing it through the pots reduced the toxicity measurably for all

Table 20. Effect on Potted Plants of Passing Illuminating Gas and Certain of Its Unsaturated Hydrocarbon Constituents Through the Soil for a Period of 30 Minutes

Plant	Vol. of gas in cu. ft.	Relative degree of injury to roots caused by different gases *						
		Illuminating gas			Ethylene	Propylene	Butylene	Acetylene
		Not filtered	Filtered through					
			Water	NaOH				
Tomato	1	+++	+++	+	0	0	0	+
	4	+++†	+++†	++	++	++	++	++
Willow	1	++	++	—	0	+	+	++
	4	+++	+++	+	+	+	+	+
Maple	1	++	+	—	0	++	++	++
	4	+++	+++	+	++	+++	+++	+++
Cherry	1	++	—	—	0	+	++	++
	4	+++	—	++	+++	+	+++	+++
Silver bell	1	+	—	—	0	++	+	+
	4	+++	—	+	+	++	++	+++
Privet	1	+	—	—	0	+	+	+
	4	+++	++	+	+	+	++	+

* 0 signifies no noticeable injury, + slight discoloration, ++ noticeable discoloration and death of part or all of many roots, +++ all roots badly discolored and most slender roots killed.

† All underground parts killed.

the plants except the tomato and willow, and scrubbing it through NaOH solution reduced the toxicity markedly for all.

The investigators showed that there was a close agreement between the amount of cyanides in the volume of gas that was needed to cause a given

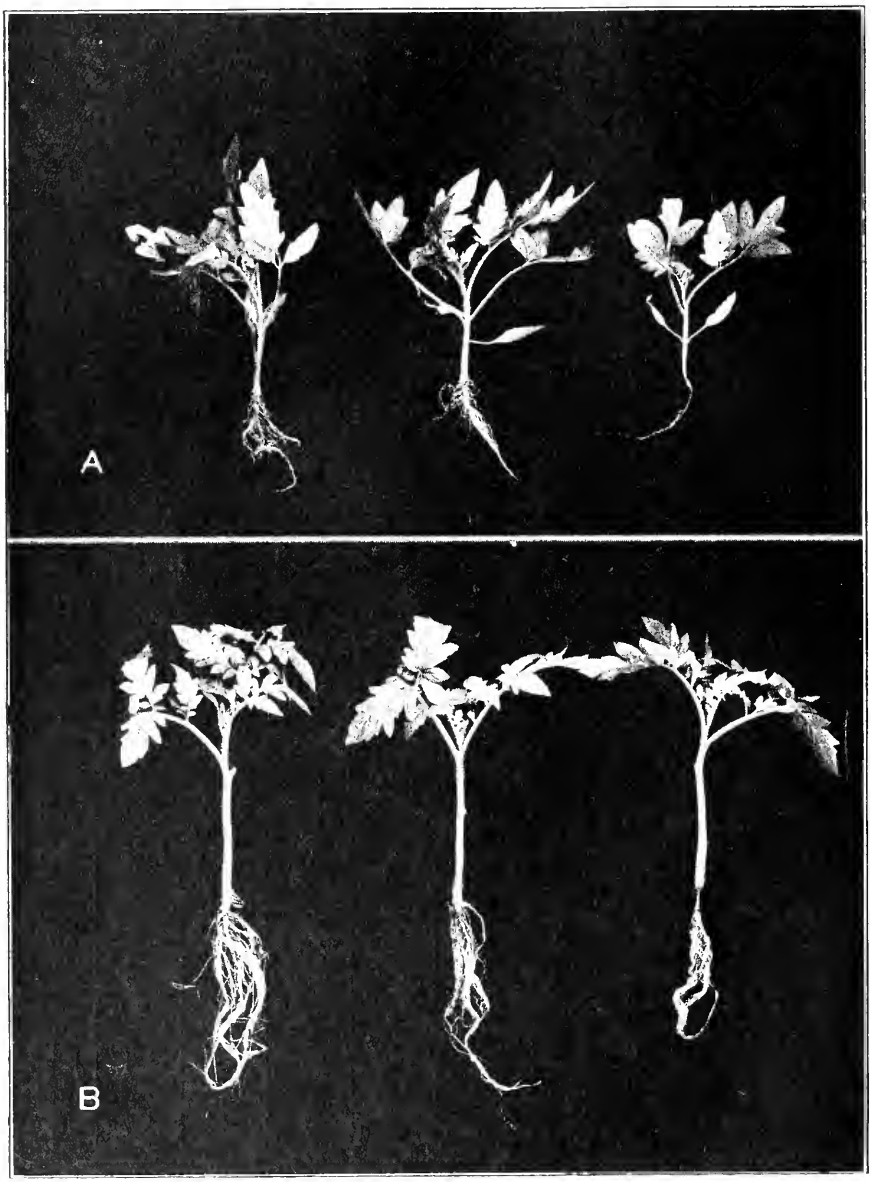

FIGURE 64. Comparison of injuries on tomato due to residues of illuminating gas left in the soil and those caused by flowing gas and residues. A, Seedlings placed in soil previously gassed. B, Roots of seedlings in soil subjected to flowing gas. *Left to right* in (A) and (B): control, one cubic foot, two cubic feet.

injury and the amount of cyanide that had to be added as a water solution of $Ca(CN)_2$ or KCN in order to produce the same degree of injury. In short, the highly toxic or tissue-killing constituent of the gas was HCN. Scrubbing the gas with water removed some of the HCN, and scrubbing it with NaOH changed the HCN to NaCN and took out most of it. The amount of cyanides in solution in water necessary to cause the collapse of a small potted tomato plant was very small, 4 to 7 mg. It is interesting to find that it took 20 to 24 times as much illuminating gas that had been thoroughly scrubbed through NaOH solution to give the same amount of killing as was produced by a given amount of unscrubbed gas. Artificial illuminating gas is always scrubbed to remove HCN, and the amount in the gas probably varies considerably from time to time with the thoroughness of the scrubbing. It is evident that if the scrubbing were thorough enough to remove the last trace of HCN, the killing of trees and shrubs by many small leaks would be avoided. With such thoroughly scrubbed gas it is probable that the killing would result from compounds such as phenol, toluene, xylene, etc.

Flowing 1 cubic foot and 4 cubic feet of each of the unsaturated gaseous hydrocarbons (ethylene, propylene, butylene, and acetylene) in general produced less injury than the unscrubbed illuminating gas, and the order of sensitiveness of the several plants changes; the tomato was little injured by any of these, even with 4 cubic feet, while the maple was considerably injured, especially by 4 cubic feet of propylene, butylene, and acetylene, the cherry by 4 cubic feet of ethylene, butylene, and acetylene, and the silver bell by acetylene. The tomato, which was the most sensitive to unscrubbed illuminating gas, was among the least sensitive to the unsaturated C-gases, and some plants which were more resistant to unscrubbed illuminating gas were most injured by the C-gases.

The results shown in Table 20 throw out of perspective the significance of the C-gases as toxic factors in illuminating gas because they make up such a small percentage of the gas. The Yonkers gas [8, p.196] contained 3 per cent of ethylene, a fraction of a per cent of propylene, and still less butylene and acetylene. If the Yonkers gas were used as the source of ethylene, one would have to flow 33 cubic feet of it through the pot to get 1 cubic foot of ethylene and many times this volume to get 1 cubic foot of each of the other three unsaturated C-gases. So far as killing roots and stems is concerned, the unsaturated C-gases can be disregarded as causes of injury from illuminating gas.

In order to get growth response from ethylene and perhaps propylene and acetylene, the experiments in Table 20 were run in the wrong way, since all the gas passed through the soil in 30 minutes. Had 1 cubic foot of ethylene or even 1 cubic foot of illuminating gas containing 3 per cent of ethylene been flowed very slowly through the pot during several days or a week, no doubt ethylene responses (leaf epinasty, yellowing of foliage, proliferation of tissues on root and stem) would have appeared. Ethylene would

have dissolved in the soil and been absorbed by the plant in sufficient amounts to induce these changes with such slow flow. These statements are in accord with the findings of the experiments of Harvey and Rose, where they flowed illuminating gas very slowly through the soil. With a very slow flow of gas, it is also probable that HCN would be disposed of by microörganisms in the soil, so it would not accumulate in tissue-killing concentrations. It is evident from what has just been said that two different

FIGURE 65. Effect on the tomato of residues left in soil by ethylene and by illuminating gas. *Left:* control. *Center:* 10 cubic feet of ethylene. *Right:* collapse of roots and lower part of stem in soil caused by the residues from three cubic feet of illuminating gas. Photographed six days after seedlings were planted in the soil.

types of injury may appear in trees and shrubs along leaking gas lines: if the leak is slight, ethylene responses will appear; if the leak is large, the root systems and plants will be killed and none of the growth responses will occur. These investigators [15] found that the carbon monoxide of the Yonkers gas, although it constituted 13 per cent of the gas, was not a factor in the injury of plants in the soil outside.

Toxicity of Gas Residues in the Soil

A comparison of Fig. 64A and B shows that the tomato plant is injured to about the same degree whether it is in the soil while the illuminating gas flows through the soil or is set in the soil immediately after gassing.

Fig. 65 shows that flowing 3 cubic feet of Yonkers gas through the soil leaves a residue that kills the root system and the lower part of the tomato plant set in the soil, but that flowing 10 cubic feet of ethylene through the soil does not leave a killing residue.

It is of interest in replanting trees and shrubs in place of those killed by illuminating gas to know how long the toxic residues remain in the soil and what are the best means of getting rid of them. Fig. 66 shows that gassed soil sealed in a can at 24° C (75° F) loses practically all its toxicity within

FIGURE 66. Effect of low temperature storage on the toxicity of soil through which illuminating gas had been previously passed and which was then sealed for one week. *Left to right:* control soil −15° C (5° F), gassed soil −15° C (5° F), gassed soil 3° C (37° F), gassed soil 24° C (75° F). The tomato plants in gassed soil previously stored at −15° C (5° F) and at 3° C (37° F) were killed, but the soil kept in the laboratory at 24° C (75° F) caused only a slight retardation of growth.

a week, whereas similar soil samples sealed at −3° and −15° C (26° and 5° F) do not lose their toxicity. At proper temperatures and other conditions for growth the microörganisms of the soil use the hydrocyanic acid — which probably is mainly cyanides because of neutralization by bases of the soil — as a nitrogen source. Leaching the soil with water removes a part of the residual toxic substance. Fig. 67 shows the growth of a tomato plant in gassed soil that has been leached with water equal to ten times the volume of the soil, in contrast to the growth of a similar plant in gassed soil without leaching, and in soil not gassed. It is evident that leaching removed a portion of the poisonous residue, but there are still substances that reduce the growth rate far below that of the check. Leaching is not a very effective way of removing the toxic residues, and it might be even less effective outside where the soil is likely to be packed and poorly drained. In practice, it is probably best to remove the gassed soil and replace it with good soil. If this is not feasible, the soil could be loosened up to the proper depth and limed if it is acid, after which two to three weeks should elapse before planting. In case of acid soils, the lime will neutralize any residual HCN and increase the activity of organisms that consume the cyanides.

FIGURE 67. Effect on growth of tomato of leaching highly toxic gassed soil with water. *Left to right:* control; soil from a 65-liter lot through which 7210 cubic feet of illuminating gas had been flowed; a similar sample of gassed soil after leaching with an amount of water equal to ten times its volume. Photographed three weeks after planting.

Addition to the Soil of Other Compounds Present in Illuminating Gas

Drip oils, *i.e.*, oils condensed from illuminating gas at low temperatures, caused the young tomato plant to collapse when 1 gram was mixed with 450 grams of soil. The injury caused by these oils could not be distinguished from injury caused by adding solutions of cyanides or passing unscrubbed illuminating gas through the soil. The weight of drip oil that had to be added to produce a given injury was more than 100 times the weight of hydrocyanic acid or cyanides necessary to produce the same degree of injury. It is not uncommon to find the soil near leaks in gas lines discolored by drip oils. They persist in the soil for a long time and of course will injure roots growing in the soil bearing them. Drip oils were more toxic than equal weights of toluene or xylene which, along with these, exist in illuminating gas in small amounts. Phenol in high concentrations killed the tomato, as did illuminating gas. It also caused a deep pink or reddish coloration of roots, stems, and leaves of the tomato and of the roots of privet and sunflower. Heavy dosages of illuminating gas produced similar color changes. The color change induced by phenol is one of the symptoms that should be looked for in diagnosing illuminating gas injury to trees and shrubs.

Diagnosis of Tree and Shrub Injury by Artificial Illuminating Gas

As we have seen, it is easy to diagnose a case of illuminating gas injury in a greenhouse. At the time the injury is occurring, reliable test plants can

be used. Several days after the leak has been stopped one can judge rather accurately from the behavior of the various kinds of plants in the greenhouse whether ethylene has been present. Some greenhouse men show great accuracy in observing and describing unusual responses in the plants they are growing. With accurate descriptions of the responses, one can judge fairly accurately whether ethylene was involved long after the injury has occurred. It is more difficult to determine gas injury to trees or shrubs outside, especially when the plant is killed quickly. No physiological responses of ethylene appear. One can locate the leak and observe the degree of injury to plants at various distances from the leak. On the outskirts of the area of injury he may find ethylene responses in plants that are not killed. One should also observe various plants within the zone of injury for the red color in the roots due to phenol.

Samples of soil should be taken from different depths and from various distances from the leak and test plants exposed to the soil samples under bell jars to detect the presence of ethylene. The samples should be taken with as little stirring as possible and kept tightly sealed until ready to be placed under the bell jars for the test. The author has often obtained ethylene responses from such tests, which of course show that gas has been present. Hitchcock, Crocker, and Zimmerman did not find chemical tests of soils for the presence of various constituents of gas of very much value in diagnosing gas leaks. Among these were treating soil extracts with bromine water to detect ethylene, etc., reactions for phenol and cyanides in soil extracts. Drip oils do often discolor the soil in the immediate region of the leak.

Leaks are more likely to develop in the winter with freezing and thawing of the ground. If there is a frozen soil crust after a big leak develops, the gas is likely to move for considerable distances under the crust in sufficient amounts to cause injury over a sizable area. If the roots are killed in the early spring the foliage may open out, but the leaves remain very small and die later. If the roots are killed in midsummer the full-sized leaves die and dry up.

Injury from Natural Gas

With one known exception natural gases do not contain ethylene or other similar growth-modifying gases and, in the one exception claimed to date, the percentage of ethylene is extremely low.[8] Natural gases [5, 22] also contain no HCN, phenol, toluene, xylene, etc. Natural gases have methane (CH_4) as the main constituent. In some, methane is the sole combustible constituent. Others contain in addition the higher homologs of methane — ethane, propane, and butane as gases, and vapors of pentane, hexane, heptane, and octane. Natural gases from some fields contain hydrogen sulphide and organic sulphur compounds. In some of these the hydrogen sulphide runs as high as 15 per cent. Hydrogen sulphide, if present, is supposed to be completely scrubbed out before the gas is de-

livered to consumer pipes. As we shall see later, hydrogen sulphide, although highly toxic to mammals, has a rather low order of toxicity to plants. Some N_2 and CO_2 are present in most natural gases. Nitrogen may run as high as 40 per cent and CO_2 over 1 per cent. Some natural gases contain helium. All these constituents except the hydrogen sulphide have relatively low toxicity to plants. If the hydrogen sulphide, when present, is completely scrubbed out, the natural gases are almost inert so far as plants are concerned. The most toxic constituents are the higher homologs of methane, especially pentane, hexane, heptane, and octane, and they are present in very low concentrations.

Solheim and Ames [31] found that certain natural gases of the northwestern United States showed very low toxicity to plants. Tomato, potato, sunflower, castor bean, and geranium plants were uninjured by 50 per cent of natural gas from the Billy Creek field in Sheridan, Wyoming, or gas from the mains at Laramie after four days of exposure. Cut carnations did not exhibit any symptoms of injury when they were exposed to 2 per cent of the gas for four days. Fuchsia petals showed slight browning and wilting when the plants were exposed to 4 to 50 per cent of the gas for one to four days.

Schollenberger [24] found that a natural gas composed mainly of methane and ethane rendered soil highly toxic to wheat and oats. Analysis of this soil showed a marked increase in soluble manganese and ammonium nitrogen, and lesser but distinct increases in sodium, potassium, and calcium. Since these changes frequently occur as a result of water-logging, puddling, or other conditions which favor reducing actions, Schollenberger concluded that the toxic effect of the natural gas in soil was due primarily to reduced oxygen pressure.

Injury to Plants in a Greenhouse by Mercury Vapor

Zimmerman and Crocker [41, 42] were asked to investigate injuries to roses in a greenhouse at Attleboro, Mass. The injuries were quite different from those caused by illuminating gas; also the injuries could not be attributed to insects or fungal and bacterial pests, for the plants were almost free from such pests and the injuries were not such as would be caused by them. The only hint at a possible source of injury was the fact that the soil in some of the benches had been treated with a solution of mercuric chloride, $HgCl_2$, to kill earthworms. The soil had also been fertilized with tankage. The injury, however, appeared in all benches in the house, regardless of whether they had been treated with $HgCl_2$ solution and tankage. Later, rather extensive experimentation led the investigators to the conclusions that the injury was caused by the $HgCl_2$ solution added to the soil of some of the benches, and that the organic matter of the soil reduced the chloride to metallic mercury, which had sufficient vapor pressure to move through the air and injure roses in untreated benches. Ratsek [23] later reported similar results from treating soils in a rose house with $HgCl_2$, but he explained the

injury to the roses in untreated benches in another way. He believed that the $HgCl_2$ passed as a vapor through the air and injured the roses at a distance.

There is considerable evidence in favor of the Zimmerman-Crocker interpretation. Organic matter reduces $HgCl_2$ to mercurous chloride,[20, p.833–834] and vegetable and animal substances[20, p.703] reduce HgCl to metallic mercury. The vapor pressure of metallic mercury is considerably higher than the vapor pressure of $HgCl_2$ throughout a considerable range of temperature, and especially at temperatures suitable for the growth of plants. Fig. 68 shows the vapor pressure of mercury, $HgCl_2$, and HgCl at various

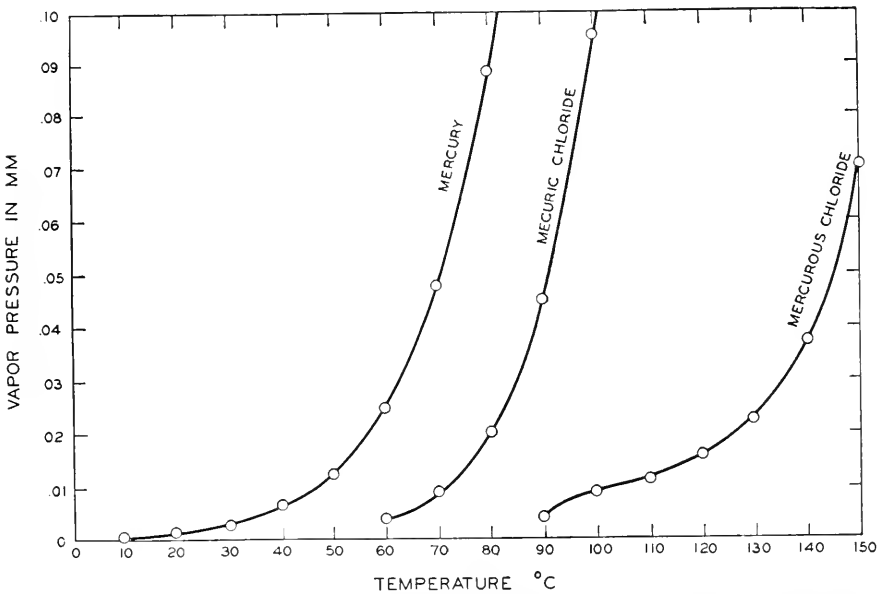

FIGURE 68. Curves showing the relationships of the vapor pressures of metallic mercury, mercuric chloride, and mercurous chloride at different temperatures. *All data taken from International Critical Tables.*

temperatures. For instance, the vapor pressure of mercury at 40° C (104° F), a temperature often attained in a greenhouse, is 66 per cent higher than the vapor pressure of $HgCl_2$ at 60° C (140° F), practically a pasteurizing temperature. The vapor pressure of mercury at 60° C (140° F) is five and one-half times that of $HgCl_2$. About one-fourth the $HgCl_2$ molecule is chlorine. At saturation at 60° C (140° F), the air will contain more than seven times as much mercury in the form of mercury vapor as in the form of $HgCl_2$ vapor. When a current of air is drawn through a column of $HgCl_2$ crystals and then over a gold foil, no mercury is deposited on the foil; but if air is drawn through a rich soil treated with $HgCl_2$ solution, mercury is deposited on the foil, showing that the soil reduces the $HgCl_2$ to the metal. If air was drawn through a bell jar containing a glass

plate atomized with $HgCl_2$ solution, no mercury was deposited on gold foil by such air. If in addition the bell jar contained a potted plant, mercury was deposited on the gold foil. The plant evidently gave off some material that reduced the chloride, or $HgCl_2$ vapor reached the plant and was reduced to the metal.

Addition of $HgCl_2$ solution to tankage injured untreated plants enclosed with it much more than did $HgCl_2$ solution added to sand or powdered charcoal. Mercurous chloride dust, which has much lower vapor pressure than $HgCl_2$, is not toxic to a growing plant with which it is enclosed. If $HgCl_2$ is added to soil and the soil enclosed with a plant, the plant is injured. Zimmerman and Crocker found that various inorganic mercury compounds as well as several organic mercurial fungicides, Dubay, Nu-Green, Semesan, and Uspulun, when added to fertile soils, gave off emanations that injured plants in untreated soils in the same enclosure. Finally, Daines [9] says that in soils where mercurials are effective as fungicides, mercury compounds are reduced by the soil to metallic mercury, which migrates in the soil as mercury vapors, and that any factor that prevents the conversion of the mercury salt to metallic mercury destroys the fungicidal effects of the mercurial.

Walker, *et al.*[36] give a similar explanation for the effectiveness of $HgCl_2$ solution in controlling club root of cabbage when applied at the time of transplanting. This is the type of evidence we have that fertile soils reduce mercury compounds to metallic mercury, and that it is vapors of the metallic mercury that travel through the air and injure the plants in untreated soil in the same enclosure. Regardless of the correctness of this interpretation of the mechanics by which additions of mercury compounds to the soil in greenhouses injure plants in untreated soils in the same house, there is no doubt that mercury compounds, organic and inorganic, must be used with caution in greenhouse soils, because of the release of mercurial vapors in the air. In the use of mercurials in outside planting the danger of injury is through the roots. The air will be kept relatively free from dangerous vapors by diffusion and wind currents.

That mercury vapor in the air is injurious to both plants and animals has long been known. The only new claim here introduced is that adding mercury compounds, even those with very low vapor pressures and solubilities, to a greenhouse soil releases mercurial vapors into the air in sufficient concentrations to injure plants throughout the greenhouse, including those in untreated soils.

Zimmerman and Crocker [42, p.172] state: "In 1797 there was made known in a letter addressed to Van Mons by Lauwerenburgh that four Dutch chemists, Deiman, Paats, Van-Troostwyck, and Lauwerenburgh,[10] had discovered the deleterious effects of metallic mercury vapors on plants. The results of 15 experiments mentioned in the letter showed that where beans, mints, or spiraea were enclosed in bell jars with metallic mercury the leaves became spotted after 24 hours, and if left exposed to the vapors

FIGURE 69. Briarcliff rose buds. A, *left to right:* 1, normal control bud; 2, bud from plant in muslin cage where the soil bearing 14 other plants had been watered with 0.05 per cent mercuric chloride; 3 and 4, buds from plants in muslin cage in which the soil bearing the plants had been watered with 0.05 per cent mercuric chloride. B, *left to right:* 1, normal bud from control glass case; 2, 3, and 4, buds from plants in glass case where the soil bearing three other plants had been watered with 0.05 per cent mercuric chloride.

for several days the plants died. In 1867 Boussingault [3] repeated some of the experiments, obtaining results comparable to those of the Dutch scientists. Since that time several German workers have discussed the poisonous effects of metallic mercury vapors, but no publications have been found which show that the air becomes contaminated where mercuric compounds are applied to the soil."

The buds of the Briarcliff rose proved especially sensitive to mercury vapor in the air. Very young buds and a region of the stems just below them were killed. In older buds the petals were killed and turned brown, the whole corolla abscissed, and stamens and pistils became black. In old buds the petals which had begun to open lost most of the pink pigment and turned brown at the edges. Fig. 69 shows the nature of these injuries. It will be noted from Fig. 69B that buds showed the same type and degree of injury whether they grew in pots to which $HgCl_2$ solution was added to the soil or in pots in the same enclosure in untreated soil. The injury in all cases was due to vapor in the air. While the buds were the most sensitive part of the plants, the leaves were also injured with more severe treatments. There was considerable variation in the sensitiveness of different varieties of roses. Of the seven varieties tested (Columbia, Templar, Killarney, Pernet, Madame Butterfly, Mrs. Calvin Coolidge, and Briarcliff), Briarcliff was the most sensitive and Templar the most resistant.

Plants of 65 different genera were injured by vapors from metallic mercury or from soils treated with $HgCl_2$ solution. Broad bean, butterfly weed, oxalis, and sunflower were especially sensitive. Aloe, croton, and sarcococca were very resistant. The sensitiveness probably depended upon the maturity and degree of dormancy of the plants at the time of treatment as well as upon the species. In actively growing peach seedlings a foot or more high, the old leaves were most sensitive and the young leaves most resistant.

The degree of injury caused by vapors from mercury or soil treated with mercury compounds depended upon several factors. Temperature was very important, as would be expected from the fact that the vapor pressure of mercury rises rapidly with the temperature. Fig. 70 shows that all the leaves are killed on peach seedlings at 75° F (24° C) by vapors from both mercury and soil treated with $HgCl_2$ solution. The injury is less severe at 60° F (16° C), slight at 50° F (10° C), and nil at 40° F (4° C). With mercury the injury increased not with the total volume of mercury in the enclosure but with the surface exposed. Covering the mercury with a layer of water 1 cm thick prevented injury.

The mercury was determined in the leaves of various plants after exposure to mercury vapor. No relation existed between the amount of mercury absorbed and the degree of injury produced in the various kinds of plants. Under a given exposure the leaves of the Briarcliff rose absorbed about one-half as much mercury as leaves of Killarney and Coolidge, and yet the leaves of the former were more injured than those of the latter.

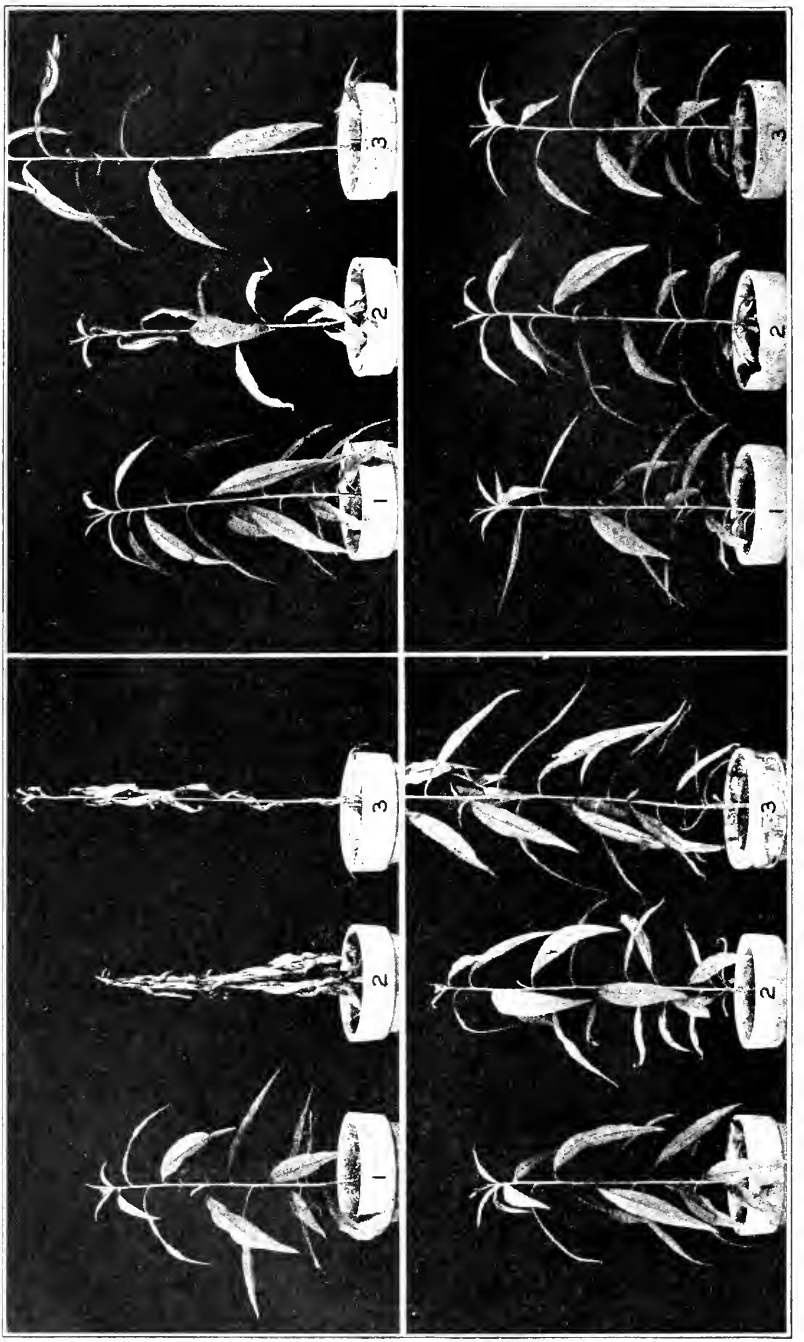

FIGURE 70. Effect of temperature on peach seedlings confined five days under bell jars as follows: 1, control; 2, with 400 cc of tankage moistened with one per cent mercuric chloride solution; 3, with 5 cc of metallic mercury in open beaker. Temperatures used; *upper left:* 75° F (°24 C); *upper right:* 60° F (15° C); *lower left:* 50° F (10° C); and *lower right:* 40° F (4° C).

The Jerusalem cherry absorbed much mercury from mercury vapor in the atmosphere but was little injured by it. Evidently there is a great difference in the resistance of protoplasm of different plants to injury by mercury.

Gray and Fuller [12] found that dry seeds of pea, corn, bean, radish, sunflower, and cucumber stored in fairly tight chambers with an open beaker of mercury for six months showed no injury. There was a slight delay if the seeds were germinated in the presence of mercury vapor, but the percentage of germination was not affected. Seedlings of these plants were injured by addition of mercury to the substratum or mercury vapor in the air. The injury was somewhat greater under the first condition. In both cases the seedlings were stunted, showed yellowing of leaves, early leaf fall, and failure of leaf development. Kincaid [16] found mercury vapor in the air toxic to germinating tobacco seeds, especially at higher temperatures and with exposure of large surfaces of the metal. Harrington [13] states that presence of mercury forces dormant Johnson grass seeds to germinate promptly at temperatures that gave no germination in absence of mercury. Giese [11] has recently emphasized the danger of laboratory workers being poisoned by mercury fumes. A cubic meter of air saturated with mercury at 25° C (77° F) contains 19.5 mg of mercury. A stream of air passing over 10 cm^2 of mercury at 25° C (77° F) at the rate of 1 liter per minute becomes about 15 per cent saturated and contains 3 mg of mercury per cubic meter. He cites researches to show that prolonged exposure of some individuals to as little as 0.01 mg of mercury per cubic meter and of many individuals to 0.25 mg results in chronic mercury poisoning. Mercury is eliminated from the body slowly and consequently accumulates with continued exposure. Giese mentions that Faraday, Pascal and other physicists and chemists suffered from chronic mercury poisoning without realizing it. Among the symptoms of mercury poisoning are irritability, headaches, and recession of the gums.

Effect of Sulphur Dioxide on Plants

Fifty years ago it was not unusual to see large areas around smelters completely denuded of plants by the large amount of sulphur dioxide released into the air. Extensive studies have been made in both Europe and America on injury to vegetation and even to animals by smelter fumes. These studies have resulted in three great accomplishments. First, very accurate methods have been developed for determining the effect of SO_2 on plants. This phase has been especially marked by the designing of accurate instruments for automatic recording of the SO_2 content of the atmosphere and for fumigating plants with regulated and automatically recorded SO_2 content. Secondly, a great deal of information is now available on the injury to plants by SO_2, the relative sensitiveness of different plants and plant parts to SO_2, and the degree of injury necessary to reduce crop yield. Finally, smelters have made use of this scientific knowledge in reducing the injury by SO_2 about smelters. They have also removed solid matter from smelter

fumes by the use of Cottrell precipitators. This prevents other toxic substances from reaching the atmosphere about the smelters and gives valuable by-products.

Research by Hill, Thomas, and associates. The advance in methods and instrumentation was started by Wells of the Selby Smelter Commission and later further developed and perfected by scientists of the Department of Agricultural Research, American Smelting and Refining Company of Salt Lake City, Utah, early by O'Gara and later by Hill and Thomas and associates. The outstanding advance in apparatus was the Thomas autometer for continuously analyzing and recording low concentrations of SO_2 in the atmosphere. This has later been adapted for measuring and recording low concentrations of many other gases and vapors in the atmosphere. The automatic recording fumigating apparatus used for SO_2 and other gases at the Institute is illustrated in Fig. 71,[27] and a full description of the apparatus and method of its operation was made by Setterstrom.[26] Thomas and associates [33] have recently improved this apparatus for long-time large-scale study of the effect of SO_2 fumigation on the nutrition and physiology of plants. The new apparatus gives control of the soil conditions as well as the atmosphere about the plants.

Besides doing so much to develop suitable apparatus, the group at the American Smelting and Refining Company has contributed much accurate knowledge on the physiological effect of SO_2 in the air on plants. Space will permit the mention of only two of the later contributions. They studied the effect of SO_2 fumigations on the rate of photosynthesis and respiration [34] as measured by the CO_2 absorbed or released by alfalfa. Heavy fumigations, 0.7 to 1.26 ppm, of durations too short to kill any tissue of the leaf reduced the rate of photosynthesis during the exposure; but immediately after exposure the photosynthesis rose to normal or greater than normal, so the net effect of such fumigations was practically zero. Light fumigations for long periods, 0.24 ppm for 3 days, 0.19 ppm for 11 days, 0.14 ppm for 39 days, either showed no effect or suggested stimulation. Even when heavy fumigations were long enough to cause extensive killing of leaf tissue, much of the photosynthetic power was restored by the development of new leaves within 10 or 15 days. The workers conclude that fumigations that do not produce visible injury (spotting of leaves) do not produce "invisible injury" (reduction of photosynthesis and yield). Thomas and co-workers,[32] in a study of sulphur nutrition of alfalfa in the improved culture chambers, found that sulphur-deficient plots were improved in yield by light SO_2 fumigations. We shall later discuss the significance to soil fertility of release of SO_2 into the atmosphere by industries.

American Smelting and Refining Company has faced realistically the problem of possible injury to vegetation by the fumes from its smelters, and has built and manned a fine laboratory to work out in detail the effect of SO_2 upon plants. They have contributed materially to improved instrumentation in plant and industrial science and to fundamental knowledge

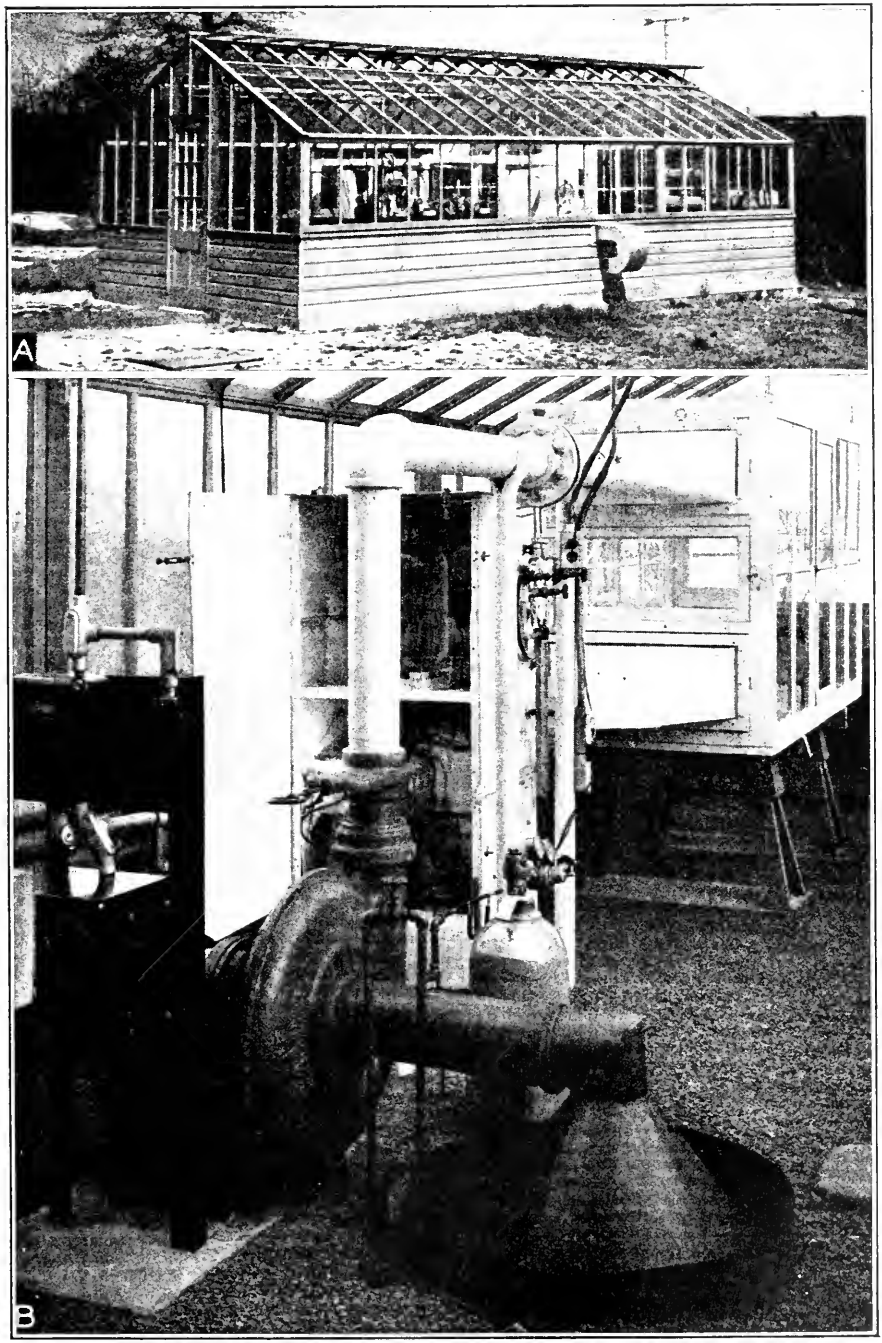

FIGURE 71. Part of the apparatus used for studying the effects of gases on plants and animals. A, Greenhouse which houses most of the apparatus. B, Close-up of scrubber and metering devices with cabinet and autometer in the background.

of plants. The findings have been made generally available to industry, to the public, and to science. Much credit is due the director, Dr. George R. Hill, for the public-spirited and thoroughgoing scientific way in which the researches have been managed, and to Dr. Moyer D. Thomas for his unique ability in designing apparatus and planning and carrying through experiments for solving difficult problems in plant physiology.

Research by National Research Council of Canada. The best case history we have of fumes from a larger smelter is that of the Trail Smelter in the upper Columbia Valley, British Columbia, about ten miles north of the international boundary. The researches were conducted by eight specialists of the National Research Council of Canada, and are published in a 447-page volume.[21] The book reports the observations and experiments of this group and reviews the earlier literature on the subject. Part I (206 pages, including several inserted two- or three-page plates) covers the field studies. The introduction gives the history of Trail Smelter, the amount of SO_2 emitted by it yearly from 1900 to 1935, and also in 1937, SO_2 abatement methods adopted, and history of the international tribunal dealing with claims of United States farmers for SO_2 injury. Other subjects covered by the first part are: SO_2 content of the atmosphere in industrial regions, including the Trail region; symptoms of SO_2 injuries on plants; and the effect of the SO_2 content of the air on the sulphur content of trees and shrubs, on the acidity, base-exchange capacity, and sulphur content of soils, and on the growth in diameter of trees. Part II describes numerous fumigation experiments on forest and crop plants, including a study of the effect of environmental factors on susceptibility of barley and alfalfa to SO_2 injury. It discusses the effect of SO_2 fumigation on stomatal behavior; on crop yield when the fumigation is in tissue-killing and sub-killing dosages; and on photosynthesis, respiration, and chemical composition of plants. The Thomas recorders were used extensively in these investigations and modifications of the Hill and Thomas fumigating apparatus were used throughout the fumigating experiments.

This work has added many accurate data and sound generalizations to our knowledge of the effect of SO_2 on vegetation. Space will permit the mention of only two of the generalizations. The "invisible injury" findings of Hill and Thomas were confirmed, that is, there was no reduction in crop yield unless the fumigations were sufficient to kill leaf tissue. Ethylene is an ideal gas for producing "invisible injury" because it is a growth inhibitor and does not kill tissue, as pointed out in the last chapter. It will, however, reduce growth rate only so long as it is in the air surrounding the plant. The SO_2 in the leaves was oxidized to sulphates, apparently completely in light fumigations and partially in heavier fumigations. It is the unoxidized SO_2 or sulphites that kill leaf tissue. Leaves of evergreens growing in the SO_2 belt some distance from Trail showed three to four times the normal content of sulphur without any effect on the color of the leaves and without any retardation in growth. It is likely that the oxidation of SO_2 to sulphates accounts for the lack of "invisible injury."

Experiments at Boyce Thompson Institute. Beginning in 1930, Zimmerman and Crocker [40, 43] made a study of the effect of SO_2 on more than 30 species of plants representing more than a dozen families and including farm, garden, ornamental, and wild species. The experiments were carried on under a considerable range of conditions as to SO_2 concentration, duration of fumigation, light, moisture, and other factors. These experiments were made before automatic self-recording fumigating apparatus was available. The concentration of the SO_2 was adjusted by flow meters and the concentration of SO_2 checked in the fumigating chamber by periodic analyses of air samples.

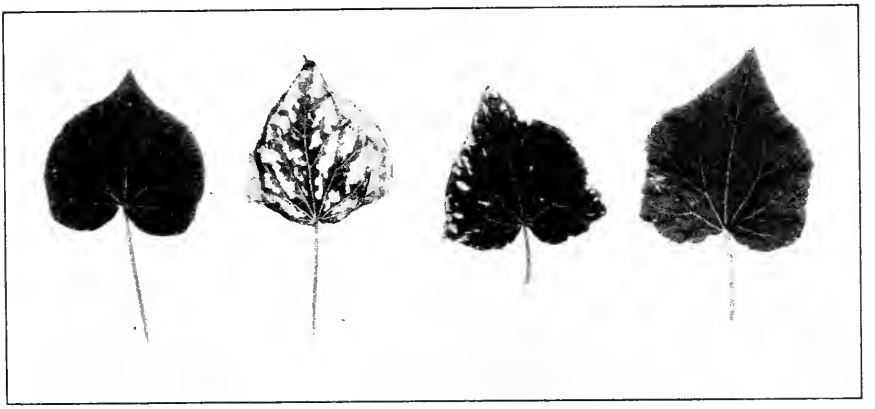

FIGURE 72. Buckwheat leaves from plants treated for six hours with various concentrations of sulphur dioxide gas. *Left to right:* (1) control, (2) 1.0 ppm, (3) 0.7 ppm, (4) 0.5 ppm.

The experiments led to the following conclusions. There is a great range in the sensitiveness of different species of plants: buckwheat proved the most sensitive; it showed killing of leaf tissue in 0.46 ppm with seven hours' fumigation. Some species of orchids were very resistant, withstanding 60 ppm for several hours without injury. The cereals and various weeds were sensitive and carnations, gardenias, and rhododendrons were rather resistant. The leaves were injured, but not the stems and buds. In dicotyledons middle-age leaves were more sensitive than young or old leaves; interveinal parenchyma was more injured than the veins, and small veins more than large ones. Fig. 72 shows the leaf injury on buckwheat by 1.0, 0.7, and 0.5 ppm with six hours' exposure. The plates (some colored) in the Trail Smelter book show the type of injury caused in leaves by SO_2, and the Report of the Selby Smelter Commission contains colored plates of leaf injury by SO_2. The Trail report speaks of marginal as well as veinal killing of leaf tissue in dicotyledons.

Both duration of exposure and concentration of the SO_2 were important in determining the extent of injury. Sulphur dioxide is very soluble in plant tissues and accumulates with time. In subtoxic concentrations time

is not a factor. As the National Research Council of Canada group found in such concentrations, the SO_2 is oxidized to less harmful sulphates before enough accumulates to kill the tissue. With responses from ethylene the situation is quite different. Here time of exposure is of prime importance. Ethylene has low solubility in plants and consequently does not accumulate with time. Moreover, it must be present until the response occurs and the response ceases soon after the ethylene disappears from the surrounding air. Wilted plants were more resistant than similar plants that were turgid. The difference was considered to be due, at least in part, to the condition of the stomata. The investigators believe that low air humidity and low water content of the soil make plants more resistant by modifying the water content of the plant. Plants fumigated at night showed more resistance than similar plants treated during the day. Plants also gained in resistance if they were placed in the dark two hours before they were fumigated in a dark case. Shading while plants were being exposed to the gas was not effective.

If this work was important at the time it was done in furthering the physiological point of view in SO_2 injury, it has since been greatly outdistanced even in this respect by the extensive and very accurate researches by Hill, Thomas, and associates, and by the National Research Council of Canada. Later work by Setterstrom and Zimmerman [29] confirmed the earlier work of Zimmerman and Crocker and added the following facts: plants are more resistant at 40° C (104° F) than at higher temperatures, and also when previously grown in good rather than in poor light and when exposed to full light rather than in deep shade (65 per cent or greater reduction of light intensity). The sulphate nutrient supply and previous treatment with SO_2 did not affect the susceptibility if in the latter case sufficient time was allowed for recovery from previous treatment.

Sulphur Dioxide Content of Air at Boyce Thompson Institute.

The SO_2 content of the air at Boyce Thompson Institute [25, 28] was recorded continuously with minor interruptions for two years, November 1, 1936 to November 1, 1938, by use of the Thomas recorders.

The average readings including zero readings were 0.033 ppm for the first year and 0.035 ppm for the second. The maxima were 0.75 ppm for the first year and 0.53 ppm for the second. Both the averages and the maxima are almost identical with those at Northport, Washington [21, p.35] during the growing season, where claims were made for injury to crops and forests. The high SO_2 content of the air at Yonkers was in the winter when plants outside were not growing.

Setterstrom and Zimmerman [28, p.178] conclude: "Correlation of SO_2 concentrations with the wind direction indicates that the sulphur dioxide comes largely from New York City (15.4 miles SSW to Times Square which marks the approximate center of the metropolitan area). A study of the relationships between concentrations of sulphur dioxide of the atmosphere

and of the air of a greenhouse shows that greenhouse concentrations are approximately 90 per cent of atmospheric when ventilators are partly open, 60 per cent when ventilators are closed. The fact that the many plants grown throughout the year in the Institute greenhouses are considered comparable to plants grown in areas where there is no sulphur dioxide, is an indication that exposure to sulphur dioxide in the prevailing concentrations and durations has no unfavorable effect on plant life."

Sulphur Dioxide of Atmosphere as a Sulphur Source for Plant Nutrition

Setterstrom, Zimmerman, and Crocker [30] found that sulphur deficiencies in the soil for growth of alfalfa could be supplied in part by fumigating the air with non-marking concentrations of SO_2. The Cruciferae, which are very rich in organic sulphur, gave negative results. As we have already seen, Thomas and associates [32] found later that sulphur deficiency for alfalfa could be supplied in part by SO_2 fumigation.

Available sulphur is deficient [1, 7] in some soils for maximum yield of protein-rich legumes like alfalfa. Indeed, it is so deficient in some soils in Washington, Oregon, and Idaho that sulphur additions will increase alfalfa yields as much as five-fold. As measured by crop needs in general, sulphur supply of soils is about equal to phosphorus supply, and as measured by the needs of high-sulphur crops like certain legumes and crucifers, the sulphur supply in the soil is below the phosphorus supply. Moreover, sulphur in the form of sulphates is leached from the soil in large amounts, whereas phosphorus is held rather tenaciously. In the United States enormous amounts of sulphur, largely in the form of SO_2, are released into the atmosphere by smelters and by industrial and home consumption of coal and other fuels. This finally reaches the soil by being washed down by rain or absorbed directly by the soil or vegetation. This averages many pounds per acre per year for the whole country, and of course is high near industrial and population centers and very low in remote rural sections. This replenishment of sulphur in the soil may be an important factor in soil fertility around big population centers. Together with the later use of acid phosphate, which is about half calcium sulphate, this may account for the fact that gypsum [6] was very effective on clover in colonial days but has far less effect in eastern United States today. Also it is possible that fumes from the Trail Smelter may be improving and not injuring the yields of alfalfa in the upper Columbia Valley in the United States. This is made probable by the fact that soils in neighboring valleys both east and west are deficient in sulphur for maximum alfalfa production.

Effect of Sulphur Dioxide on Animals

Men and animals, like plants,[37] are subjected to sulphur dioxide fumes. In the atmosphere of smoky cities sulphur dioxide sometimes reaches concentrations of 10 ppm. Workers engaged in the manufacture of refrigerants

are exposed to 30 to 100 ppm at times. The disaster of the Meuse Valley in 1930 during six days' fog when 63 people died has been attributed to sulphur dioxide, which was estimated to reach concentrations as high as 38 ppm. It has since been questioned whether these deaths were due to SO_2 and whether it reached anywhere near such a concentration. There are many other statements in the literature concerning the effect of SO_2 in the air on health, most of them not well backed by facts. We seem long on assumptions and short on measurements of the effect of SO_2 in various concentrations and for various durations upon animals. The earlier measurements of the effects of SO_2 on animals were on the dosage basis, that is,

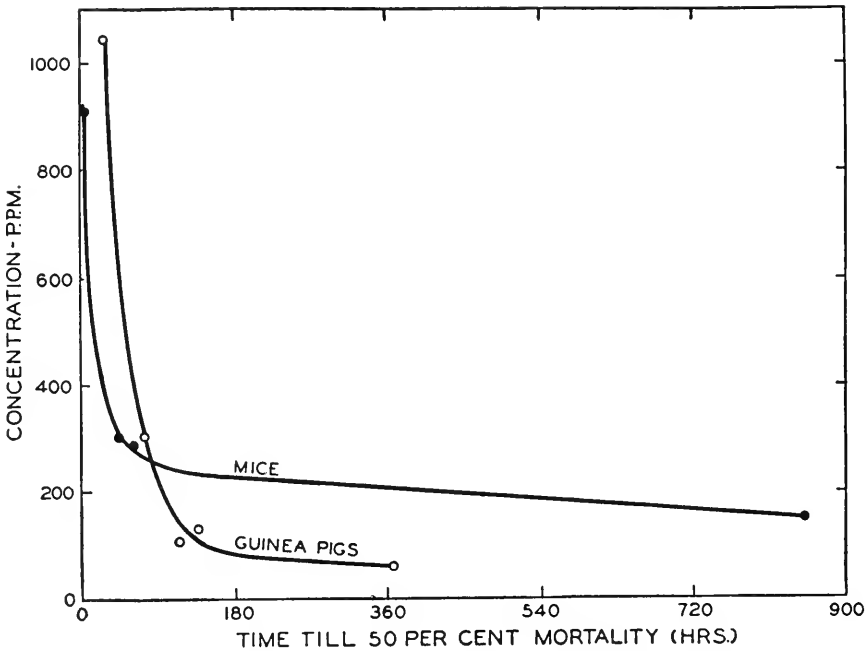

FIGURE 73. Time till 50 per cent mortality *versus* concentration toxicity curves for mouse and guinea pig in various SO_2 concentrations.

the animal was enclosed in a case and a single dose added, generally a large one, and the effect observed. Sulphur dioxide is absorbed readily by the walls of the case and more readily by the animal. Some later experiments were performed by the continuous air flow method but the concentration was not regulated and recorded accurately.

Weedon, Hartzell, and Setterstrom [37] used the accurate continuous flow fumigation chambers at the Institute to study the effect of SO_2 on guinea pigs, mice, grasshoppers, and cockroaches. The concentrations used were 10, 35, 65, 100, 150, 300, and 1000 ppm and the duration of exposures ran up to more than 1000 hours in some cases. No significant mortality or signs of distress occurred in healthy animals in 33 ppm, even with 400 or

500 hours of exposure. One realizes the great resistance of animals as compared with the most sensitive plants when it is stated that leaf tissue in buckwheat is killed by seven hours' exposure to 0.46 ppm, though animals are not injured by 500 hours' exposure to 33 ppm. At high concentrations a longer total exposure to gas was necessary to cause death when the SO_2 treatments were given in intermittent doses.

Fig. 73 shows the length of exposure to various concentrations of SO_2 necessary to kill 50 per cent of mice and guinea pigs. Above 200 ppm the guinea pigs are more resistant than the mice, and below that concentration the mice are much more resistant than the guinea pigs. Fifty per cent of the mice were still alive after nearly 900 hours in 150 ppm. The susceptibility of both grasshoppers and cockroaches approximated that of the mice.

Symptoms in vertebrates at higher concentrations of SO_2 were lethargy, nasal catarrh, lachrymation, coughing, conjunctivitis, difficult breathing, distension of abdomen, weakness, and paralysis of hindquarters. In the highest concentration insects cleaned their mouth parts and antennae, showed lack of coordination of muscular movements, and paralysis of posterior legs. The authors describe the internal pathologic changes in mammals resulting from high dosages: [37, p.323] "Pathologic changes in vertebrates include general visceral congestion of slight to moderate degree, slight to moderate edema of the lungs with hemorrhages at higher concentrations, acute dilation of the right heart at higher concentrations, gross distention of the stomach with multiple ulcers and hemorrhages at the higher concentrations, distention of the gall bladder except at lowest concentrations."

Effect of Hydrogen Sulphide on Plants

In connection with spray injury from lime sulphur, McCallan and associates [17] ran continuous-flow fumigation experiments with hydrogen sulphide on 29 different species of plants. The fumigations were carried on in shaded glass cases outside during the summer. The duration of the fumigation was five hours in each case and was carried on at midday. The mean temperature was 74° to 81° F (23° to 27° C) and the relative humidity from 82 to 100 per cent in the several experiments.

The symptoms of injury were scorching of young shoots and leaves and basal and marginal scorching of next older leaves with the older and mature leaves unaffected. This differs strikingly from the injury by SO_2 which kills the parenchyma of the middle-aged leaves in lower fumigations while the young and mature leaves are more resistant and the stems uninjured.

The authors describe further results of their experiments: [17, p.196–197] "The different species varied widely in their response: carnation, purslane, Boston fern, apple, cherry, peach, strawberry, and coleus showed no appreciable injury at concentrations below 400 ppm; pepper, rose, nasturtium, castor bean, gladiolus, sunflower, buckwheat, and cornflower, slight to moderate injury at concentrations from 40 to 400 ppm; and soybean,

Turkish tobacco, aster, kidney bean, cucumber, tobacco (*N. glauca*), salvia, poppy, tomato, clover, radish, calliopsis, and cosmos, slight injury below 40 ppm and severe injury and death above 400 ppm. Temperature is as important as concentration, injury increasing rapidly with increases in temperature. In some cases wilted plants appear less sensitive to hydrogen sulphide injury than normal turgid plants. Plants tested for lime sulphur injury (aster, buckwheat, sunflower, and tomato) showed symptoms identical with those produced by hydrogen sulphide."

Here again injury by H_2S differs from injury by SO_2 in two respects: the minimum concentration of H_2S for injury to plants is many times that for injury by SO_2, and wilting of plants furnishes less protection against H_2S than it does against SO_2. We have already seen that the degree of stomatal opening is an important factor in SO_2 injury. Hydrogen sulphide may enter through the cuticle rather than through the stomates, or the young tissues may be much more susceptible to H_2S injury than older tissues.

Effect of Chlorine Gas and Chlorinated Water on Plants and Animals

The staff of the Institute has been called in on several cases of injury to plants by chlorine gas escaping from tanks used to treat water for swimming pools and on injury to plants by fumes from laundries. The question whether there is enough residual chlorine in the tap water of a city to injure greenhouse plants watered with it, and water plants and fish living in it, is a perennial question.

Our observations and unpublished experiments indicate that chlorine gas in the air affects plants much as does SO_2. The middle-aged leaves are most sensitive and the spotting of the leaves is similar to that caused by SO_2; also, as reported later in this chapter, Cl_2 is much more toxic to leaves in clear than in cloudy weather as is SO_2, indicating further similarity of behavior between these two gases. It will also be observed that Cl_2 in the air spots leaves in even lower dosages than SO_2. It is also more toxic to animal pathogens and about equally toxic to plant pathogens. Our unpublished experiments likewise proved leaves more sensitive to Cl_2 than to SO_2.

Zimmerman and Berg [39] ran extensive experiments on the effect of chlorinated water on land and water plants and on goldfish. Land plants proved rather resistant to chlorinated water, but water plants were sensitive. The authors summarize their results on plants as follows: [p.47–48] "None of the species of plants grown in loam soil and watered, syringed, or watered and syringed with chlorinated water having 50 ppm or less of chlorine in water, were injured or retarded when grown in pots in cold frames, on open benches in the greenhouse, or under bell jars in the greenhouse. Chlorine concentrations of 100 and 150 ppm injured or retarded some plants but had no effect on others. Concentrations of 200 and 300 ppm always produced some degree of injury to the tops, but had no

appreciable effect on roots. Concentrations of 500 and 1000 ppm retarded the emergence of seedlings, burned and usually killed the tops and roots of plants eventually. A combination of watering and syringing generally produced greater and more rapid injury than watering the soil alone. Syringing the tops usually was somewhat less injurious than watering the soil with the same solutions. Injury from all treatments was in most cases more serious and more rapid in the greenhouse than in open cold frames, and worse under bell jars in the greenhouse than on the open greenhouse bench.

"Tops of plants grown in a mixture of equal parts of loam and sand were retarded in top growth by solutions of 50 ppm and 100 ppm but not by 5 ppm. Roots were never injured in this soil. Plants grown in sand were retarded in root and top development by solutions of 5, 50, and 100 ppm chlorine, the degree being somewhat in relation to the concentration of chlorine in the solution. Chlorinated water applied to sand and to loam soil for three weeks had no significant effect on acidity of the media.

"Roots from tomato cuttings in chlorinated water were retarded in size directly as the chlorine concentration increased from 10 ppm, but they were not affected by 5 ppm. Cut flowers were not affected by free chlorine in water up to 10 ppm. Fifty parts per million injured gerbera and snapdragons but not gladiolus or roses. *Cabomba* and *Elodea* were discolored by chlorinated water containing 3 ppm in one week when the water was refreshed daily. A concentration of 5 ppm produced injury in two days and death in four days."

From what has been said above, it is evident that Cl_2 in the air is much more toxic to land plants than when in water solution; it takes less than 1 ppm by volume of Cl_2 in the rare medium, air, to spot more sensitive plants, whereas it requires several ppm by weight in the dense medium, water, to injure these plants. The Cl_2 enters the tissue of land plants through the stomates much more readily as a gas than it does through the general cuticular structure in solution. The greater injury from keeping plants sprayed with chlorinated water under bell jars or in greenhouses as compared with open air was due to gas escaping from solution and being held about the plants by the enclosures so it could enter through the stomates. Since water plants, such as *Cabomba* and *Elodea*, are poorly cutinized, Cl_2 in solution enters the plant readily.

Concerning the effect of chlorinated water on goldfish the authors say: [39, p.48] "Under the conditions stated in the text goldfish were killed by chlorine solutions of 1.5, 2.0, and 3.0 ppm where the water was changed daily. Where water was constantly renewed, concentrations of 1.0 and 1.5 ppm were toxic. Fantail variety appeared to be less resistant than the Common variety of goldfish, while the Common variety was less resistant than the Shubunkin variety. Aquatic plants appeared to counteract in some degree the toxic effects of chlorinated water on fish."

The authors point out that Yonkers city water at the tap frequently contains 0.5 ppm of residual Cl_2, occasionally 1.0 ppm, and rarely 1.5 ppm.

Hence it is probable that the free chlorine in city water supplies sometimes reaches a concentration that will injure or kill fish and even water plants, but it is questionable whether it ever contains enough free chlorine to be dangerous for watering or syringing land plants.

Comparative Effect of Five Toxic Gases on Plants and Animals

Several members of the Institute staff [2, 18, 19, 35, 38] used our continuous-flow fumigation apparatus to study the relative toxicity of the following five gases upon various animals, plants, and plant organs: ammonia (NH_3), chlorine (Cl_2), hydrogen cyanide (HCN), hydrogen sulphide (H_2S), and sulphur dioxide (SO_2). Each gas was studied at concentrations of 1, 4, 16, 63, 250, and 1000 ppm of the air and the periods of exposure were 1, 4, 15, 60, 240, and 960 minutes. The full set of experiments produced a great number of detailed facts, many of which are of interest. Space demands, however, that we must present only the general findings with a few of the more interesting details in connection with some of the organisms.

Fig. 74 shows the number of minutes of exposure required for the highest concentrations of the gases used, 1000 ppm, to kill one-half of the various organisms (LD50) insofar as 960 minutes was sufficient to accomplish this end. One must realize in this figure that, due to the limited page size, the time is recorded as a geometric series on the ordinate rather than as an arithmetical series. If the figure were made on the arithmetical basis and each minute given the same space as the first minute, the figure would be about 45 inches high and the points in the upper part would be very much farther apart, increasingly so as the top of the figure is approached. With this in mind, let us see what the figure shows.

Beginning with the eight actively growing plant pathogens, it is evident that they vary greatly from each other in their resistance to the several gases. They are arranged from left to right in their increasing order of resistance. These pathogens are in general most readily killed by SO_2. The one exception is *Rhizoctonia tuliparum*, which proved a little more sensitive to Cl_2 than to SO_2. Chlorine is the next most toxic. Then follow NH_3, H_2S, and HCN in succession, with no significant difference between the last two. Each of the last two failed to kill 50 per cent of four of the eight plant pathogens even in 960 minutes. In the case of the two animal pathogens tested, Cl_2 proved by far the most toxic, with SO_2 second. The other three gases showed low toxicity. Sclerotia of fungi and seeds proved resistant. These reproductive organs are well protected by non-living coats. Soaked seeds, of course, were more sensitive than dry ones.

Green leaves were, on the whole, the most sensitive to the five gases of any organisms tested, with Cl_2 the most toxic, SO_2 next, followed by NH_3 and HCN, and with H_2S least toxic by a large margin.

As has already been mentioned, Cl_2 and SO_2 both caused interveinal spotting of the blades of the leaves. Ammonia in much higher dosages brought about spotting of the leaves, but the spots were often black instead

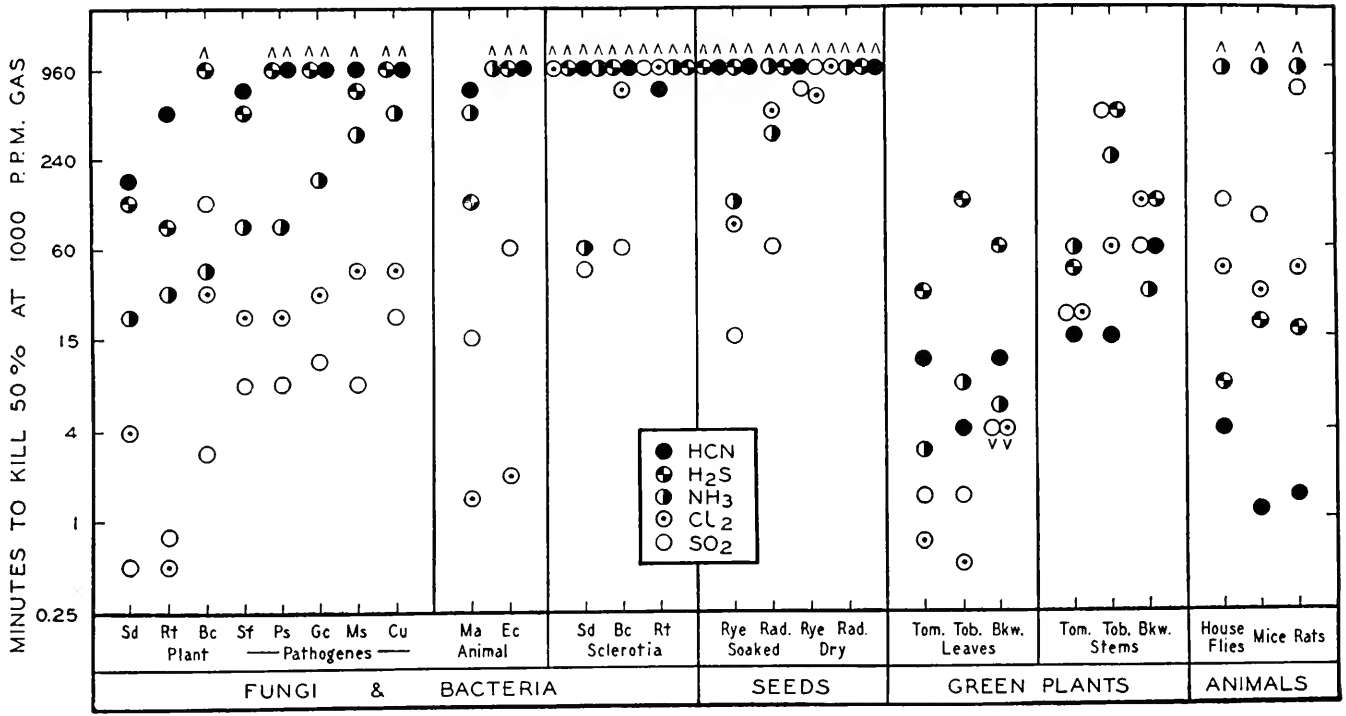

FIGURE 74. Comparison of LT50 values in minutes at 1000 ppm for different gases and organisms. Abbreviations of organism names *from left to right as follows:* Plant pathogene cultures — *Sclerotium delphinii, Rhizoctonia tuliparum, Botrytis* sp. (*cinerea* type), *Sclerotinia fructicola, Pestalotia stellata, Glomerella cingulata, Macrosporium sarcinaeforme,* and *Ceratostomella ulmi;* animal pathogene cultures — *Monilia albicans* and *Escherichia coli;* plant pathogene sclerotia — *Sclerotium delphinii, Botrytis* sp. (*cinerea* type), and *Rhizoctonia tuliparum;* seeds — soaked rye and radish, dry rye and radish; green leaves — tomato, tobacco (*Nicotiana glutinosa*) and buckwheat; green stems — ditto; animals — houseflies, mice, and rats. Symbol ∧ indicates greater than, and ∨ less than.

Table 21. Relative Toxicity of Gases and Sensitivity of Organisms, Significant Difference with Odds of 20:1 or Greater, Between Each Group

A. Order of Toxicity of Gases to Classes of Organisms

Fungi and Bacteria	Seeds and Sclerotia	Green Leaves	Green Stems	Animals
1. SO_2, Cl_2 2. NH_3 3. H_2S, HCN	1. SO_2 2. Cl_2, NH_3, HCN, H_2S	1. Cl_2 2. SO_2 3. NH_3, HCN 4. H_2S	No difference	1. HCN 2. H_2S 3. Cl_2 4. SO_2 5. NH_3

B. Order of Sensitivity of Classes of Organisms to Gases

Ammonia	Chlorine	Hydrogen Cyanide	Hydrogen Sulphide	Sulphur Dioxide
1. Leaves	1. Leaves	1. Animals	1. Animals	1. Leaves, Fungi and Bacteria
2. Stems, Fungi and Bacteria	2. Fungi and Bacteria, Stems, Animals	2. Leaves, Stems	2. Leaves, Stems	2. Stems, Animals, Seeds and Sclerotia
3. Seeds and Sclerotia,* Animals	3. Seeds and Sclerotia	3. Fungi and Bacteria, Seeds and Sclerotia	3. Fungi and Bacteria, Seeds and Sclerotia	

* No significant difference between Seeds and Sclerotia and group 2.

of yellow or reddish brown due to the presence [4] of tannins in the leaves. Darkness also protected leaves from injury by NH_3 but to a lesser degree than it did against SO_2. The petioles of the younger leaves [35] were first killed by HCN. In the higher dosages Cl_2 and SO_2 showed marked acidulation of the leaves, Cl_2 being more effective than SO_2, whereas H_2S caused only slight acidulation. Ammonia made the leaves more alkaline. The pH of the soil was lowered by Cl_2 and SO_2 and raised by ammonia. Stems were much less sensitive on the whole than leaves, with little if any significant difference between the several gases.

The toxicity of the five gases to houseflies and mammals shows a great difference in effectiveness with no overlapping of results. They rank as follows: $HCN > H_2S > Cl_2 > SO_2 > NH_3$.

Table 21 shows (A) order of toxicity of gases to classes of organisms, and (B) order of sensitivity of classes of organisms to the five different gases based on the highest concentration used, 1000 ppm. In general, Cl_2 and SO_2 are most toxic to plants and HCN and H_2S to animals.

Table 22. Time Till 50 Per Cent Mortality of Gas-Treated Animals (In Minutes)

Gas	Animal	Concentration, ppm			
		1000	250	63	16
NH_3	Flies	> 960			
	Mice	> 960			
	Rats	> 960			
Cl_2	Flies	45	240	840	> 960
	Mice	28	440	> 960	
	Rats	53	440	> 960	
HCN	Flies	3.3	< 8	8.2	48
	Mice	1.2	5.1	66	> 960
	Rats	1.4	8.7	40	> 960
H_2S	Flies	7	> 960		
	Mice	18	410	804	> 960
	Rats	14	> 960	> 960	> 960
SO_2	Flies	120	720		
	Mice	132	786	> 960	
	Rats	910	> 960		

Up to now we have discussed the effect of only one concentration, 1000 ppm, with only the time of exposure varying. If the total dosage (time × concentration) were a constant for producing a given physiological effect, this would be adequate at least for one kind of organism. Calculating from the figures in Table 22 will show that the product law does not hold. There is another reason why various concentrations as well as various times

must be considered when one organism is compared with another; flies, for instance, resist 1000 ppm of HCN more than twice as long as mice or rats, but with 63 ppm mice and rats endure HCN five to seven times as long as flies. At 16 ppm neither rats nor mice showed any deaths or even signs of injury during 960 minutes, while 50 per cent of the flies were killed in 48 minutes. For SO_2 we have already mentioned that guinea pigs resisted higher concentrations better than mice, but that mice were much more resistant to low concentrations. It is certain that flies could be killed in the presence of mammals by use of HCN in perfectly regulated low concentrations for long periods. It is regrettable that several other insects were not included in these studies to see how generally very low regulated concentrations of HCN proved fatal to insects.

Those that are interested will want to read the original articles, especially for details on symptoms and internal pathological changes caused in animals and on types of injuries produced in green plants by the several gases.

Summary

As we saw in the previous chapter, ethylene is the constituent of artificial illuminating gas that injures plants in greenhouses when this gas seeps through the soil and into the houses. Hydrocyanic acid is the most deadly constituent of artificial illuminating gas to plants growing outdoors near leaking gas pipes. Most natural gases have very low toxicity to plants because they contain no ethylene or other unsaturated hydrocarbons, and no HCN or other highly toxic gases. Some natural gases contain H_2S which might injure plants if the gas were not thoroughly scrubbed.

Mercuric chloride, calomel, or organic mercury fungicides must be used with caution on soils in greenhouses or other enclosed spaces because the soils reduce these compounds to metallic mercury which has sufficient vapor pressure, especially at higher growing temperatures, to injure plants throughout the enclosed space. Use of mercury in respirometers or to seal apparatus may vitiate experiments in plant physiology either by injuring or stimulating the plants. The health of laboratory workers is endangered by exposure of large surfaces of mercury to the air, as in the case when pellets of mercury are allowed to lie on the floor or laboratory tables.

Injuries from SO_2 from smelters have led to the development of accurate apparatus for recording the SO_2 concentration in the air and for fumigating plants and animals in continuous-flow chambers with the SO_2 regulated accurately and continuously recorded. The Department of Agricultural Research of the American Smelting and Refining Company did much to develop this apparatus and contributed greatly to knowledge of the physiological effect of SO_2 on plants. The National Research Council of Canada in connection with Trail Smelter injury added much to our understanding of the effect of SO_2 on vegetation. Boyce Thompson Institute added to the knowledge of the physiological effect of SO_2 on plants and animals. The most delicate plants are injured by 0.46 ppm of SO_2 with seven hours'

exposure. Animals endure 33 ppm for 500 hours without injury. Sulphur dioxide kills the leaf parenchyma of the medium-aged leaves, thus cutting down assimilation, but there is no reduction of assimilation or growth if no tissue is killed, that is, there is no "invisible injury." Darkness and partial wilting increase the resistance of plants to SO_2, partly at least by closing the stomates. Many continuous records have been made of SO_2 content of the air about industrial and population centers. At Boyce Thompson Institute the average and highest annual concentration of SO_2 in the air, largely blowing in from New York City, is about the same as that in the upper Columbia Valley at Northport, Washington, during the growing season, where injury from the Trail Smelter is claimed. The highest concentration of SO_2 in the air about cities is during the winter when most coal is being burned. Finally, the SO_2 given off by industries and cities aids soil fertility by replenishing sulphur deficiency of the soil.

Hydrogen sulphide differs from SO_2 in several ways as to its effect upon plants; it requires a much higher concentration to injure plants, 40 to 400 ppm; it kills the young leaves and stems rather than spotting middle-aged leaves, and its toxicity is not so greatly reduced by darkening and by wilting the plants.

Chlorine acts much like SO_2 on plants and spots them in even lower concentrations. Chlorinated water has relatively low toxicity for land plants when used either for syringing or watering them. Chlorinated water bearing 1.0 to 1.5 ppm of chlorine will kill fish, and water plants are a little less sensitive. City water supplies may at times contain enough chlorine to injure fish and water plants.

By use of the continuous air-flow method, a study was made of relative sensitiveness of plant and animal pathogens, sclerotia, seeds, green plants, and houseflies, rats, and mice to the five gases, Cl_2, HCN, H_2S, NH_3, and SO_2. Chlorine and SO_2 showed high toxicity to pathogens and other gases low toxicity. Sclerotia and seeds were little injured by any of the gases. Green leaves were very sensitive to these gases, and the gases showed the following order of toxicity: $Cl_2 > SO_2 > NH_3 > HCN > H_2S$. Green stems were more resistant than leaves, with no significant difference in the degree of toxicity of the five gases. Animals were readily killed by HCN and H_2S and the order of toxicity for animals was $HCN > H_2S > Cl_2 > SO_2 > NH_3$.

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CHAPTER 6

Plant Hormones

by P. W. Zimmerman

The mysterious forces which regulate the growth and movement of plants have always been a subject of major interest to botanists. For many years efforts were centered around essential mineral elements in fertilizers and soil, with the thought that properly balanced nutrient solutions might lead to an understanding of growth regulation. While plants grew at varying rates according to the kinds and amounts of minerals supplied, there was no evidence that tropisms, correlation of organs, flowering, and maturation are regulated by fertilizers. The effect of the growing stem tip on growth of other organs, the bending of stems toward light, the capacity of plants to right themselves when placed in a horizontal position, the production of adventitious roots, and the polarity of shoots and roots were phenomena which were not controlled by mineral nutrients. There was, perhaps, something made by the growing plants, natural substances, which regulate growth.

The results of recent investigations take away some of this mystery and indicate that, as in the animal kingdom, growth, movement, and maturation of plants are regulated by chemical substances (hormones) produced by the organism itself. In fact such substances, extracted from plants and animals and re-introduced into normal tissue, cause hormone-like responses. Physiologically active chemicals were prepared synthetically in the laboratory to take the place of natural hormones. As with animals, a single active compound has several different effects on plants. For example, a single treatment of a growing plant with α-naphthaleneacetic acid may cause cell elongation, resulting in curvature of stems and epinasty of leaves, proliferations involving cell division and induction of adventitious roots, inhibition of buds, and regulation of rate of growth. These are all hormone-like responses. The term "hormone" was borrowed from the animal field, where it referred to a regulating substance produced in a particular ductless gland but having its effects on organs or tissues some distance away. In plants the terminal bud produces a substance which regulates growth of axillary buds. Botanists have a number of terms used more or less synonymously with the word "hormone" — growth substance, growth regulator, phytohormone, and auxin. The term "hormone" should be reserved for natural substances, but it has a popular appeal and has been used loosely.

Many controversial views were held before the modern growth substance

concepts were established. In order to present a brief historical picture, a few of the outstanding contributions from the time of Darwin are cited.

Darwin [8] in 1881 showed that the coleoptiles of *Phalaris* and *Avena* curved toward the light and that when only the tip was unilaterally illuminated the influence traveled downward. When the tip was shaded, the remaining stump was unable to make a phototropic response. This definitely showed that the tip of the coleoptile was a place of great importance in connection with phototropic curvatures in plants, though Darwin did not recognize the influence as being of a chemical nature.

The beginning of the chemical substance idea with proof to support it goes back to 1907 when Boysen-Jensen [1, 2, 3] started his classic experiments to show that the stimulus (chemical substance) could cross a discontinuity in the coleoptile of *Avena*. He found that when excised coleoptile tips were replaced on the stump with a layer of gelatin, phototropic curvatures resulted after unilateral illumination of the tip, as with normal coleoptiles. That is, a substance which was formed in the tip drained into the gelatin and then diffused through this non-living material into the stump, where it accelerated growth on the dark side, causing bending toward the lighted side. Boysen-Jensen also showed that he could intercept the substance by inserting a small piece of mica into the coleoptile. If the mica was inserted on the illuminated side, phototropic curvature occurred normally; if inserted on the dark side, very little or no bending occurred. Similar experiments were performed with geotropically stimulated coleoptiles. If mica was inserted in the horizontally placed organs on the upper side of the tip, negative geotropism resulted, as in normal coleoptiles; if the mica was inserted on the lower side, little or no bending occurred. The interpretation which Boysen-Jensen put upon the results of these experiments was that the stimulus originating in the tip was of a chemical rather than a physical nature, and that it acted in regulating growth. He thought there was an increased transmission of the growth-promoting substance on the dark side — a view which is still tenable.

Paál [32, 33] from 1914 to 1918 confirmed Boysen-Jensen's results and further showed that if an excised coleoptile tip was replaced on one side of the stump, growth was accelerated on that side, resulting in curvature. For this response no special stimulation of the tip was necessary, thus showing that the tip was continually making the growth hormone in the dark also. Paál also demonstrated that the stimulus passed through an interposed gelatinous membrane 0.1 mm in thickness between the tip and the stump of the coleoptile. He concluded from his experiments that the transmission of the phototropic stimulus was brought about by means of a diffusible substance.[33, p.431]

Stark [37] in 1921 made the next big advance by investigating the transmission of phototropic, traumatotropic, and haptotropic stimuli. He expressed the sap from coleoptiles and mixed it with agar. Out of the agar plate, blocks were cut and placed unilaterally on decapitated coleoptiles. A sub-

stance drained out of the block into one side of the coleoptile, retarding growth and causing curvature.

Seubert [35] in 1925 extended the experiments of Stark by infiltrating agar with substances of both plant and animal sources — diastase, malt extract, saliva, etc. — demonstrating the existence of both accelerating (causing curvatures) and inhibiting substances.

The field was further advanced from 1920 to 1934 by Purdy,[34] Söding,[36] Cholodny,[5, 6, 7] Went,[39, 40, 41] Dolk,[10] Zimmerman, Crocker, and Hitchcock,[47, 48] Boysen-Jensen,[4] Laibach *et al.*,[31] Thimann and Went,[38] and Kögl *et al.*[30]

Hitchcock [14] in 1935, experimenting with indole acids, demonstrated that β-indoleacetic acid and β-indolepropionic acid induced curvatures, proliferations, and adventitious roots when applied to intact plants. Hitchcock also found phenylacrylic (cinnamic) and β-(phenyl)-propionic acids to be physiologically active when applied to intact plants.

Zimmerman and Wilcoxon [64] in 1935 brought to light seven new hormone-like substances, giving support to the assumption that there are many physiologically active compounds, both natural and synthetic. Table 23 gives a list of hormone-like substances known in 1935. Two acids in this list, β-indolebutyric and α-naphthaleneacetic, were pointed out as the most effective root-inducing substances. Many practical applications have been made with these and they still rank among the most important.

In 1939 Zimmerman, Hitchcock, and Wilcoxon [56, 63] listed a total of 54 different growth substances which showed activity when applied to plants in the vapor form. The greatest possibility for locating large numbers of active acids came when Zimmerman and Hitchcock [58] in 1942 showed that substituted phenoxy and benzoic acids were active and that this activity varied with kind, number, and location of the substituent groups or atoms.[44, 45, 46, 59] This is illustrated in Table 24. Table 25 shows a long list of active substituted phenoxy acids.

Of the halogen substituents, chlorine and bromine caused approximately the same degree of activation. Iodine substituents in phenoxy acids did not activate to the same degree as bromine and chlorine. However, iodine substituted in the ring of benzoic acids made very active molecules.[44, 45, 46] Chlorine substituted in the ortho position to make 2-chloro-3,5-diiodobenzoic acid furnished a molecule that acted very much like 2,3,5-triiodobenzoic acid.

Methods and applications. Physiological activity in substances or extracts was first detected by use of the *Avena* coleoptile as a test object in a dark room. The method is somewhat complicated and requires a considerable amount of equipment. Since it has been described elsewhere [42] it need not be repeated.

A simple method perfected in the Boyce Thompson Institute laboratories requires only a growing plant in light or dark. The young tomato plant, which has long been a standard test object for detecting the presence of

Table 23. A List of Physiologically-Active Acids Known and Available in 1935

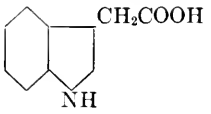
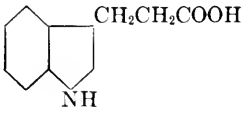
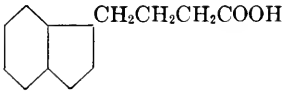
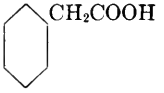
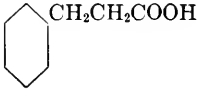
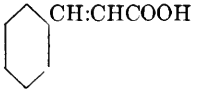
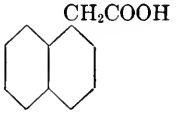
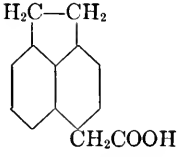
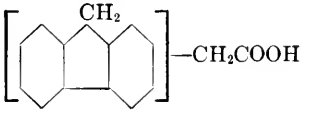
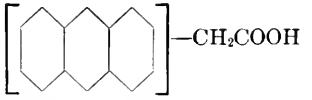
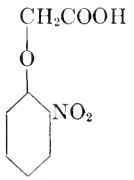
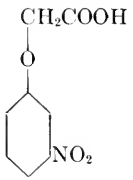
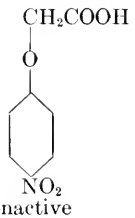
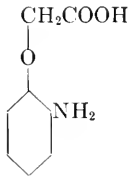
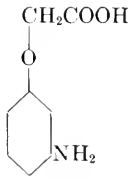
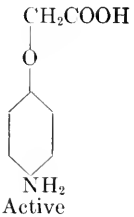
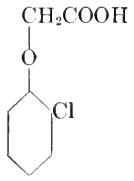
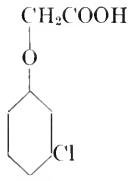
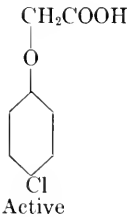
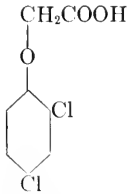
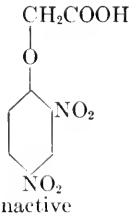
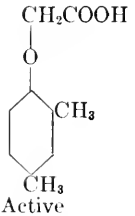
	3-Indoleacetic acid
	β-(3-Indole)-propionic acid
	γ-(3-Indole)-*n*-butyric acid
	Phenylacetic acid
	β-(Phenyl)-propionic acid
	Phenylacrylic acid (cinnamic acid)
	α-Naphthaleneacetic acid
	Acenaphthene-(5)-acetic acid
	Fluoreneacetic acid
	Anthraceneacetic acid

Table 24. Dependence of Activity for Cell Elongation Upon the Position of Substituents, Groups, or Atoms

 Inactive	 Active	 Inactive
 Inactive	 Inactive	 Active
 Active	 Active	 Active
 Active	 Inactive	 Active

ethylene gas, is a satisfactory species. The coleoptile can be used only to detect the cell-elongating power of a substance. With one treatment the tomato plant detects the capacity of a chemical to induce cell elongation, cell division, adventitious roots, and formative effects. The time required varies with the response. Detection of cell elongation requires only from 20 minutes to two hours, cell division 48 to 72 hours, initiation of roots five to ten days, and formative effects four to ten days. The four types of response are illustrated in Figs. 75 and 76. If the chemical does not induce at least one of these responses, it is listed as inactive.

Table 25. Comparative Activity of Phenoxy and Substituted Phenoxy Derivatives of the Lower Fatty Acids. Tested as Lanolin Preparations

Substances	Cell elongation (epinasty). Threshold concn. mg/g	Modification of tomato leaves. Threshold concn. mg/g
Phenoxyacetic acid	20	Inactive
α-(Phenoxy)-propionic acid	5	5
α-(Phenoxy)-*n*-butyric acid	5	5
2-Chlorophenoxyacetic acid	1	0.25
α-(2-Chlorophenoxy)-propionic acid	1	Inactive
α-(2-Chlorophenoxy)-*n*-butyric acid	1	Inactive
3-Chlorophenoxyacetic acid	0.5	Inactive
α-(3-Chlorophenoxy)-propionic acid	0.5	Active
α-(3-Chlorophenoxy)-*n*-butyric acid	0.5	Inactive
4-Chlorophenoxyacetic acid	0.25	0.06
α-(4-Chlorophenoxy)-propionic acid	0.5	Inactive
α-(4-Chlorophenoxy)-*n*-butyric acid	1	Inactive
2,4-Dichlorophenoxyacetic acid	0.015	0.003
α-(2,4-Dichlorophenoxy)-propionic acid	0.5	Inactive
α-(2,4-Dichlorophenoxy)-*n*-butyric acid	0.5	Inactive
2,4,5-Trichlorophenoxyacetic acid	0.06	Inactive
α-(2,4,5-Trichlorophenoxy)-propionic acid	0.03	Inactive
α-(2,4,5-Trichlorophenoxy)-*n*-butyric acid	0.1	Inactive

The simplest method known for testing new chemicals is by means of the lanolin preparation made by mixing 10 to 20 mg of the substance with one gram of lanolin. When these are thoroughly mixed, a small amount is applied with a glass rod to the upper side of a young tomato leaf and to one side of the adjacent stem. The angle between the stem and the leaf before treatment is usually near 45 degrees. If the chemical is active, causing cell elongation, the leaf moves downward and the stem curves away from the treated side, thus increasing the degree of the angle (Fig. 75). The same plant is kept for 10 to 12 days to determine the effect on cell division, root-inducing activity, and formative effects. If activity is indicated by the first test, the chemicals are then studied in comparison with a standard, such as α-naphthaleneacetic acid, which is active when used at 0.001 per cent in lanolin.

Comparisons can also be made by applying the chemical to the soil of the potted plant. For example, 1 to 10 mg of an active chemical in 50 cc of water applied to the soil will cause the entire plant to show an epinastic response. This then is usually followed by the three other responses described above.

Induction of adventitious roots. To date practically all the recorded growth substances which induce cell elongation (causing epinasty or stem curvatures) are active also for inducing adventitious roots. This capacity may be associated with the power to induce cell division. It is a fact that growth substances which induce mature cells to make further growth also

FIGURE 75. Tomato plants showing induced cell elongation and cell division. A, *left to right:* control plant; three plants treated on the upper side of a leaf petiole with lanolin preparations containing three different concentrations of α-naphthaleneacetic acid (0.0005 per cent, 0.00025 per cent, and 0.0001 per cent, respectively) which causes cell elongation, the degree of response increasing with increasing concentration. Photograph taken after 24 hours. B, *left:* control plant; *right:* lanolin preparation containing 1.5 per cent α-naphthaleneacetic acid applied around the upper end of the stem causing increased cell division and swelling of leaves and entire stem. Photograph taken after 48 hours.

stimulate cell division. The actual initiation of root primordia involves some form of growth regulation which is not well understood. Root primordia usually are associated with proliferations involving stimulated cell division. Several types of induced rooting are shown in Fig. 77.[54]

The many growth substances do not all have the same degree of root-inducing power, and there are many qualitative differences in the induced responses. From a concentration standpoint alone the requirements vary from 0.1 mg/l or less for α-(2,4-dichlorophenoxy)-propionic acid to 40 mg/l for β-indolebutyric acid. Again the species vary in their capacity to respond. For example, *Ligustrum* (privet) responds to α-naphthaleneacetic acid but not to β-indolebutyric acid; but *Evonymus* (strawberry bush) is just the opposite, being sensitive to β-indolebutyric acid and not to α-naphthaleneacetic acid. The usefulness of a root-inducing preparation can be extended and made to cover a wider range of species by including two or more

FIGURE 76. Plants showing two different responses to "plant hormones." A, *Kalanchoe* plants. *Left:* control plant; *right:* treated around the stem near the tip with 1.0 per cent β-indolebutyric acid to induce adventitious roots. B, Tomato shoots. *Left:* control plant; *right:* growth showing modification after the tip had been sprayed with a solution containing a methyl substituted phenoxy acid. Many variations of this can be induced with different substances having a formative influence.

growth substances. Considerable attention has been given to comparative activity [17, 19] and effects obtained with mixtures of root-inducing substances.[20]

Propagation of plants. Substances detected by means described in the preceding paragraphs may or may not have value for practical propagation. For example, β-indoleacetic acid is a very effective substance for inducing epinasty when applied to tomato plants. It is not, however, as effective for inducing roots as β-indolebutyric acid, α-naphthaleneacetic acid, or many of the substituted phenoxy acids. The best root-inducing substances are determined only by testing various substances and concentrations. By this method it has been found that species of plants vary in their sensitivity to the different substances. Generally speaking, β-indolebutyric acid and α-naphthaleneacetic acid together cover practically all the species require-

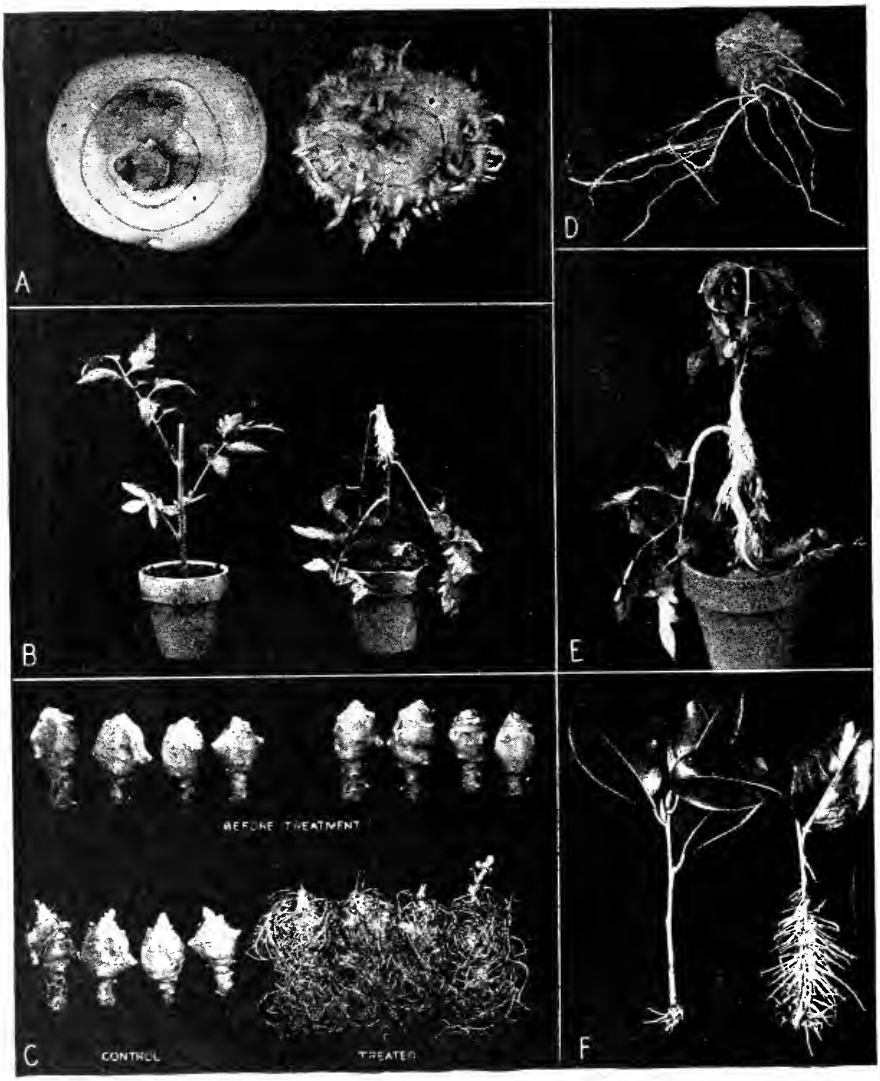


FIGURE 77. Adventitious roots induced with plant hormones. A, *Gladiolus* corms showing (*left*) normal condition; (*right*) adventitious roots induced with β-indolebutyric acid. B, Tomato plants with tops removed. *Left:* control having the cut surface treated with pure lanolin; *right:* lanolin preparation containing 1 per cent β-indolebutyric acid applied to cut surface of stump. C, *Helianthus tuberosus* tubers showing control *on left;* and *right:* tubers growing adventitious roots 20 days after having been immersed 48 hours in a solution of α-naphthaleneacetic acid (50 mg/liter of water). D, An internal piece of potato tuber induced to root by treatment with β-indoleacetic acid solution [*see publication by Guthrie*[13]]. E, Tomato plant treated with the first preparation of α-naphthaleneacetic acid used as a hormone. F, *Camellia* cuttings showing natural rooting from callus on non-treated control (*left*); and *right:* roots induced along stem of cutting dipped into a mixture of β-indolebutyric acid + α-naphthaleneacetic acid, equal parts, making a total concentration of 20 mg per cc of water.

ments. In some cases substituted phenoxy acids show considerably more activity for inducing roots than these two, but they have not been thoroughly tested for practical purposes.

Three methods of treating stem cuttings are to immerse the basal end of the cutting in the given concentration of the chemical in water solutions; to dip the basal end of the cutting into a powder preparation containing a

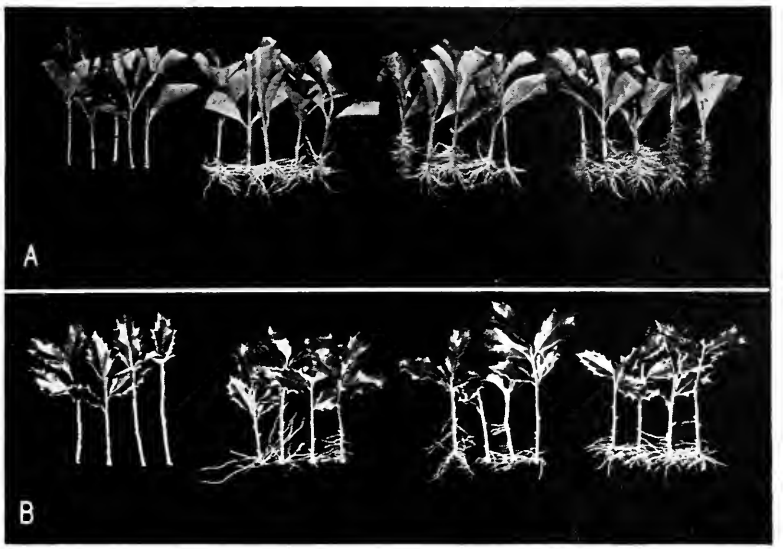


FIGURE 78. Propagation with aid of root-inducing chemicals. A, *Evonymous* cuttings showing increased rooting with increasing concentrations of β-indolebutyric acid. *Left to right:* non-treated control, next three treated at base with solution of β-indolebutyric acid — 0.5 mg/liter, 1.0 mg/liter, and 2 mg/liter, respectively. The basal end of cuttings were immersed in the solution for 24 hours, then planted in rooting medium. Photograph taken after 20 days. B, *Ilex* cuttings showing the effects of the growth substance alcoholic dip method. *Left to right:* control dipped in 50 per cent alcohol; basal end dipped in 5 mg per cc of 50 per cent alcohol; 10 mg; and 20 mg, respectively. Cuttings were in rooting medium 33 days before being photographed.

given amount of the chemical; or to dip the basal end of the cutting into an alcoholic solution of the substance or a concentrated water solution of the substance. In a 24-hour immersion treatment, β-indolebutyric acid is used at a concentration of 0.5 to 80 mg of the acid per liter of water, the optimum varying with the species (Fig. 78A). α-Naphthaleneacetic acid is effective over a range of 0.5 to 60 mg of the acid per liter of water. Halogen-substituted phenoxy compounds must be used in low concentrations. For example, α-(2,4-dichlorophenoxy)-propionic acid is effective over a range of concentrations from less than 0.1 to 10 mg/l, the exact requirement varying with the species.

A second method, which has been found particularly effective, is known as the solution dip method. One to 10 mg of β-indolebutyric acid in 50 per cent alcohol makes an effective range of concentrations which can be used

on a large number of species. The basal end of the cutting is dipped directly into the alcoholic solution and planted immediately in the rooting medium. Similarly, a soluble salt of α-naphthaleneacetic acid may be used in water instead of alcohol (Fig. 78B). This is a simple method since it does not require preliminary soaking before planting.

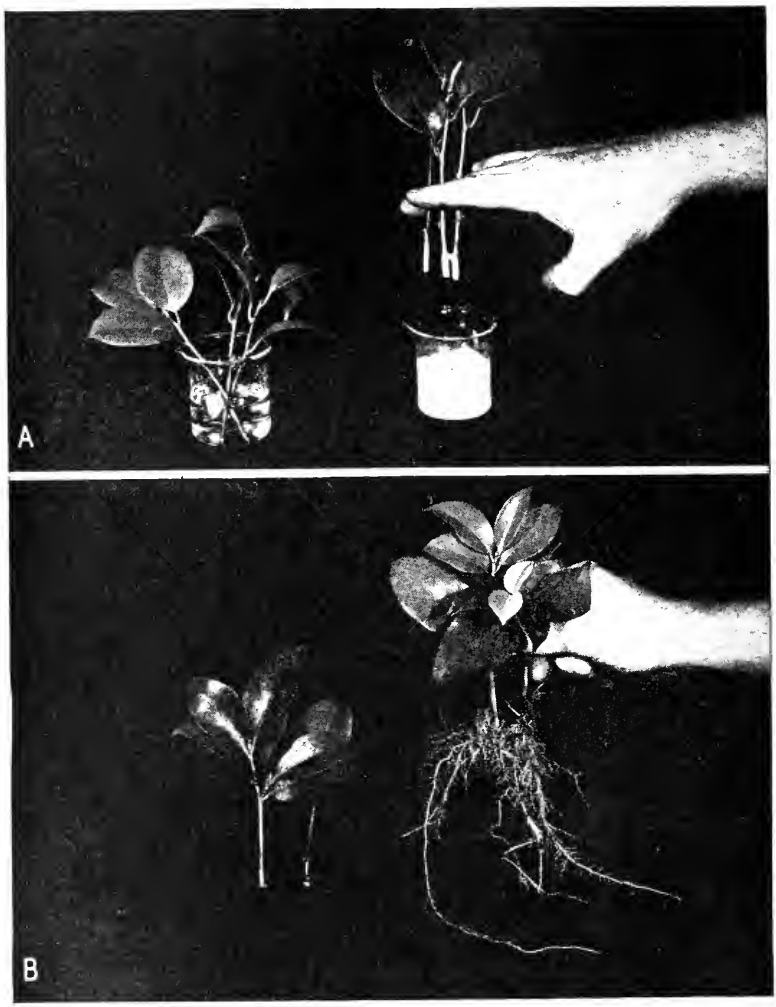


FIGURE 79. The powder dip method for propagating cuttings with growth substances illustrated with *Camellia* species. A, Moist *Camellia* stems dipped into powder preparations containing 10 mg of β-indolebutyric acid per g of talcum powder. B, *Left:* control treated with pure talcum powder; *right:* rooted cuttings which had been treated with talcum powder preparation containing 10 mg of β-indolebutyric acid per g of talc.

The third and the most extensively used method at the present time is the powder dip method (Fig. 79). This involves a mixture of 1 to 10 mg of β-indolebutyric acid, α-naphthaleneacetic acid, or other substances per

gram of talcum powder in which the basal end of the cutting is dipped and then planted in the rooting medium. Enough of the mixture clings to the basal end of the stem to induce roots the same as the other treatments.

All three of the methods described have their supporters among the amateurs and commercial growers. It is generally recommended that interested growers familiarize themselves with all the methods and then select the one which is most satisfactory. Also, for the average grower it is better to use a recommended commercial preparation than to attempt to measure and prepare the substances in small quantities. Details for propagating plants are given in several Boyce Thompson Institute papers.[19, 21–29]

β-Indolebutyric acid, which accelerates callus formation, has been found effective also for accelerating union between the scion and the stock where the grafting method is used. The basal end of the scion is dipped into a water solution containing 50 to 80 mg of β-indolebutyric acid per liter of water and then grafted as usual.

Lily scales which are used for growing bulbils may be treated by any of the methods described for cuttings and planted in the usual way. β-Indolebutyric acid accelerates rooting of the scale, thus keeping it in good condition until the bulbils are formed. Though various claims have been made, it has not been proved that any of the known growth substances to date initiate shoot buds.

Saintpaulia leaves used for propagation purposes can be induced to form many roots by treatment with well-known growth substances. In no case, however, has acceleration of buds been proved. Generally, bud initiation is inhibited by the treatment.

Preharvest apple drop. The practical use of growth substances has been greatly extended by the work of Gardner *et al.*[11] in connection with abscission layers and premature falling of fruit. Of all the substances listed in Tables 23 and 24, it appears that α-naphthaleneacetic acid is the most effective substance for preventing preharvest apple drop. The water solution containing 10 to 50 mg of α-naphthaleneacetic acid per liter of water is sprayed on the entire tree at the time the apples begin to fall prematurely. The chemical prevents the separation of the abscission layer, thus causing the apples to stay on the tree. It has been reported that trees normally losing 50 to 75 per cent of their apples before harvest time have been reduced to 5 to 10 per cent drop. Many of the active substances listed in this chapter are effective for preventing abscission of leaves, but so far none has proved as effective for preharvest apple drop as α-naphthaleneacetic acid. Not all varieties of apples respond alike. For example, the McIntosh variety is very resistant, the results being negative in practically all reports. Williams variety is perhaps among the most sensitive, the treatment preventing practically all the apples from dropping. The effectiveness for other varieties fluctuates between these two extremes. Variations are also reported for a given variety in different locations over the country.

Inhibition of growth. The use of chemicals for inhibiting growth is often as important as for accelerating growth. The same chemicals which stimulate root growth (cause cell elongation, cell division, etc.) may be used also to inhibit growth. Hitchcock [15] in 1935 tested seven substances for comparative effectiveness in inducing epinasty. He listed them in decreasing order of activity as follows: α-naphthaleneacetic, β-indoleacetic, β-indolebutyric, β-indolepropionic, phenylacetic, β-(phenyl)-propionic, and phenylacrylic (cinnamic) acids. The same order holds for inhibition. When Hitchcock applied the substances to the cut surface of decapitated stems, axillary buds down along the stem were inhibited. The buds nearest the cut surface (that is, nearest the chemical) were inhibited most. Lanolin preparations of two unsaturated hydrocarbon gases, ethylene and propylene, applied to the cut surface also inhibited the uppermost buds.

Sprouts and seedlings treated with α-naphthaleneacetic acid were first accelerated in growth and then inhibited (Fig. 80). The stems later increased in diameter but not in length, and the buds failed to develop.[56, 63, 64] Stem cuttings of some species, treated with α-naphthaleneacetic acid to induce basal roots, showed inhibition of buds up along the stem after roots were well established.

Roots differed from stems in response to growth substances by being inhibited immediately without a preliminary acceleration period.[49] Aerial roots of *Cissus*, which normally elongate 5 to 10 inches within a 24-hour period, practically stopped when a lanolin preparation containing 0.1 to 1.0 per cent α-naphthaleneacetic acid was applied at the tip.

Guthrie [12] showed that methyl α-naphthaleneacetate applied as a vapor was effective for inhibiting buds of potato tubers. He placed layers of filter paper impregnated with the ester among the stored tubers, and effectively prevented growth. About 500 mg of methyl α-naphthaleneacetate per kg of potatoes were sufficient. Denny, Guthrie, and Thornton [9] improved the method and worked out other methods for preventing potato tubers from sprouting by use of methyl α-naphthaleneacetate. Dusting potatoes with a talcum powder preparation of the ester was found to be effective. Though not fully tested, the new aerosol-growth substance method has been effective when used in the laboratory.

Another use for bud inhibition is to delay flowering of fruit trees until danger of frost is past. This is done by spraying in the previous summer and autumn when the buds are forming. A solution containing 100 mg of α-naphthaleneacetic acid per liter of water applied in August with a sprayer delayed flowering for two to three weeks. Some delay was accomplished also by spraying branches when the buds started showing color in the spring, but much higher concentrations were required. It was predicted that tropical fruits and others could be staggered throughout the year by this method. Flowering shrubs which have one flush of flowers in the spring might also be staggered in time of flowering.

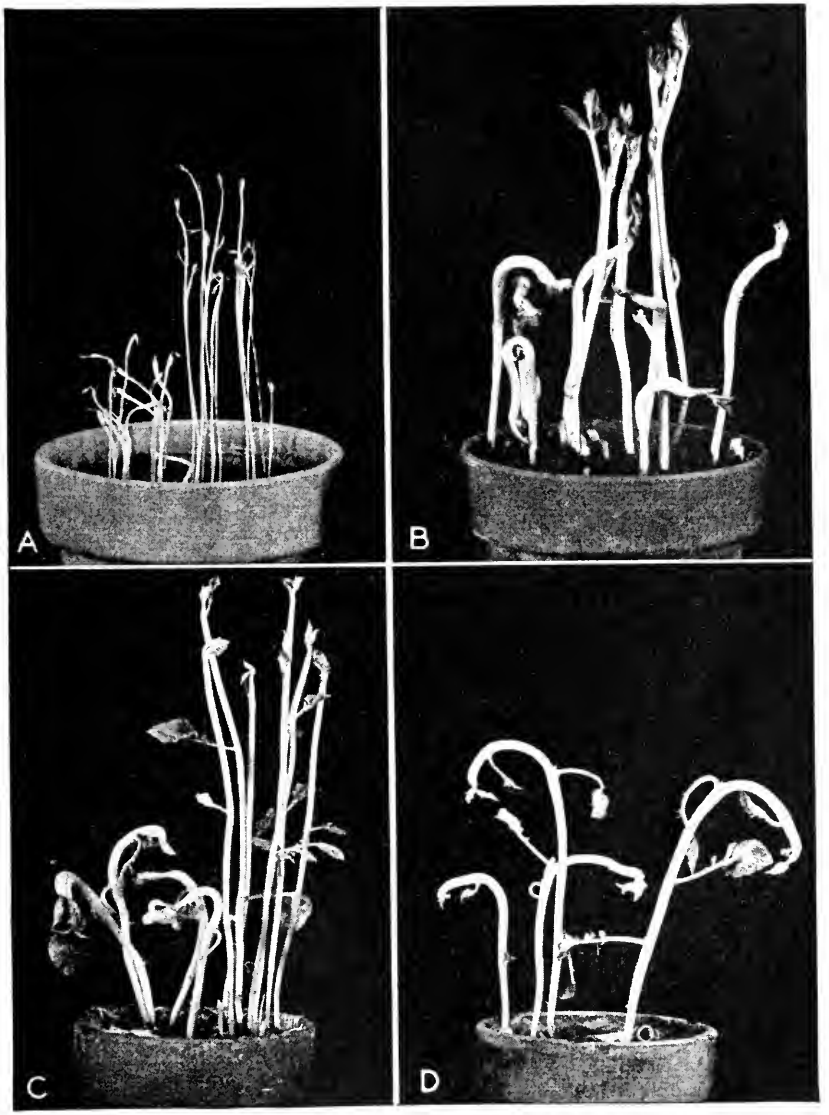


FIGURE 80. Inhibition of growth followed by increase in diameter of stem. A, Sweet pea seedlings showing controls (*right*), and seedlings treated at the tip with lanolin preparation containing 1 per cent α-naphthaleneacetic acid. B, Windsor bean seedlings showing upright controls, not treated, and treated seedlings which are inhibited and swollen. C, Potato sprouts showing upright controls (*right*) and curved and swollen sprouts resulting from treatment with lanolin preparation containing about 1 per cent β-indoleacetic acid. D, Potato sprouts showing inhibition of growth and swelling after treatment with lanolin preparation containing about 1 per cent α-naphthaleneacetic acid. Controls shown in C.

Fruit set and seedless fruit. A long list of growth substances capable of causing fruit set without pollination of the flower is given in Table 26. In addition to inducing seedless fruit (Fig. 81), these chemicals may, under certain conditions, increase the size of fruit resulting from pollinated

Table 26. Growth Substances Active for Parthenocarpy, Fruit Set of Tomatoes, and Activity or Inactivity for Modification of Leaves. Applied as Spray to Flower Clusters

Phenoxy acids	Effective range of concentrations for fruit set in mg/l of water	Activity or inactivity for modification of leaves
α-(Phenoxy)-propionic acid	100–200	Active
α-(Phenoxy)-*n*-butyric acid	100–200	Active
2-Chlorophenoxyacetic acid	200–300	Active
α-(2-Chlorophenoxy)-propionic acid	25–50	Inactive
α-(2-Chlorophenoxy)-*n*-butyric acid	50–200	Inactive
α-(2-Methylphenoxy)-propionic acid	50–100	Inactive
α-(3-Chlorophenoxy)-propionic acid	50–200	Inactive
α-(3-Chlorophenoxy)-*n*-butyric acid	Active	Inactive
4-Chlorophenoxyacetic acid	50–100	Active
α-(4-Chlorophenoxy)-propionic acid	50–200	Inactive
α-(4-Chlorophenoxy)-*n*-butyric acid	50–200	Inactive
2,4-Dichlorophenoxyacetic acid	5–10	Active
α-(2,4-Dichlorophenoxy)-propionic acid	50–100	Inactive
α-(2,4-Dichlorophenoxy)-*n*-butyric acid	50–100	Inactive
2,4-Dimethylphenoxyacetic acid	300–450	Active
α-(2,4-Dimethylphenoxy)-propionic acid	300–450	Active
2,5-Dichlorophenoxyacetic acid	25–100	Inactive
α-(2,5-Dimethylphenoxy)-propionic acid	100–300	Inactive
α-(2,5-Dimethylphenoxy)-*n*-butyric acid	Active	Inactive
3,4-Dimethylphenoxyacetic acid	Active	Active
α-(3,4-Dimethylphenoxy)-propionic acid	300–500	Active
2,4,5-Trichlorophenoxyacetic acid	25–100	Inactive
α-(2,4,5-Trichlorophenoxy)-propionic acid	10–50	Inactive
α-(2,4,5-Trichlorophenoxy)-*n*-butyric acid	25–100	Inactive
2,4,5-Trimethylphenoxyacetic acid	25–100	Active
2,4,6-Trichlorophenoxyacetic acid	Active	Active
β-(2,4,6-Trichlorophenoxy)-β′-chlorodiethyl ether	Active	Active
β-Naphthoxyacetic acid	50–100	Active
β-Naphthoxypropionic acid	50–100	Active
β-Indolebutyric acid	500–1,000	Inactive
2,5-Dichlorobenzoic acid	100–300	Active

flowers.[60, 61] In the latter case the resulting fruit may be partially seedless, but once the eggs are fertilized the substances do not inhibit their growth. Under certain conditions only the wall of the fruit may be stimulated by the chemical; as a result, it develops more rapidly than the gelatinous pulp. When this happens there may be a space between the pulp and the wall. Although the flavor of the fruit may not be impaired, this condition makes the fruit unsatisfactory for commercial purposes.

Several methods for applying growth substances to tomato flowers have been developed. Perhaps the simplest is the application of spray to the

flower cluster with an atomizer. When two or more flowers are open, the entire cluster may be treated to set the fruit on both buds and open flowers. The recommended concentration for the chemical in solution is shown in Table 26. Spreaders may be used in the solution but they are not necessary.

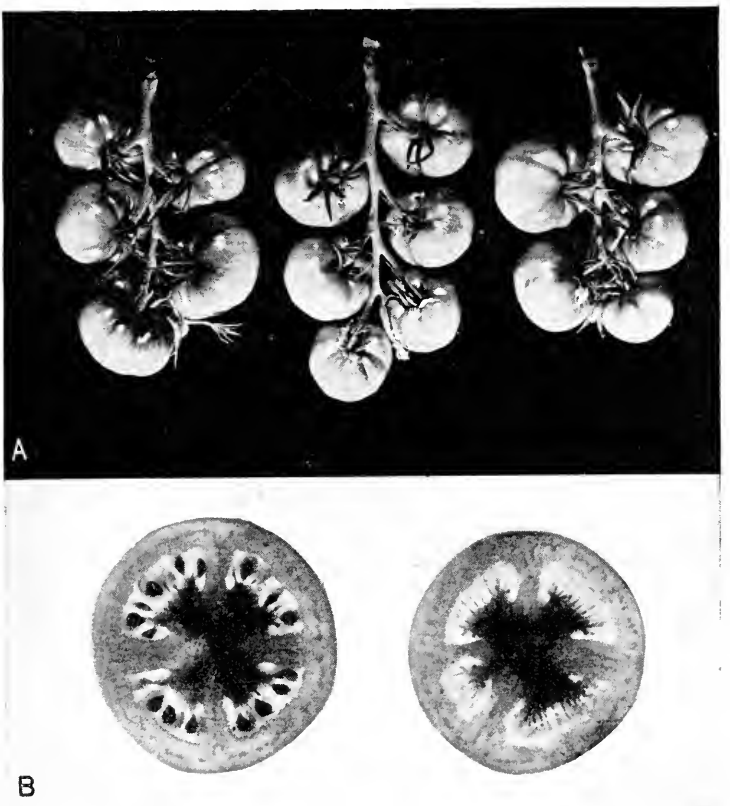


FIGURE 81. Induced fruit set and seedlessness. A, *Left to right:* normal tomato fruit resulting from pollinated flowers; parthenocarpy and seedlessness resulting from treatment of flower cluster with 2,4-dichlorophenoxyacetic acid (5 mg/liter) when three flowers were open; parthenocarpy and seedlessness resulting from treatment of flower clusters with 2,5-dichlorobenzoic acid (100 mg/liter). B, *Left to right:* cross section of normal control with seeds; cross section of seedless tomato induced with β-naphthoxyacetic acid (100 mg/liter).

Also lanolin emulsion has been tested and found effective, but is no more satisfactory than water solution. In fact there is some indication that the emulsion brings about some undesirable effects, such as scarring the surface of the tomato or increasing blossom end rot. Carbowax (polyethyleneglycol 0.5 to 1 per cent) is a good spreader and is fully as effective as lanolin emulsion. It has been recommended for fruit sprays. In spraying the flower cluster it is well to apply the solution to the peduncle as well as to the open side and back of the flowers. For a high percentage of fruit set it

has been found that spraying only open flowers causes approximately 100 per cent set.

Another method of applying growth substances is to remove the style with small scissors and apply a lanolin preparation directly to the cut surface. For this purpose 0.1 to 0.3 per cent of β-indolebutyric acid in lanolin has been recommended. Less of other chemicals is required, as follows: β-naphthoxyacetic and propionic acids 0.05 per cent, α-(2-chlorophenoxy)-propionic acid 0.01 per cent. 2,4-Dichlorophenoxyacetic acid is not recommended for this method since it causes modification of leaves even when used in very low concentrations.

The vapor method of treating flowers throughout the entire house is effective, but inhibition of growth of the entire plant after the treatment may result. If used, the ethyl or methyl esters of β-naphthoxyacetic acid or α-(2-chlorophenoxy)-propionic acid are recommended. Twenty-five to 50 mg per 1000 cubic feet of space vaporized by means of a warm electric plate are recommended. The heat should be applied so that the vapor rises slowly in the course of an hour. The air should be agitated by means of an electric fan.

The aerosol method of applying growth substances has recently been emphasized. It is a kind of modification of a fine spray. One of the best methods of applying aerosol is to place the growth substance with sesame oil in the cylinder with Freon (dichlorodifluoromethane). The internal pressure resulting is approximately 150 pounds. The mixture, dispensed through a small aperture, makes a mist or fog which affects the entire plant. When used in a greenhouse of 2000 cu. ft. capacity with tomato plants having open flowers, 50 to 450 mg of ethyl α-(2-chlorophenoxy)-propionate were found sufficient to set fruit on all the plants. The aerosol method is subject to the same objection as the vapor method.

2,5-Dichlorobenzoic acid applied to the soil of potted tomato plants induced seedless fruit. One to 5 mg in 50 cc of water applied to the soil of a 4-inch pot were sufficient to induce fruit set of open flowers and also flowers growing on the plant for one month thereafter. This method has more theoretical than practical value at the present time.

The formative influence of growth substances. A new line of plant hormone research was started when it became known that some substances had a formative influence on growth, modifying organs in size, shape, pattern, and texture.[20, 43, 44, 45, 46, 57, 58, 59] Formative effects were evident on new growth which occurred within days or weeks after the plant was treated. This was in contrast to induced curvatures due to cell elongation which took place within a few minutes after treatment. Fig. 82 illustrates a type of formative influence which 4-chlorophenoxyacetic acid has on the growth of tobacco plants.

Three groups of chemicals, β-naphthoxy, substituted chlorophenoxy, and substituted benzoic acids, were outstanding for their formative influence,

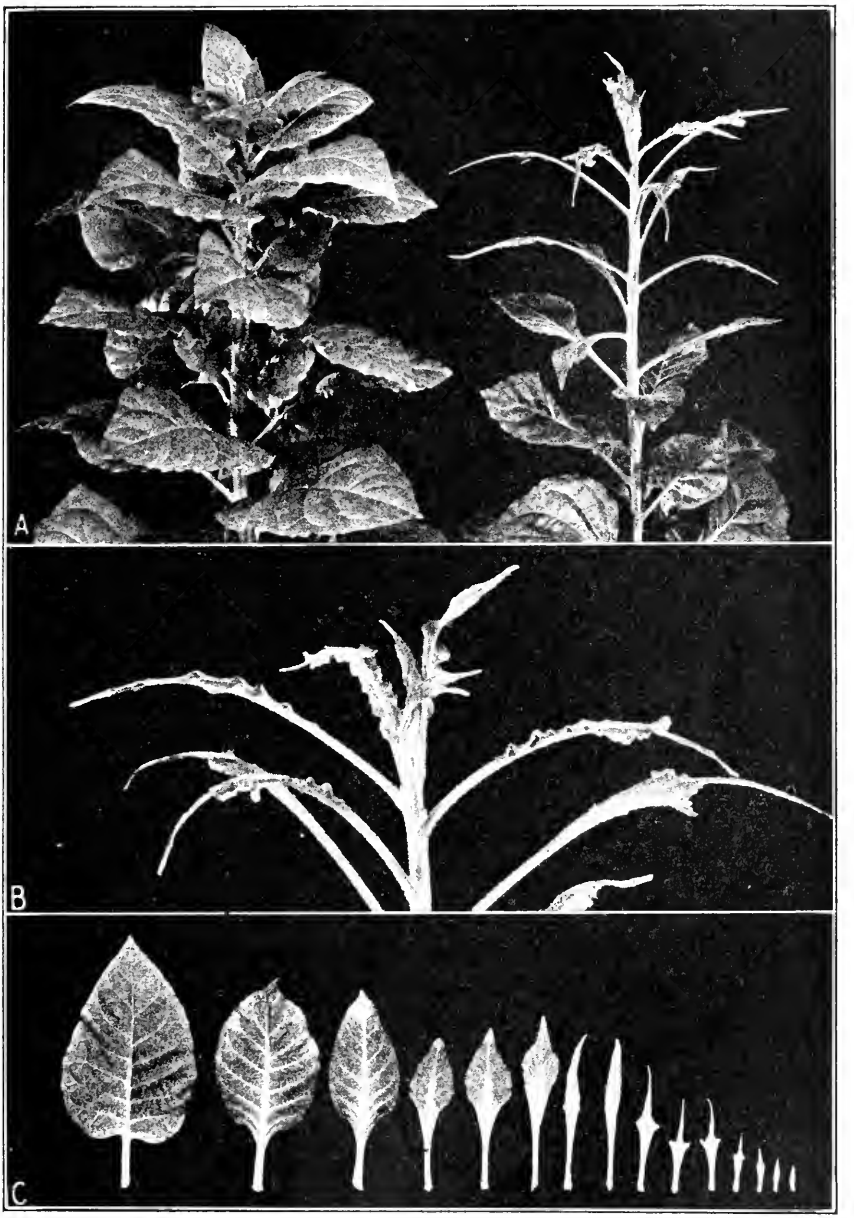
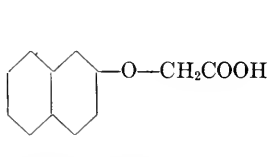
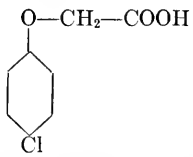


FIGURE 82. A, Tobacco plants to show formative effects induced with 4-chlorophenoxyacetic acid 200 mg/liter applied at the tip when the plant was approximately 10 inches in height. *Left:* control. B, Enlargement of plant in A. C, Series of leaves taken from base to tip of treated plant.

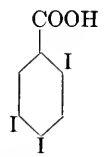
in addition to many other hormone-like effects. The following graphic formulas illustrate the structure of one acid in each group:



β-Naphthoxyacetic acid



4-Chlorophenoxyacetic acid



2,4,5-Triiodobenzoic acid

Table 27. Comparative Physiological Activity of Xylenoxy Compounds

Xylenoxy acids and esters	Cell elongation and epinasty of tomato leaves. Threshold concn. mg/g of lanolin	Modification of leaves. Threshold concn. mg/g of lanolin
2,5-Dimethylphenoxyacetic acid	Active 5	Inactive at 20 mg/g
α-(2,5-Dimethylphenoxy)-propionic acid	Active 0.5	Inactive at 20 mg/g
α-(2,5-Dimethylphenoxy)-*n*-butyric acid	Active 5	Inactive at 50 mg/g
α-(3,5-Dimethylphenoxy)-malonic acid	Inactive	Active
3,5-Dimethylphenoxyacetic acid	Inactive at 20 mg/g	Active 10
α-(3,5-Dimethylphenoxy)-propionic acid	Inactive	Active 0.25
α-(3,5-Dimethylphenoxy)-*n*-butyric acid	Inactive	Active 50
β-(3,5-Dimethylphenoxy)-*n*-butyric acid	Inactive at 20 mg/g	Inactive at 20 mg/g
2,4-Dimethylphenoxyacetic acid	Active 0.5	Active 0.25
α-(2,4-Dimethylphenoxy)-propionic acid	Active 0.5	Active 0.5
α-(2,4-Dimethylphenoxy)-*n*-butyric acid	Active 20	Active 20
3,4-Dimethylphenoxyacetic acid	Active 1	Active 1
α-(3,4-Dimethylphenoxy)-propionic acid	Active 1	Active 0.25
α-(3,4-Dimethylphenoxy)-*n*-butyric acid	Active 5	Active 5

The degree of activity varied with the groups or elements substituted, with the number and location of substitutions, and in higher homologs with the place of attachment of the chain to the ring of the molecule. The comparative influence of groups and elements and the location of the substitution are illustrated in Table 24. The activity referred to in this case is the power to induce cell elongation. When a formative influence is involved, still other differences are evident. The illustration shows that the NO_2

group brings about activity for cell elongation when substituted in the meta position of the benzene ring, and the NH_2 group in the para position; chlorine activates the molecule in the ortho, meta, or para positions. To date it has not been possible to predict physiological activity by study of the molecular configuration alone — biological assay is necessary. The study of configuration in relation to activity, however, has helped to locate a large number of active compounds. Table 25 shows a long list of phenoxy acids in relation to activity and inactivity for cell elongation and formative influences. Table 27 [62] shows a similar list of xylenoxy acids, and Table 28 derivatives of benzoic acid.

Table 28. Active and Inactive Derivatives of Benzoic Acid

Substances	Cell elongation	Formative effects
Benzoic acid	Inactive	Inactive
2-Iodobenzoic acid	Inactive	Inactive
3-Iodobenzoic acid	Inactive	Inactive
4-Iodobenzoic acid	Inactive	Inactive
2,4-Diiodobenzoic acid	Inactive	Inactive
3,5-Diiodobenzoic acid	Inactive	Active
2-Bromo-3-nitrobenzoic acid	Active	Active
2-Chloro-5-nitrobenzoic acid	Inactive	Active
2-Amino-5-chlorobenzoic acid	Inactive	Inactive
2,3,5-Triiodobenzoic acid	Inactive	Active
2-Chloro-3,5-diiodobenzoic acid	Inactive	Active
2-Amino-3,5-diiodobenzoic acid	Inactive	Inactive

The formative influence of derivatives of benzoic acid [59] is illustrated in Fig. 83. Some of these compounds modify the flowering habit and change the correlation of organs.

In addition to the nature of the molecule, the constitution of the receptor tissue in the plant is important. First, the genetic constitution of the tissue plays an important part, and secondly, the location in the organ and the age of the tissue are determining factors.

Though in the same family group, tomato and potato tissue do not respond alike to a given substance, due perhaps to the difference in their genetic constitution. Apple and lilac stem cuttings can be induced through chemical treatment to produce adventitious roots in the spring of the year, but not in autumn or winter. Though the tissue is receptive at an early age, the capacity to respond to the chemicals is soon lost. With still other species young tissue does not respond to chemical treatment, whereas older tissue is susceptible. Many other illustrations could be given to indicate that there are complex internal and external influences playing upon the living protoplasm and that the sum total of these regulates the growth and development of the plant.

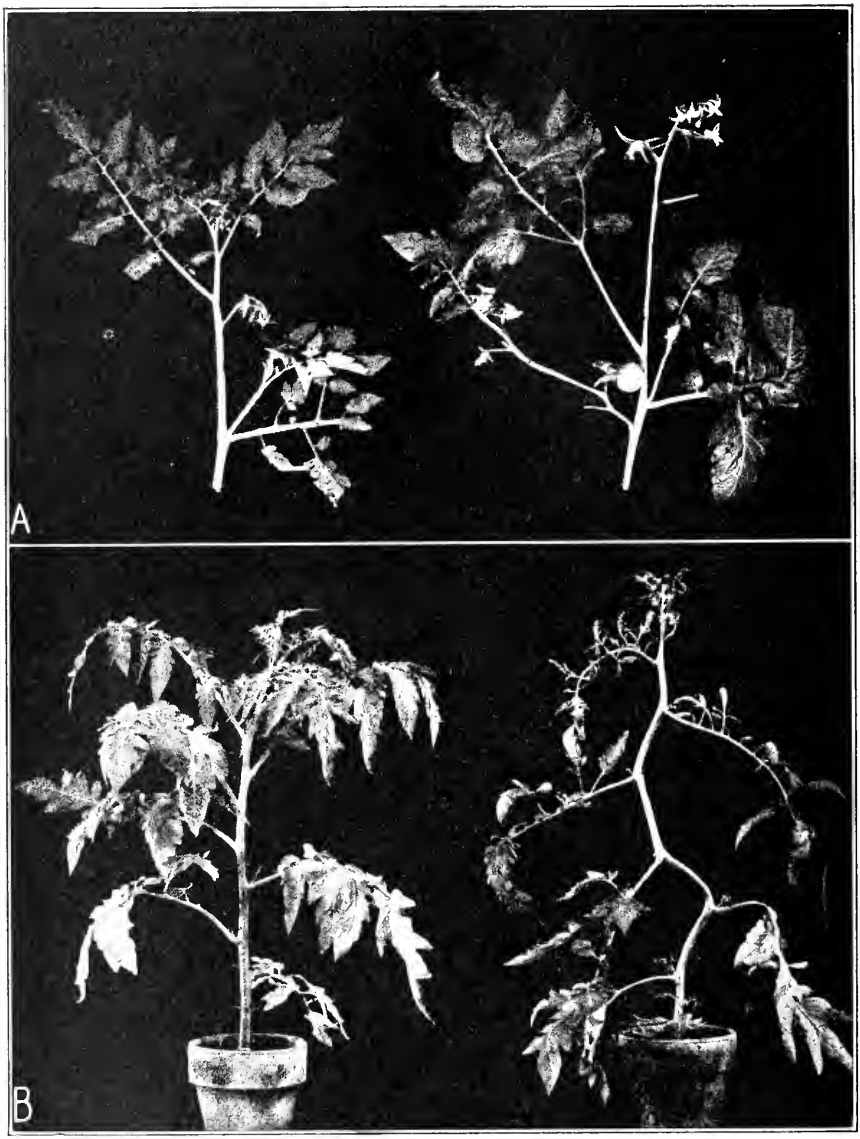


FIGURE 83. Tomato shoots and plants to show the effect of 2,3,5-triiodobenzoic acid on configuration, flowering habit, and correlation of organs. A, *Left:* control; *right:* upper portion of main shoot of plant 51 days after 1.25 mg of the substance had been added to the soil. Note that the terminal shoot ended in a flower cluster with an abnormal pedicel. Arrow points to node-like structure. B, *Left:* normal plant; *right:* plant showing response to the substance 21 days after 5 mg had been added to the soil. The plant was approximately 6 inches in height when treated. Note curvature of stem at nodes and modification of new leaves which were formed after treatment.

There are ample facts to prove the existence of formative influences exerted by hormone-like substances, but we are far from understanding the mechanism through which the chemicals act. It seems logical to assume that the nucleus is the stable element, and that the "normal" form of the plant results from this stable element acting in combination with the "complex influences" from the cytoplasm. If one considers the cytoplasmic influence the less stable, then it may be assumed that the modifications in form may be brought about by the action of a chemical environment upon the cytoplasm. Each chemical constitutes a different environment and therefore permits different potentialities of the protoplasm to develop.[44, 46]

Other Subjects of Interest Involving Plant Hormones

Only brief discussions are included. If details are needed, the citations will be helpful.

Absorption and movement of growth substances. The controversial issues on direction of movement of hormones were somewhat moderated when it was demonstrated that roots can absorb growth substances from the soil.[16] The fact that the active substances were absorbed and translocated throughout the plant was indicated by the response of the aerial parts. The rate at which the substances were absorbed and moved in the plant varied with the rate of transpiration, increasing with increasing transpiration. Applied at the tip of the stem by means of sprays or lanolin preparations, the substances moved downward, causing a systemic response. When applied to the middle of the stem the substances caused a response above and below the treated region. In short, it was shown that growth substances can move in all directions through the plant.

Light and dark effects. Young growing tomato plants maintain their power to respond to growth substances while in light and for a period of hours or days (depending on size and age) when transferred to a dark room. They first lose their capacity to right themselves while in darkness, when placed in a horizontal position. When this condition first occurs, the plants may use synthetic substances to bring about tropic responses. Later, however, young plants lose their power to respond to either natural hormones or synthetic substances. Plants attached to storage organs (potatoes, Jerusalem artichoke, etc.) or having storage tissue in the pith (tobacco) maintained their power to respond to growth substances for more than 40 days after being placed in a dark room.[50]

Tropic curvatures. Natural tropic curvatures, such as bending of the stem toward light or away from the earth, are assumed to be due to unequal distribution of the natural hormones.[52, 53] Stems treated unilaterally with preparations of growth substances underwent pronounced negative bending when in darkness or when light came from all directions. If, however, the plants were allowed to make natural tropic responses before the synthetic growth substances were applied, the final responses were greatly modified. When the substance was applied opposite the side that natural

hormones were assumed to accumulate, the degree of curvature was greatly reduced; when they were applied on the other side, the curvature was greatly increased.

Anesthesia. In Chapter 4 (p. 150) there is a discussion of ethylene-induced anesthesia in plants. It need only be mentioned here that a condition can be induced with growth substances which in many respects resembles ethylene-induced anesthesia. The most effective growth substances for anesthesia are α-naphthaleneacetic, β-naphthoxyacetic, β-naphthoxypropionic, 2,4-dichlorophenoxyacetic, 2,4,5-trichlorophenoxyacetic, and α-(2,4,5-trichlorophenoxy)-propionic acids.

Activation of cinnamic acid with ultraviolet light. *Trans*-cinnamic acid is not an active growth substance. If, however, it is exposed to ultraviolet light, an active substance results. The light changes the *trans* to the *cis* form, which is active. During the investigation of this subject it was found that the vapors of the substance were active and induced responses similar to ethylene.[55] The vapor method has since become of considerable importance.

Natural influences. Evidence continues to accumulate to show that the growing plant produces natural hormones and that these vary with environment and orientation of the plant organs. Potted plants which were placed in a horizontal position gave a pronounced geotropic response. When the pots were again set upright, the stems recovered from the first response but the leaves developed epinasty.[52] The final effect was as if synthetic hormones had been applied.

Unusual physiological responses were also induced on intact plants by capping with black cloth.[18] Capping the tomato plant for 3 to 14 days is assumed to bring about an over-production of natural hormones which causes epinasty of leaves, swelling of stems, proliferations (including intumescences), inhibition of growth, initiation of roots, and a disturbance of correlation of organs.

Comparative effectiveness of acids, esters, and salts. The part of the molecule which brings about a physiological response is still unknown. It may be the entire molecule or one of its component parts. It has been determined, however, that the application of acids, esters, and salts brings about similar results. For solutions it is convenient to use acid and salts, while for vapor treatment it is best to use the esters, due to their high vapor pressure.[51]

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CHAPTER 7

Dormancy in Buds

A rest period is general for buds of tubers, bulbs, and woody plants. This is especially true of temperate-zone plants. The duration of dormancy in buds of potato tubers varies from 9 to 12 weeks, depending on the variety, when stored at air temperatures. It is not shortened by storage at low temperatures but is shortened [51] considerably by storage at 35° C (95° F), as well as by storage with high moisture.[72] Low-temperature storage is very significant in shortening the rest periods of buds of many bulbs, such as the gladiolus. It is also important in inducing flowering in bulbs and in certain biennial [2] and perennial plants. After having plants of *Crassula rubicunda* about the greenhouse for years without flower production, Arthur induced profuse flowering by exposing the plants to low temperature for a few months. Flower induction by low-temperature exposures has been termed [2] thermoperiodism. When seeds are made to produce plants that flower earlier by exposing them to low temperatures the process is referred to as yarovization or vernalization. The physiological changes involved in thermoperiodism and yarovization are probably similar. Buds of temperate-zone trees and shrubs go into dormancy in late summer and are thrown out of this condition by the cool weather of fall, winter, and spring. As in low-temperature after-ripening of seeds, temperatures between 0° and 10° C (32° and 50° F) are effective [5] for buds of various kinds of trees and shrubs, but higher temperatures and temperatures below freezing are not.

Dormancy in Potato Buds

Attempts have been made to force dormant buds by the use of anesthetics [68, 69, 70] without great success. As we have already seen, ethylene and certain plant hormones which are good anesthetics inhibit bud growth, at least as long as they are in contact with the buds. McCallum [52] found ethyl bromide especially effective in overcoming dormancy of potato buds. He exposed the tubers to 1 to 2 cc of the chemical for 24 hours in a 5-liter air-tight jar. Appleman [1] found that buds of potato tubers grew more rapidly if the tubers were kept moist. He concluded that moisture prevented the development of the corky periderm which reduced the oxygen supply to the buds. Thornton,[72] in a paper that was awarded the A. Cressy Morrison Prize as an outstanding paper in biology in 1938 by the New York Academy of Sciences, confirmed Appleman's result that potato

buds lose their dormancy quicker in the moist than in the dry condition, but gave quite the opposite interpretation. He found by anatomical studies that moisture favored the rapid development of periderm which, in turn, cut down the oxygen supply to the buds and favored growth; and that freshly harvested tubers germinated rather promptly under 5 to 10 per cent oxygen pressure rather than at normal pressure. Peeling the tubers about the eyes shortened the rest period because it led to the rapid development of effective periderm. Two to 10 per cent of oxygen eliminated apical dominance, so that several buds grew from each eye instead of one bud from one of the apical or, seed end, eyes. Increasing the oxygen above 20 per cent prolonged the dormant period. This is one of few cases on record where reducing oxygen pressure below the normal favors growth of flowering plants. Certain seeds are favored in germination by reduced oxygen pressure. Perhaps storage at 35° C (95° F) [51] hastens after-ripening of potato tubers by increasing the development of corky periderm.

About the time the Institute opened we had an inquiry from Bermuda for some means of throwing freshly dug potatoes into immediate growth. Bermuda had been using seed grown in Long Island, which had been harvested early enough in the summer to after-ripen before planting time in Bermuda in early October. This seed was unsatisfactory; it became infected with virus diseases during the season due to the abundance of insect vectors on Long Island. It was estimated that the virus diseases carried in the tubers reduced the yield about 50 per cent. Northern-grown seed could be obtained which was practically free from leaf roll and mosaic. However, it was harvested in September and was not ready to grow for some weeks after planting time in Bermuda.

The Institute decided to organize a project to study dormancy in buds much as it was studying dormancy in seeds. Dr. F. E. Denny, who had recently developed the ethylene method of coloring citrus fruits, was asked to head the project. Later a grant from the Herman Frasch Foundation for Research in Agricultural Chemistry enabled the Institute to add two well-trained biochemists, Drs. John D. Guthrie and L. P. Miller, as associates on the project. These three scientists, with assistants, carried on the project under the Frasch grant for eleven years, and it has been continued with a slight change in the staff for nine additional years. As a result of these studies some very effective chemicals have been found for throwing buds out of dormancy and for inducing or maintaining dormancy. Much has been learned about the metabolic changes brought about by the dormancy-modifying chemicals in buds and the plants to which the buds are attached. In this chapter we can cover only a few of the points of more general interest. The details are published in more than seventy-five different articles.

Chemicals That Force Dormant Buds

Ethylene chlorhydrin and thiocyanates. In his original study of the effect of 224 different chemicals for forcing dormant potato buds, Denny [6, 8]

found two chemicals, ethylene chlorhydrin ($ClCH_2CH_2OH$) and thiocyanates (K, Na, or NH_4) that are very effective in throwing potato buds into prompt growth. The first proved especially desirable because there is a wide margin between forcing dosage and toxic dosage; moreover, as it

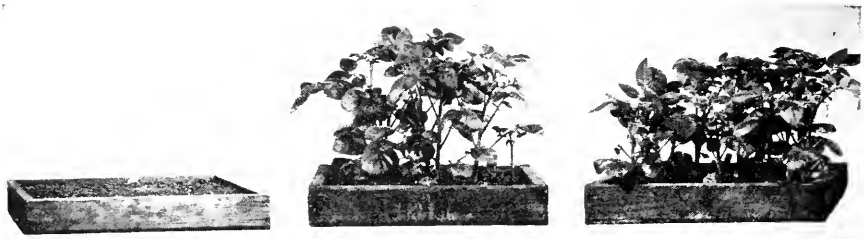

FIGURE 84. Effect of dip treatment of potatoes with 40 per cent ethylene chlorhydrin after dipping tubers left in a closed container 22 hrs. *Left to right:* check, dipped in H_2O; dipping solution, 15 cc per liter; dipping solution 30 cc per liter.

is soluble in water and has a high vapor pressure, it can be used either for dip or vapor treatment. The thiocyanates have a narrow margin of dosage and must be used by a soak treatment. Fig. 84 shows the effect of ethylene chlorhydrin and Fig. 85 the effect of sodium thiocyanate upon the growth of dormant potato tubers. These treatments completely eliminate the need of the rest period, that is, the dormant tubers treated with the chemicals grow with as high vigor and apparently give as high crop yields as time-after-ripened tubers; unlike the hormone-like chemicals discussed in previous chapters, they do not produce any formative modifications in the resulting plants, such as partial or complete anesthesia, extra root formation, modification of leaves and stems, and parthenocarpy. They merely activate the normal growth of dormant buds.

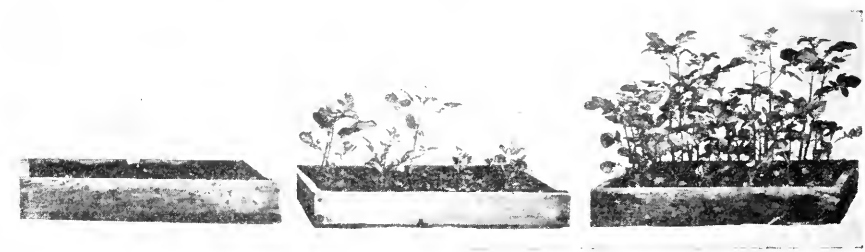

FIGURE 85. Effect of sodium thiocyanate solution upon the germination of freshly harvested potato tubers. Cut tubers soaked one hour. *Left to right:* check (H_2O); ¼ per cent; 1 per cent.

By treatment of dormant tubers just after harvest, new sizable tubers can be produced before dormant untreated tubers have germinated (Fig. 86), so that several crops of potatoes can be produced in a year. This has been very useful to potato breeders, who can hurry tuber multiplication by eliminating the rest period and to plant pathologists who are studying potato

diseases, especially those carried in the tubers. It is now common practice to force northern-grown seed potatoes in the greenhouse during the winter to determine whether the seed is virus-free. This gives a preview of the next year's crop. This control of tuber dormancy is also useful in practice, not merely because it enables southern growers of the fall crop to produce

FIGURE 86. Second crop of potato tubers in same year in Institute gardens from tubers treated with ethylene chlorhydrin. (*Left*) Check lot not treated.

from northern-grown disease-free tubers, but also to produce a second crop from the small tubers of a first crop.

Many other chemicals were found more or less effective in forcing dormant potato tubers, but ethylene chlorhydrin and the thiocyanates showed advantages over the others for one or more of the following reasons: high effectiveness in forcing; wide margin between forcing and killing dosages; or cost of the chemical for treatment. Among the other chemicals that Denny early found to be more or less effective were di- and trichloroethylene,

carbon disulphide, ethylene dichloride, xylene, and ethyl bromide. The last was very effective, as McCallum found, but it is rather expensive and must be used as a vapor, since it is not soluble in water. Later, Miller [54, 55] found a number of sulphur compounds more or less effective in breaking the dormancy of potato tubers: ammonium dithiocarbamate, thiosemicarbazide, hydrogen sulphide, ethyl mercaptan, ethyl disulphide, and

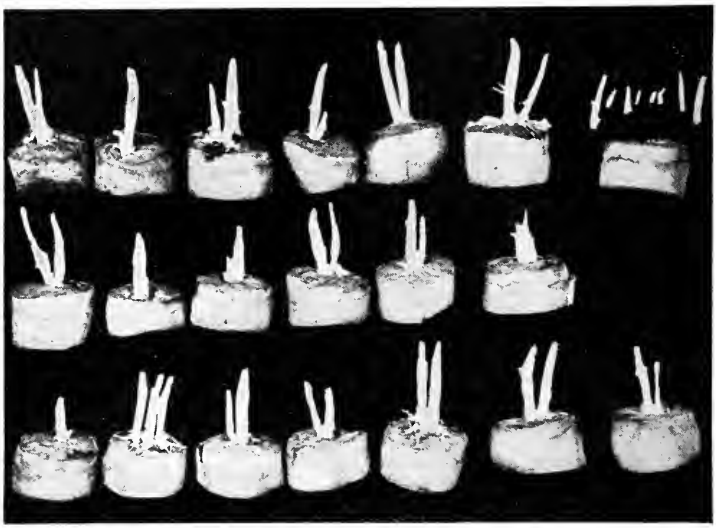

FIGURE 87. Individual eyes of Bliss Triumph potato tubers cut out with a large-sized cork borer and later soaked one hour in 3 per cent thiourea solution. Note that chemical forced the growth of several buds from most of the eyes.

several others. Guthrie [42] synthesized what he believed was thiocyanohydrin ($CH_2SCN \cdot CH_2OH$), a compound resembling both of the very effective compounds mentioned above, and found it effective in breaking tuber dormancy. Ethyl carbylamine and glutathione [45, 46] were also effective. Thornton [71] found 10 to 60 per cent of carbon dioxide in combination with 21 or higher percentages of oxygen effective in forcing dormant buds of potatoes. Since carbon dioxide was effective with normal or higher than normal oxygen, the action of carbon dioxide was not due to anaerobiosis. None of these compounds shows advantages that would lead them to displace ethylene chlorhydrin and the thiocyanates as bud forcers. Denny [25] has also found a combination treatment with ethylene chlorhydrin followed by thiocyanate of advantage in greenhouse tests for virus in potato tubers. This gives approximately 100 per cent germination, which is necessary in these tests.

Thiourea as a bud forcer. Thiourea has proved to be a fair bud-forcing chemical, but it differs from the other effective chemicals in that it breaks up the growth correlation between the several buds of an eye and between the eyes in a seed piece. In normally after-ripened tubers and in dormant

tubers treated with ethylene chlorhydrin or thiocyanates only one bud generally grows from one apical eye of the seed piece. Thiourea may force the growth of all the bud primordia in an eye. This may be as high as eight. Fig. 87 shows the effectiveness of thiourea [7] in inducing the growth of several buds from a single potato eye. Fig. 88 indicates the effect of thiourea in breaking up the correlation between the several eyes of the

FIGURE 88. Seed pieces of non-dormant Early Ohio potato. *Top row:* soaked in 2 per cent thiourea one hour before planting. *Bottom row:* soaked in water one hour before planting. Note that thiourea forced the growth of buds from eyes throughout the length of the seed piece and that water induced growth of only one bud from an eye at the seed end.

potato, so that eyes all along the seed piece from the seed end to the stem end produce multiple sprouts. We have noted above that reduced oxygen pressures [72] had similar effects on eye and bud correlations in potato tubers. In western Nebraska and in Colorado the potato tubers grow too large for economical use as seed. Treating the seed pieces with the proper dosage of thiourea gives more than one stem from the seed piece with a correspondingly greater number of smaller tubers per hill, but with the same yield per acre. In this way treatment of tubers with thiourea has proved of service to seed-potato growers.

Thiourea as an antioxidant. Denny observed that the cut surfaces of seed pieces of potatoes treated with thiourea remained white for a long time after they were planted, whereas cut surfaces of seed pieces treated with other bud-forcing chemicals, or not treated, turned brown very readily. A further study showed that thiourea interfered with the oxidation system in the tuber that produced browning. Denny [14, 20, 24] concluded that it was the peroxide in the system that was inactivated or destroyed

rather than the peroxidase. Recent work [33] indicates that thiourea also inactivates the oxidase system in fruits that destroys vitamin C.

Fig. 89 shows the effectiveness of thiourea in preventing the browning of sliced apples and apple juice. If the thiourea is placed in the juice immediately upon pressing, no browning occurs; also the early browning of the juice is completely or almost completely reversed if the thiourea is added within an hour after pressing, but the later oxidation in the juice is irreversible. The amount of thiourea required to prevent browning is very small. Sliced fruit is dipped momentarily in a 0.1 per cent solution and then drained, after which each pound of fruit retains about 0.027 gram of thiourea. If used to treat the juices, the thiourea content is less than 0.05 gram per pound. The same treatment is quite as effective in preventing sliced apricots, bananas, nectarines, peaches, pears, and plums from browning. In peaches a 0.05 per cent solution is effective. It has been suggested that thiourea dip be substituted for sulphur dioxide treatment for drying, canning, and quick-freezing of fruits. Thiourea is also excellent for treating sliced fruits for salads and desserts.

Thiourea [32] has lower acute toxicity dosage for mammals than table salt, and life-time feeding of mammals [48] with dosages many times that which can be consumed in treated fruit has no effect on either the weight or life span of the animals. For several years [4] it has been known that plants of the cabbage family cause thyroid enlargement, *i.e.*, are goitrogenic. It has also been found [49] that allyl thiourea, or some similar compound released from mustard oils by glucoside-splitting enzymes, causes this goitrogenic action and that various organic sulphur compounds (thiourea, thiouracil, etc.) in high doses have goitrogenic action. Astwood [3] used thiourea and thiouracil as therapeutic agents against hyperthyroidism. The initial doses of 1 to 2 grams of thiourea a day were used until the basal metabolism was reduced to the proper level and then 0.5 of a gram a day to maintain it. The effect of the thiourea ceases soon after its use is discontinued. When 1 to 2 grams per day were fed to normal individuals for 13 to 17 days, no change in metabolism was noted. Very recently [34] it has been observed that rats fed a diet containing 0.5 per cent thiourea for 12 or more days are able to endure an atmospheric pressure of 200 mm of Hg, equivalent to an altitude of 32,000 feet, for two hours, whereas the majority of those not fed thiourea were killed by a like exposure to low pressure. Leblond [50] found that rats given 1 per cent of thiourea in their drinking water for three months endured an atmospheric pressure of 100 mm of Hg much better than did the controls. These are extremely high doses. It has been suggested that aviators might be able to endure rarefied air better if given thiourea in their diet. The therapeutic and goitrogenic doses are enormous compared with the intake obtainable from eating treated fruits. Even if all the largest annual apple and peach crops the United States has ever produced were treated with thiourea to prevent browning and were all eaten by the people of the United States, each person would get on the

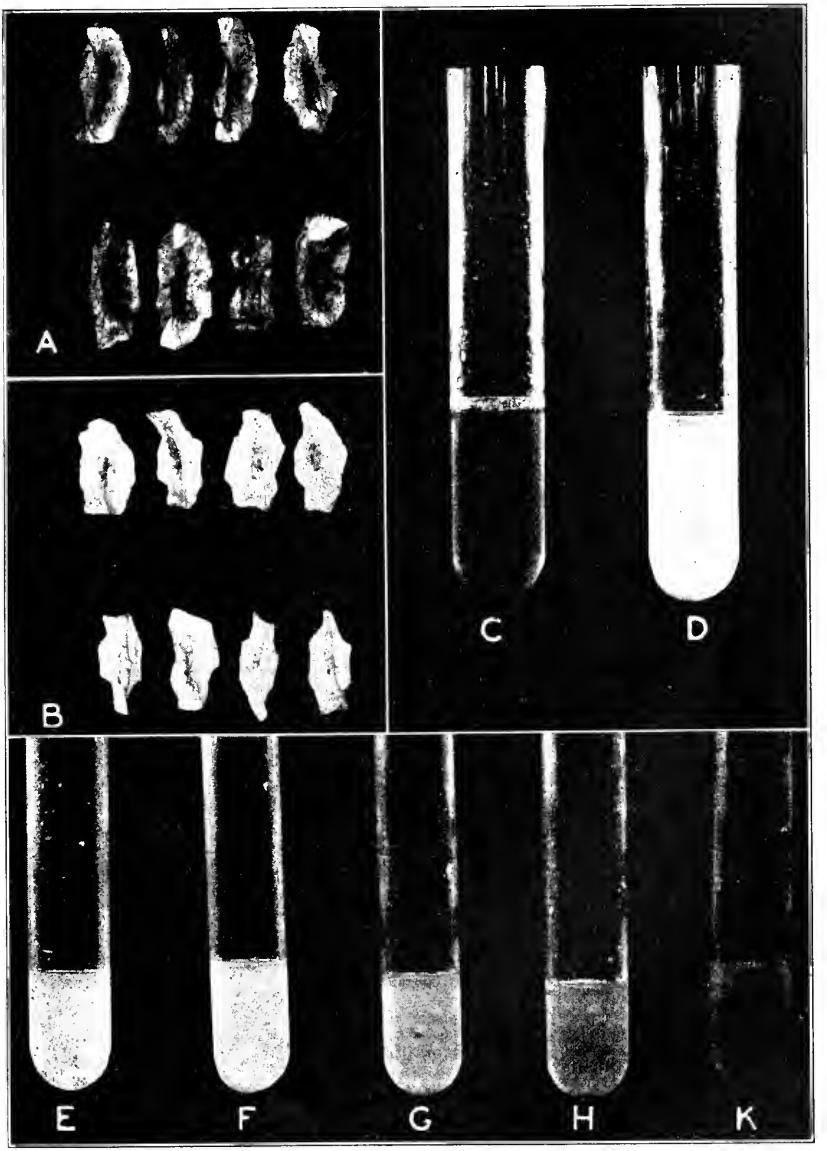

FIGURE 89. A, Slices of McIntosh apple allowed to dry in air. B, Slices of McIntosh apple soaked one minute in 0.1 per cent thiourea solution and allowed to dry in air. C, Brown apple juice, check lot. D, Brown apple juice decolorized by adding 1 mg of thiourea per 10 cc of juice. E, Thiourea added to juice at once after pressing; F, after being exposed to oxidation in air for 15 minutes before adding thiourea; G, for one hour; H, for two hours; K, for four hours. Residual liquid after decolorizing action of thiourea shows increasing amounts of an unreducible pigment as duration of the preliminary oxidation was increased.

average only 2 grams of thiourea in a year. Only a minor fraction of these crops would ever be treated with any antioxidants. The chances are poor that any individual would get as much as a gram of thiourea in a year by

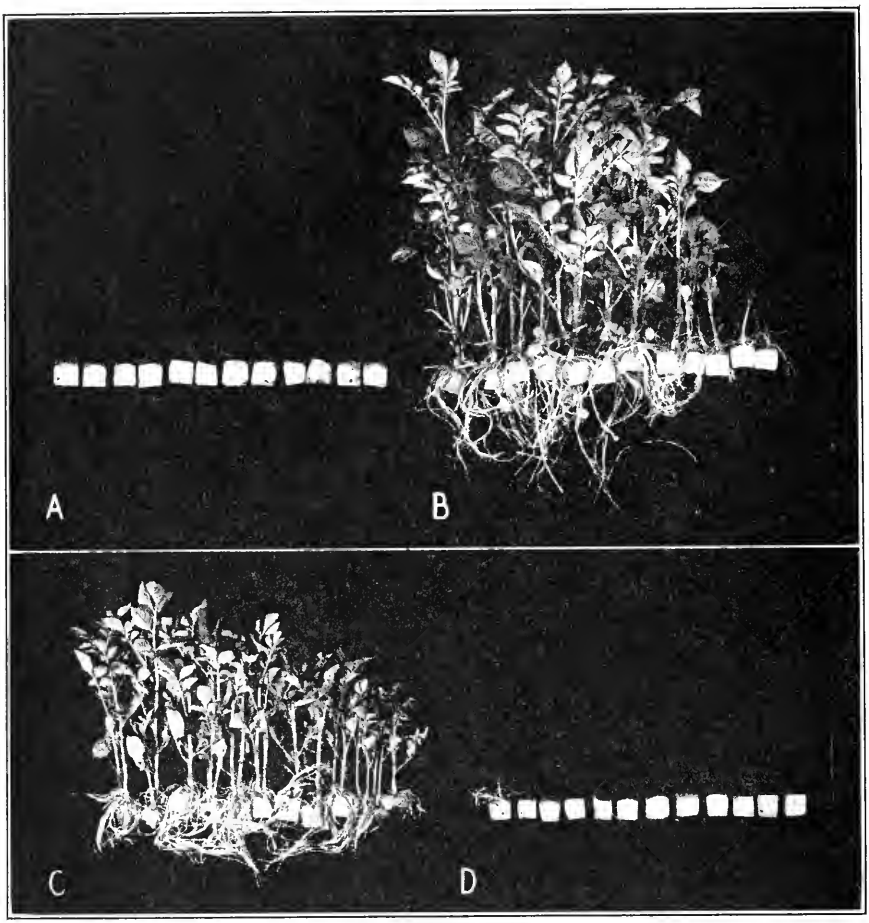

FIGURE 90. Inducing dormancy in potato tubers with the potassium salt of α-naphthaleneacetic acid and then breaking it with ethylene chlorhydrin. A, Pieces treated with the potassium salt of α-naphthaleneacetic acid, 100 mg per liter for 4 days. B, Control pieces treated with water. C, Pieces like those shown in A treated with ethylene chlorhydrin, 24 hr. dip, 25 cc of 40 per cent per liter. D, Pieces like those in A, treated with water.

eating fruit treated with it. A year's dose from treated fruit would be less than a daily dose for goitrogenic or therapeutic effects and is far below the threshold for either goitrogenic or therapeutic effects. In spite of these facts the United States Food and Drug Administration prohibits the use of thiourea as an antioxidant on fruit.

A Chemical That Inhibits Potato Buds

In previous chapters we have mentioned that ethylene and other hormone-like chemicals inhibit bud growth. Guthrie [43, 44] found α-naphthaleneacetic acid and its salts and esters very effective in inhibiting the growth of buds of the potato. Fig. 90 shows the effectiveness of the potassium salt of this acid in inhibiting potato buds. It also shows that ethylene chlorhydrin throws potato buds out of chemically induced dormancy, though perhaps not as effectively [22] as it does out of natural dormancy. With the

FIGURE 91. Irish Cobbler potato tubers stored in paper bags for five and one-half months after treatment with different dosages of the methyl ester of α-naphthaleneacetic acid applied in talc powder. Temperature varied from 10° to 16° C (50° to 60° F). Dosages (above): check without talc, check with talc only; (below, left to right): 25 ppm of weight of tuber, 50 ppm, 100 ppm of methyl ester.

use of these two chemicals, potato buds can be thrown into and out of dormancy almost at will. The methyl ester of α-naphthaleneacetic acid [22] is desirable for use for potato storage because it has sufficient vapor pressure to insure its entrance into the buds; the most efficient way to apply it to the tubers is in a talc or clay dust on the surface of the tubers at 55° F (13° C) or above so that the chemical will have sufficient vapor pressure.

Fig. 91 shows the effect of different dosages of this chemical in maintaining dormancy in potato buds. It is evident that 25 parts of the chemical to a million parts of the tubers by weight is sufficient to maintain complete dormancy. It is possible that even a lower dosage applied by this method will be effective, especially if the treatment is made at room temperature. It has been shown that only 5 ppm of the chemical is absorbed by the tubers when the dosage is 100 ppm, and of the chemical absorbed four-fifths is held in the skin. Unpublished experiments at the Institute have shown that α-naphthaleneacetic acid and its salts and esters have low toxicity for

mammals. It has been shown that tubers treated with this chemical can be stored for a year or more in bins at 10° C (50° F) or a somewhat higher temperature without sprouting or shrivelling. To prevent sprouting and shrivelling by cold storage for a year the temperature could be very little above 6° C (43° F). Such low storage temperatures are not only expensive and often unavailable but they lead to transformation of much of the starch of the tuber to soluble sugars. As we shall see later, tubers that contain considerable reducing sugars give dark brown potato chips. The world's annual potato crop is approximately seven and one-half billion bushels a year. Any improvement in storage that will give a net average saving of one cent a bushel is worth seventy-five million dollars a year.

Dormancy of Gladiolus Corms and Cormels

Forcing Dormant Corms by Chemical Treatment and Temperature Storage

Corms of some varieties (Souvenir, Maiden's Blush, and Alice Tiplady) of gladiolus [9, 15, 17] could be successfully forced one week after harvest with ethylene chlorhydrin vapors. The dosages were 3 to 4 cc of 40 per cent solution per liter of the enclosure containing the corms and the exposure was two to four days. Ethylene and ethyl ether were not effective. Corms of the Halley variety did not respond to the treatment until one month after harvest; and the corms of Remembrance did not respond to chemical treatment at any time after harvest, but after-ripened with sufficient period of storage without treatment. To insure good flowering of fall-forced corms natural light may have to be supplemented with artificial light. In many varieties the chemical treatment was successful only after a cold storage period of three to six weeks at 5° C (41° F). High-temperature storage (30° to 35° C, 86° to 95° F) was not effective if applied immediately after harvest, but had good forcing action for some varieties after the corms had been kept at room temperature for 52 days before they were transferred to high temperatures. Because of the great variation in behavior of corms of different varieties, both as to depth of dormancy and factors that overcome dormancy, it is evident that each variety must be studied separately to determine the best forcing methods. In some varieties chemical treatment is effective soon after harvest; in others it is effective only after a period at proper storage temperatures, and in still others proper storage alone is the best method of forcing. The attempts at chemical forcing of corms to date should not be considered final. It is possible that other chemicals can be used in combination with ethylene chlorhydrin or still other chemicals found that will give perfect forcing soon after harvest.

Ethylene chlorhydrin treatment [12, 47] increased peroxidase, catalase, pH, sulphydryl, soluble organic nitrogen, and sucrose and decreased the reducing sugar of the corms. Most of these changes occurred regardless of the dormancy of the corms, and there is no evidence that any of them hold a causal relation to the breaking of dormancy.

Maintaining Gladiolus Corms in the Dormant Condition

Gladiolus corms can be kept sound and in the dormant condition [18] for 18 months or more, depending upon the variety, by storing the freshly harvested corms in moist soil at room temperature or preferably at 27° C (80° F). Corms stored in this condition have a very low rate of respiration [19, 73] and consequently use up stored foods very slowly, which permits of long survival. When these corms are taken from the soil and placed in respirometers at the storage temperature, the respiration begins to rise within a few hours and reaches a maximum after 20 to 30 hours, after which it gradually falls back to the original low rate. The rise in respiration is 5-fold, 10-fold, 30-fold, or even larger. Such corms may be placed in soil again and continue in the dormant condition for weeks longer, the duration depending upon the state of dormancy and the variety. Corms stored in soil for longer periods, as mentioned above, are in very delicate equilibrium so far as dormancy is concerned. They grow readily when treated with ethylene chlorhydrin or after exposure to low temperatures (0° to 5° C, 32° to 41° F), for a few hours.[21] We have already seen that a brief period of chilling various dormant seeds in a germinator throws them into active growth.

Dormancy in Gladiolus Cormels

Gladiolus cormels are generally more dormant than the corms, and the depth of dormancy increases with decrease in size of the cormels. Treatment of cormels of five varieties (Alice Tiplady, America, Halley, Remembrance, and Souvenir) with ethylene chlorhydrin forces them out of dormancy.[27, 28] The greatest forcing action was not immediately after harvest

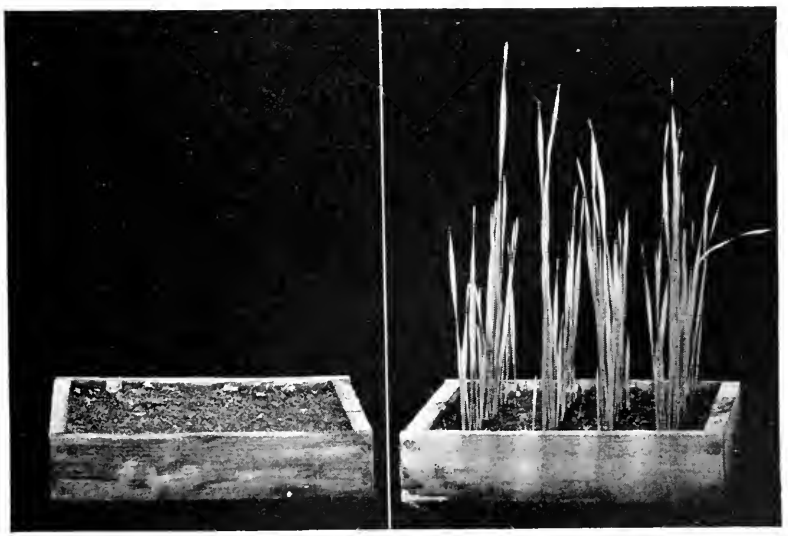

FIGURE 92. Gladiolus cormels, variety Souvenir, stored at room temperature until January. *Left:* untreated control. *Right:* 100 grams of cormels sealed in a Mason jar with 1.5 cc of 40 per cent ethylene chlorhydrin for four days before planting.

but one to three months later. Fig. 92 shows the forcing action of ethylene chlorhydrin on dormant cormels. By April cormels of many varieties after-ripen in storage and do not need chemical treatment to induce growth. Storage of cormels of these varieties at 3° to 10° C (37° to 50° F) overcame the dormancy more readily than higher storage temperatures.

Cormels of seven varieties (Giant Nymph, Mr. W. H. Phipps, Dr. E. F. Bennett, Mrs. F. C. Peters, Minuet, Willbrinck, and Golden Measure) are especially dormant.[16] When these cormels are stored at the best low temperature until May most of the cormels of each variety fail to produce plants during the summer when planted in soil without treatment. Proper ethylene chlorhydrin treatment in the spring increased the number growing by many-fold in three varieties and by 100 to 200 per cent in two varieties. Two varieties could be forced by ethylene chlorhydrin only after this long period of cold storage.

It is important to get a high percentage of germination of cormels of gladiolus in order to have them develop as quickly as possible into flowering-sized corms. Fig. 93 shows the great increase in size and number of corms

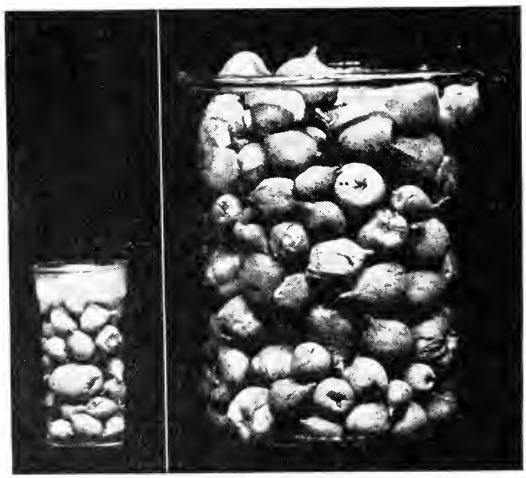

FIGURE 93. Yields obtained in the autumn from 150 gladiolus bulblets, variety Minuet, stored over winter at 15° C (59° F) and planted in the spring. *Left:* bulblets soaked 3 days in H_2O and planted. *Right:* bulblets soaked 3 days in H_2O, then exposed to vapor of ethylene chlorhydrin, using 1 cc of 40 per cent ethylene chlorhydrin per 100 g of bulblets, weight before soaking.

produced during the summer when a given number of cormels of a dormant variety are treated with ethylene chlorhydrin in the spring. This is the chief way of multiplying flowering stock of the variety. But in general the grower does not care to have them germinate until time for spring planting outside. The situation is different with dormant corms for they are often forced for winter flowers. Cormels of varieties that are only moderately dormant should be stored at a low temperature, about 5° C (41° F), until spring and planted without treatment. Cormels of very dormant varieties should be similarly stored and treated with ethylene chlorhydrin before planting in the spring. In case of new very choice varieties it is possible that by combination of temperature and chemical treatment one could get two growing periods during the year, one in the greenhouse and

another outdoors, and hasten greatly the production of flowering corms. Denny is using other chemicals in combination with ethylene chlorhydrin, seeking even more effective forcing action.

Denny [23] gives the following simple description for forcing dormant cormels: "The amount of chemical to use depends upon the amount of bulblets to be treated, *e.g.*, seven drops of the chemical per ounce, one and one-fourth teaspoonfuls per pound, or one pint per 100 lbs of bulblets. The amounts do not need to be exact, but reasonable care should be taken. For small lots, glass fruit jars with wide mouth are used as containers. The bulblets are weighed and put in the jars; several varieties may be included in one jar if the bulblets of the different varieties are tied in cheesecloth bags properly labeled. On top of the bulblets lay a small piece of paper toweling and on top of this place a piece of cheesecloth (in a loose pile) containing the right amount of the ethylene chlorhydrin. A little practice will show how large the piece of cheesecloth is required to take up the liquid without serious dripping. The piece of paper will absorb any excess drops. Then seal up the jar and let it stand at room temperature, approximately 70° to 75° F, for four days. The bulblets are then removed from the jar and are ready for planting. If the weather is unfavorable the treated bulblets may be placed in paper bags and planted when the weather is favorable. A delay of a week before planting will do no harm.

"With small quantities treated in glass jars, the distribution of the vapor to all parts of the jar during the four-day period of treatment seems to be good. If a large quantity, say 100 lbs, is to be treated in large containers such as ash-cans, it is recommended that a wire screen core be placed in the center of the ash-can with the bulblets poured into space between the can and the screen. The cheesecloth containing the ethylene chlorhydrin can then be suspended from the top of the screen into the central core space, and such a procedure will assist in getting penetration of the vapors to all parts of the can.

"After the bulblets have been soaked, the excess chemical should be rinsed off with two or three changes of water. This is to avoid over-treating and to prevent the bare hands from contact with the chemical in planting, a precaution which is probably unnecessary but which may be worth taking if large amounts of bulblets are to be planted by hand."

Forcing Dormant Buds of Deciduous Trees and Shrubs

The buds of deciduous trees and shrubs can be forced in the fall by treatment with ethylene chlorhydrin vapors, thus eliminating the necessity of a low-temperature period for after-ripening the buds. Several other chemicals (propylene chlorhydrin, ethylene dichloride, vinyl chloride, carbon tetrachloride, etc.) proved more or less effective. These, like ethylene chlorhydrin, could be applied as vapor which is desirable for treating trees and shrubs because of the difficulty involved in soak treatment. On the whole,

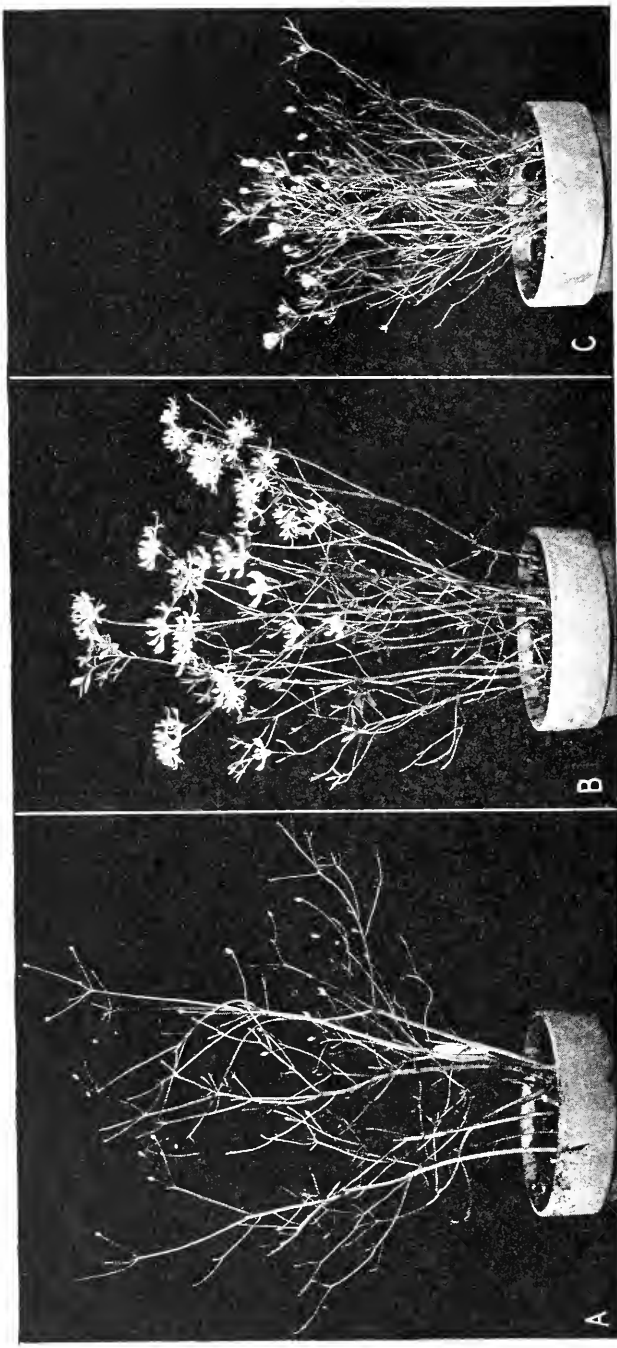

FIGURE 94. *Azalea nudiflora* L. A, Check plant, not treated; photographed January 17. B, Exposed 24 hours to vapors of ethylene chlorhydrin, 6.7 cc of the 40 per cent solution per 100 liters of space; treated December 23, photographed January 17. C, Exposed 24 hours to vapors of ethylene chlorhydrin, 0.75 cc of the 40 per cent solution per 100 liters of space; treated December 23, photographed January 17.

however, ethylene chlorhydrin proved most desirable; it is cheap, effective, and has a relatively wide dosage margin between forcing action and toxicity. Fig. 94 shows [30] the effectiveness of ethylene chlorhydrin vapors in forcing bloom in *Azalea nudiflora*. The plants are simply sealed in containers with the required amount of ethylene chlorhydrin for 24 hours and the chemical allowed to volatilize and enter the buds. From the figure it is evident that 6.7 cc of 40 per cent ethylene chlorhydrin per 100 liters of

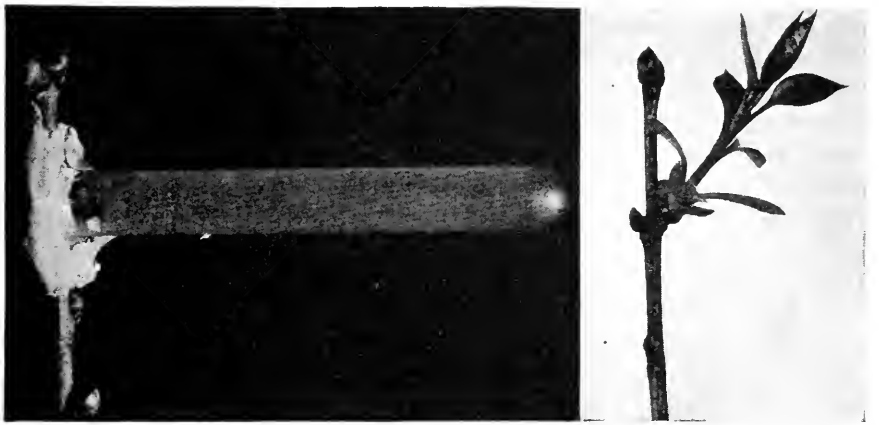

FIGURE 95. *Left:* method of treating an individual dormant lilac bud with ethylene chlorhydrin; a drop or two of the chemical is placed in the test tube and the tube sealed over the bud with modelling clay. *Right:* later growth of a bud treated in this way.

space is adequate for forcing blooming and that 0.75 cc has some forcing action. This treatment is effective in forcing both flower and foliage buds of deciduous plants. Besides azalea, lilac, flowering almond, Bechtel's crabapple, and *Deutzia* respond well to the treatment. Of those treated only the snowball, *Viburnum tomentosum*, failed to respond. It is likely that all deciduous forms can be forced by the proper concentration and time of treatment or by the combination of a cold period followed by chemical treatment. A more thorough study is needed of these and many other forms in order to make the method highly useful to practical growers. The chemical is toxic to leaves; consequently its use may be limited to deciduous forms not in foliage.

The seat of dormancy [31] seems to be in the individual buds rather than in the plant as a whole. Fig. 95 shows the method of treating an individual dormant bud of lilac and the later growth of the treated bud. (Note that the effect of the treatment is strictly local; the opposite bud on the same stem one-fourth of an inch away remains dormant.) The roots and stems of the plant are not dormant but are able to furnish the bud the necessary water and other nutrients at any time that the bud is out of dormancy and ready to grow. As we have already mentioned under *Dormancy in seeds*, buds that are only partially after-ripened due to insufficient period

of low-temperature exposure grow with low vigor or are dwarfish. This may happen if the forcing chemical is used in too low a dosage, but if the chemical is used in sufficient dosage the vigor of growth is the same as that induced by adequate low-temperature exposure.

Various physiological and chemical changes [26] were induced in dormant lilac buds by optimum forcing dosages of ethylene chlorhydrin. There was a marked increase in catalase, and an increase in water content and soluble nitrogen compounds, but no significant change in amylase. There was marked increase in respiration ranging from 20 to 100 per cent from early to later stages. These changes were greater in the buds than in the twigs.

It is possible that even more effective bud-forcing methods may be found by mixing other effective chemicals with ethylene chlorhydrin. This is especially hopeful if the mixtures show more than additive or synergistic effect. Denny is investigating this possibility. In the case of trees and shrubs it is desirable to find effective chemicals that can be added to the soil and reach the buds through the roots and stems. It is possible that if such chemicals can be found, buds of evergreens can be forced without foliar injury. This is a good deal to hope for, since the leaves have rapid transpiration and may accumulate more of the chemical than the dormant buds.

Metabolic Changes Induced by Chemicals That Force Dormant Potato Buds

Extensive studies were made of the effects of bud-forcing chemicals upon the metabolism of potato tubers. These studies were directed at answering two questions. In the soak treatment the seed pieces absorbed large quantities of ethylene chlorhydrin, and upon exposure of the seed pieces to the air the chemical disappeared from them faster than could be accounted for by evaporation alone. Explaining the disappearance of ethylene chlorhydrin led to the discovery that not only this but several other foreign and more or less toxic chemicals, when absorbed by plants, are tied up with glucose to form glucosides or with other sugars to form other glycosides which are, on the whole, less toxic to plants than the chemicals themselves. The second object of the metabolic studies was to see whether some metabolic change or changes brought about by the several forcing chemicals would explain why the chemicals changed the buds from the dormant to the active conditions. While no definite positive answer was gained for this question, since various forcing chemicals showed opposite effects on such basic processes as respiration, the studies as a whole added much to our knowledge of plant metabolism and the modification of metabolism by chemicals.

Plants Transform Certain Toxic Foreign Chemicals into Less Toxic Glycosides

Miller [59] early showed that the disappearance of ethylene chlorhydrin in treated potato tubers was due in part to the chemical being transformed

to a β-glucoside by the living tubers. Extracted juice did not form the glucoside. He found later [60] that corms of gladiolus when treated with ethylene chlorhydrin likewise transformed the chemical to a glucoside and identified the glucoside as β-(2-chloroethyl)-*d*-glucoside. This [61] was proved identical with the glucoside formed in the potato tuber. Wheat plants [65] furnished with ethylene chlorhydrin in nutrient solution synthesized the same glucoside.

When gladiolus corms [62] were exposed to the vapor of *o*-chlorophenol, much of the chemical was absorbed and later transformed by the corm into β-*o*-chlorophenyl-gentiobioside. In this case the chemical was tied up with a disaccharide, gentiobiose, instead of glucose. It seems probable that the chemical induces the formation of the disaccharide, gentiobiose, as well as the glycoside, since there is little if any gentiobiose in untreated corms. Tomato roots [63] respond similarly to *o*-chlorophenol. When gladiolus corms [66] were fumigated alternately with ethylene chlorhydrin and *o*-chlorophenol, both β-glucoside and β-gentiobioside were formed simultaneously in the corm. The foreign chemical, or aglycon, added determines the sugar with which it ties up. In this case the ethylene chlorhydrin was tied up as a glucoside and the *o*-chlorophenol as a gentiobioside.

When growing tomato plants [64] were supplied with trichloroethyl alcohol, chloral hydrate or chloral cyanohydrin, β-trichloroethyl-gentiobioside accumulated in both the tops and roots of the plants. The first chemical is built into the glycoside without modification; chloral hydrate is reduced before it is installed in the glycoside, and chloral cyanohydrin is first hydrolyzed and then reduced before becoming a part of the glycoside. Tomato plants normally contain little or no gentiobiose, so the synthesis of this sugar may be induced as well by the chemicals.

Not all of Miller's work on inducing the synthesis of foreign glycosides in plants by feeding them unusual chemicals can be discussed here, but one other piece of research should be described. When tobacco plants are supplied with chloral hydrate in the nutrient solution, both a β-glucoside and a β-gentiobioside are formed. The roots store up only the latter and the leaves accumulate both glycosides. An analysis of one set of leaves showed that the two glycosides constituted 13 per cent of the dry weight of the leaves. This shows the marked degree to which the organic chemical composition of plants can be modified by supplying plants with a foreign chemical. Probably further research in this direction will show that even greater accumulation of foreign compounds in plants is possible. In this work in every case the foreign chemical synthesized, so far as known, is a glycoside. Can plants be induced to synthesize other foreign chemicals, such as alkaloids, when supplied with organic chemicals not ordinarily found in them? This work suggests the possibility of further researches in this field that might be of great scientific interest and practical value.

It has long been assumed that formation of glucosides or glycosides is a means plants have for detoxication of poisonous products of their own

metabolism. The fact that foreign toxic chemicals, when supplied to living plants, are also tied up as less toxic glucosides or glycosides tends to confirm this explanation. Because there are many biologists who are allergic to teleology we have to be careful just how we word this conception. The fact is established, however, that some toxic products of metabolism and some foreign toxic chemicals, when supplied to plants, are tied up as parts of less toxic glycosides. How the plants acquired this synthetic power is another question. Did those that lacked this power commit suicide with their own poisons or by absorbing foreign poisons and those that had the power persist in spite of these poisons, or did the power to tie up self or foreign poisons into less toxic glycosides come about in some other way?

Other Metabolic Changes Caused by Bud-Forcing Chemicals

Fig. 96 shows several metabolic changes [67] brought about by treating intact dormant potato tubers with one of the very effective bud forcers,

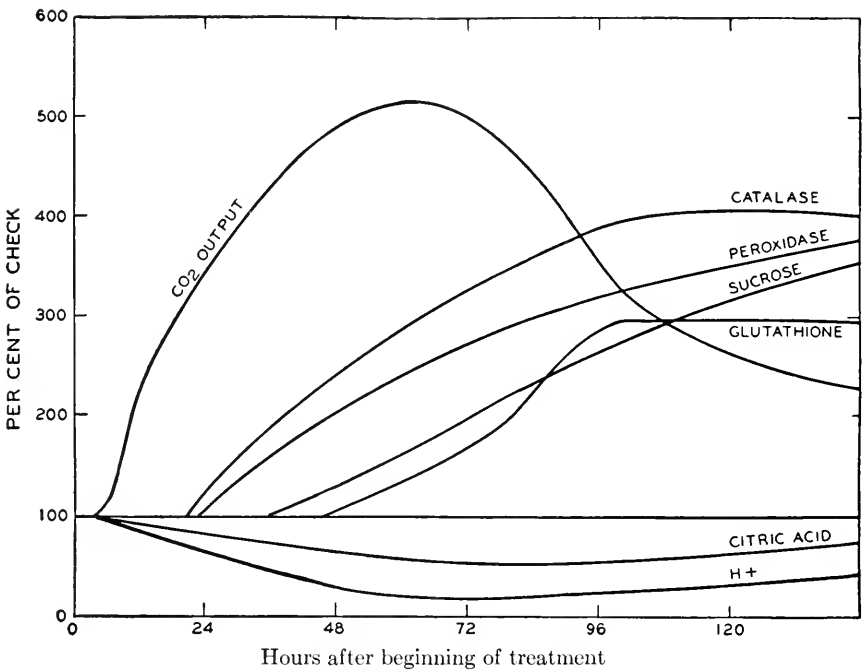

FIGURE 96. Some effects of ethylene chlorhydrin vapor on the metabolism of potato tubers.

ethylene chlorhydrin. It is evident that respiration increase as measured by carbon dioxide output is one of the earliest changes induced by ethylene chlorhydrin treatment; it is also the one showing the greatest magnitude of change, over 400 per cent of increase 65 hours after the beginning of treatment. After the maximum is reached the rate falls rather rapidly and con-

tinuously, so that after 144 hours it is about 125 per cent above the control and after a longer period it equals the control.

The decrease in citric acid and H-ion concentration [38] starts at the same time as the increase in respiration, and they reach their minimum at about the same time that respiration reaches its maximum. Citric acid is no doubt one of the substrata for respiration and its oxidation reduces the H-ion concentration.

The H-ion concentration is still further reduced by the consumption of both the sulphate and nitrate radicals,[67] probably in the synthesis of the tripeptide, glutathione. The content of these radicals begins to fall when the rise in glutathione begins and continues with the rise in glutathione, as would be expected if they were used up in the synthesis of the latter.

The two enzymes, catalase and peroxidase, begin to rise some hours after the start of the respiration rise, and continue to do so for many hours after respiration has reached its maximum. Catalase reaches its maximum before the 144th hour, while peroxidase is still rising at this hour. The increase in content of these two enzymes does not hold a causative relation to respiration rise.

The rise in sucrose content starts still later and continues at a nearly uniform rate to the end of the determinations. It is quite apparent that the respiration rate is independent of concentration of sucrose present as a substrate for oxidation, and that no positive causal relation exists between respiration intensity and sucrose content. Of course with rise in sucrose content there is a fall in starch content, but the percentage fall in the latter is rather small because starch constitutes a large percentage of the weight of the tuber.

The rise in glutathione content was the latest of the changes recorded in the curve. This rise started at the 48th hour and continued a little beyond the 96th hour, after which it remained constant at nearly 200 per cent above the check.

Metabolic Changes Induced by Ethylene Chlorhydrin Compared With Effects of Other Chemicals Including Other Bud Forcers

Respiration. It might be thought that the great increase induced by these chemicals in the fundamental process of respiration explains their bud-forcing action, but such seems not to be the case, as further facts show. Treatment of potato tubers [56, 58] with many other chemicals (ethylene bromide, hydrogen sulphide, acetaldehyde, hydrocyanic and hydrochloric acids, ethyl mercaptan, alkyl, alkylene, and alkylidene halides, etc.) shows practically the same respiration curves as does ethylene chlorhydrin. Hydrocyanic acid has only moderate bud-forcing action, and hydrochloric acid less. Methyl, ethyl, and isopropyl alcohols,[57] which have moderate bud-forcing action, decrease respiration of the tubers. When intact potato tubers are treated with vapors of both ethyl alcohol and ethylene chlorhy-

drin the vapors counteracted each other so far as the respiration is concerned. Cutting the tubers [56] into seed pieces increases the respiration rate enormously but does not force the dormant buds. Perhaps the best evidence that this early chemically-induced flush in respiration is not causally related to bud forcing is the fact that it precedes bud growth and has receded to normal considerably before bud growth begins. No doubt after bud growth begins there is a second great rise in respiration.

It has long been known that in darkness various succulents, *Bryophyllum*, cacti, etc., do not oxidize the sugars completely to carbon dioxide and water but partly to the organic acid stage; hence citric, malic, and oxalic acids accumulate in such plants during darkness. Under illumination such plants complete the oxidation of these acids. Guthrie [39] has shown that treating *Bryophyllum* leaves in darkness with ethylene chlorhydrin vapors induces them to oxidize citric and probably malic acid, in this way rendering the tissues less acid. This is similar to the effect of ethylene chlorhydrin on potato tubers.

Enzymes. Since starch is the main food storage in the potato tubers, investigators seem justified in asking whether bud-forcing chemicals are effective by increasing the activity of the amylase already present in the tuber ("direct effect"), or by increasing the amount of amylase produced by treated tubers ("indirect effect"). Possible correlations between either of these effects on amylase and the bud-forcing action of chemicals were sought. In the main, the good bud-forcing chemicals [13] did not increase the activity of either plant or animal amylases *in vitro*. Potassium thiocyanate [53] did increase the activity of animal amylase in low pH, had no effect in intermediate pH, and inhibited that action in high pH. Also hydrocyanic acid,[11] slightly effective as a bud forcer, increased the amylase activity of undialyzed potato juice. Neither of these throws any light on bud-forcing action.

Ethylene chlorhydrin treatment [11] of tubers led to a later great increase in the amylase activity of the tubers. Treatment of tubers with sodium thiocyanate, another good bud forcer, led to some increase in the amylase activity of the tubers if the tubers were not too dormant; but often dormant tubers showed no increase in amylase activity when treated with this chemical. Denny concludes that the bud-forcing action of chemicals cannot be explained either on the direct or indirect effect on amylase activity.

Dormant tubers were treated with ethylene chlorhydrin, sodium thiocyanate, and thiourea,[29] and the later effect of these treatments was determined on the catalase and peroxidase activity and reducing power of the juice of the tubers. The last was determined by the power of the juice to reduce methylene blue, indophenol, iodine, in phosphotungstic reagents. The increase in catalase and peroxidase activity began about 24 hours after the treatment, a little earlier with ethylene chlorhydrin than with the other two treatments; also the former chemical gave much greater increases than

the last two. None of the chemicals increased the enzyme activity of the extracted juice of untreated tubers, so their effect was in inducing the tubers to form more enzymes or more active enzymes. While the thiocyanate increased the formation of catalase, it inhibited its activity in the extracted juice until the chemical was partially dialyzed out. The enzyme changes were greater in the tissue nearer the eyes than in that more distant from the eyes, but the treatment of tubers with eyes removed gave some increase in the enzymes. While there was a general correlation between increased enzyme activity and the sprouting response, this correlation was not very close. Sodium thiocyanate and thiourea were much less effective in increasing enzyme activity than would be expected on the basis of the favorable sprouting response. Ethylene chlorhydrin treatment gave much greater increase in the enzyme activity in whole tubers than in cut tubers, although it forced sprouting much better in the latter.

Sugars. Potato tubers treated with ethylene chlorhydrin, thiocyanates, or thiourea [10] showed marked increases in sucrose but no significant changes in reducing sugar. Many of the other chemicals [56, 67] that brought about a great increase in respiration of potato tubers also caused marked increases in sucrose, but the latter followed the respiration increase by many hours. In many cases the sucrose of the treated tuber was lower than that of the check when the respiration was at maximum. It is evident that the rise in sucrose does not account for the breaking of the dormancy, for ethyl alcohol, which is a fair breaker of dormancy, causes a fall in respiration and little change in sucrose; and acetone, which does not break the dormancy, causes a considerable rise in sucrose.

Permeability. Freshly harvested tubers or seed pieces of tubers [36] were treated with the most effective sprout-inducing dosages of ethylene chlorhydrin, potassium thiocyanate, and thiourea, and the electrical conductivity of the tissue and the leaching of electrolytes from the tissue were later measured. Ethylene chlorhydrin treatment produced small but significant increases in the conductivity of the tissue and in the leaching of electrolytes from the tissues when placed in water. Potassium thiocyanate treatments produced changes somewhat smaller, but similar to those produced by ethylene chlorhydrin, while no significant change was produced by thiourea threatment. Here again the change in permeability induced by the three bud-forcing chemicals is not in proportion to their sprouting effects and does not furnish an adequate explanation for bud forcing.

Synthesis of glutathione and related changes. Ethylene chlorhydrin treatment [45] of potato tubers increases the glutathione content of the tubers as much as six-fold. There are two possible sources of the increased glutathione: induced hydrolysis of proteins in the tuber, and induced synthesis of the physiologically significant tripeptide. Guthrie [38, 41] thinks the second is the method of origin, for sulphuric and nitric acids decrease in the tubers parallel with glutathione increase. The two acids furnish the sulphur and nitrogen respectively necessary for the synthesis. Since more

than enough sulphuric acid disappears to account for the glutathione increase, some other sulphur compound is also synthesized.

The bud-forcing chemicals [37] that did not contain sulphur, ethylene chlorhydrin, ethyl alcohol, etc., increased glutathione, which contains bivalent sulphur, much more than do the compounds containing bivalent sulphur, thiocyanates, thiourea, etc. Hydrocyanic acid seems to be an exception. It has about the same forcing action as ethyl alcohol, but unlike the latter causes only a slight increase in glutathione. On the whole, compounds containing bivalent sulphur seem to be important in bud forcing, whether the forcing compounds contain bivalent sulphur or induce the formation of glutathione which contains it. Although it is hard to get into the protoplasm, Guthrie [45] found glutathione effective in forcing dormant buds of potatoes, pears, and peaches, and thinks it may act as one of the effective intermediate chemicals in the forcing action of ethylene chlorhydrin. Certain yeast extracts which are rich in glutathione are good bud forcers, but other chemicals in these extracts are more effective than glutathione.

The pH of the tubers treated with ethylene chlorhydrin and other non-sulphur but effective forcing compounds [57] began to rise soon after the 24-hour treatment started. This is not caused by the direct effect of the chemical, for ethylene chlorhydrin is slightly acid due to the presence of a small amount of HCl formed by hydrolysis. The fall in acidity is due to the induced consumption of citric acid by respiration and sulphuric and nitric acids in glutathione synthesis. The maximum change in pH was reached about 72 hours after the beginning of treatment. The pH change began at the surface of the tuber and worked inward. The maximum change was near the surface and the least near the center. Pieces of tubers free from eyes showed a rise in pH when treated with the chemical. In non-dormant tubers the treatment induced less change in pH. The pH rise, glutathione increase, and the increase in reducing power of the juice [35] are correlated; the pH rise was partly due to the use of sulphuric and nitric acids in glutathione synthesis, and the increased reducing power for the juice, especially for iodine in acid solution, was due in part to the increased sulphydryl in the cysteine of the glutathione molecule.

Potato tubers held for a long time in storage gradually fall in ascorbic acid (vitamin C) content. The glutathione does not show a parallel fall. Old tubers low in ascorbic acid showed a rise in this chemical after ethylene chlorhydrin treatment.[40] The rise was especially fast and the high content was maintained for a long time if the tubers were cut in pieces and exposed to air after treatment. The rise in the cut pieces did not occur if oxygen was excluded from the surface. Fresh tubers with high ascorbic acid content showed no increase in this vitamin after ethylene chlorhydrin treatment, but the treatment maintains the ascorbic acid at the high level. There is a marked increase of ascorbic acid in cut surfaces of old untreated tubers but it is maintained for only a few days. There is no correlation between rise in ascorbic acid and sprouting.

No one metabolic change induced by effective bud-forcing chemicals throws much light on the mechanics by which these chemicals initiate the growth of dormant buds. The situation is little, if any, better if one considers all the metabolic changes. Perhaps this is to be expected since the changes studied were largely in the storage organs rather than in the growing parts of the buds themselves. Another difficulty is the fact that so many metabolic changes induced by these chemicals have been found already, and there are probably many others still to be discovered. The great number of changes make it impossible to select any one that holds a causal relation to bud forcing; indeed it is a question whether any one induced metabolic change holds such a relation. This failure of the study to connect some one metabolic change with initiation of bud growth does not subtract from the value of the study. The facts learned and principles established add much valuable knowledge on plant metabolism and on the effect of chemicals upon plant metabolism. The whole study, and especially the work on the synthesis of glycosides, shows to what a degree the chemical composition of plant organs can be modified by the introduction of a foreign chemical into plant organs.

Summary

A rest period is common for buds of tubers, bulbs, and trees of the temperate zone. Buds of potato tubers remain dormant for a period of 9 to 12 weeks in ordinary storage. The period is shortened by storage at a high temperature, 35° C (95° F), and by moist storage that favors development of cork periderm. Buds of corms and cormels of gladiolus, of many bulbs, and of trees and shrubs are thrown out of dormancy by periods of low-temperature storage, 1° to 15° C (34° to 59° F). Cormels of some varieties of gladiolus do not completely after-ripen even after six or seven months of cold storage. A low-temperature period is necessary for the initiation of flower buds in many plants or plant organs.

In the researches reported in this chapter several chemicals were discovered that throw buds out of dormancy. Ethylene chlorhydrin proves especially desirable in practice; as it is soluble in water and has a high vapor pressure, it can be used either for dip treatment or for vapor treatment. It has a rather wide margin of dosage between forcing and toxic action. Sodium, potassium, and ammonium thiocyanates in water solution are also effective as bud forcers for potato tubers. These must be used for soak treatment because of lack of vapor pressure.

Thiourea in water solution proved fairly effective as a bud forcer. It shows peculiar effects in that it breaks up the growth correlations between the several buds in an eye or the several eyes in a seed piece or whole tuber. As a result, thiourea treatment of potato tubers causes several buds to grow in each of several eyes instead of one bud from an apical eye, as occurs in storage-after-ripened tubers, or tubers treated with other bud-forcing chemicals.

Thiourea prevents the browning of cut surfaces of fruits and other plant organs by inactivating the peroxide of the tissue. One dip of the cut organs in a 0.05 to 0.1 per cent solution is sufficient to prevent the browning of fruits for drying and freezing, and for salads and sliced desserts. In high dosages thiourea has goitrogenic action and reduces hyperthyroidism, but the dosages that can be consumed in treated fruit are very far below these therapeutic doses. The lethal dose of thiourea for mammals is even higher than that of table salt.

Methyl ester of α-naphthaleneacetic acid proved very effective in inhibiting the growth of potato buds. Tubers treated with 25 mg of this ester applied in talc powder to a kilogram of tubers inhibited the growth of the buds so the tubers could be stored at 10° C (50° F) or even higher for more than a year without sprouting or shrivelling. In the fully inhibiting dosage the tubers absorb about five-millionths of their weight of the chemical and four-fifths of the chemical absorbed is held by the skin. The chemical has a low order of toxicity for mammals. This discovery should prove of great value in farm and commercial storage of potatoes.

After two to three weeks of cold storage, dormant gladiolus corms can be forced by ethylene chlorhydrin vapor treatment. The cormels are more dormant, but many varieties are fully after-ripened by storage at 5° C (41° F) during the winter. The cormels of other varieties are more dormant and, in addition to low-temperature storage during the winter, require ethylene chlorhydrin treatment for a high percentage of germination. The production of corms of desirable dormant new varieties can be greatly accelerated by ethylene chlorhydrin forcing of cormels.

Corms of gladiolus can be held dormant and in good condition for eighteen months or more if immediately after harvest they are placed and kept in moist soil at room temperature or preferably 27° C (80° F). Such corms are thrown into active growth by a few hours' exposure to 5° C (41° F).

Buds of dormant deciduous trees and shrubs can be thrown into vigorous growth in the fall by treatment with vapors of ethylene chlorhydrin, alkyl halides, and other volatile chemicals. If only one bud is exposed to the effective chemical it alone grows, showing that the dormancy dwells in the individual buds.

Even more effective bud forcers are being sought by using other chemicals in combination with ethylene chlorhydrin in the hope of synergistic action of the chemicals.

Ethylene chlorhydrin disappeared from potato tubers faster than could be accounted for by evaporation alone. It is tied with glucose in the potato tuber and gladiolus corm, forming a less toxic glucoside. Several different foreign more or less toxic chemicals, when supplied to plants, are tied up with various sugars forming less toxic glycosides. This seems to be a means that plants have of rendering innocuous poisonous products of their own metabolism and certain foreign poisonous chemicals when absorbed. When tobacco plants were furnished chloral hydrate in the nutrient solution they

synthesized glycosides in sufficient amounts to constitute 13 per cent of the dry weight of the leaves. Foreign chemicals can induce plants to change their chemical composition to a marked degree.

Treating of potato tubers with ethylene chlorhydrin and many other bud forcers, as well as some chemicals that do not force buds, cause an early many-fold increase in respiration. Methyl, ethyl, and isopropyl alcohols decreased respiration but showed some bud-forcing action. Citric acid was used as an important substratum for respiration increase so that a fall in acidity accompanied the great rise in respiration. Treatment of *Bryophyllum* leaves with ethylene chlorhydrin causes them to oxidize the citric and probably malic acids in darkness, rendering the leaves less acid. Ordinarily light causes the oxidation of these acids. Many of the chemicals cause a much later and marked increase in the sucrose content of the tubers but little change in reducing sugars. Some chemicals that are not effective as bud forcers cause an increase in sucrose.

Ethylene chlorhydrin in the main did not affect the activity of amylase, catalase, peroxidase, or reducing power of extracted juices of potato tubers. It did induce the formation of more of these enzymes in treated tubers. The thiocyanates and thiourea did not induce an increase in these enzymes that was commensurate with their bud-forcing action.

Ethylene chlorhydrin treatment of intact potato tubers increases the permeability of pieces sliced from treated tubers to electrolytes. Thiocyanates caused a slighter increase in permeability and thiourea induced no increase. The induced increase in permeability was not proportional to the bud-forcing action of the several chemicals.

Treatment of dormant potato tubers with ethylene chlorhydrin causes a later increase in glutathione amounting to as much as six-fold. Bud-forcing chemicals that contain bivalent sulphur cause much less increase. Moreover, the increase in glutathione caused by the several bud-forcing chemicals is not proportional to the bud-forcing action. The chemicals seem to induce the synthesis of glutathione rather than cause its accumulation through the hydrolysis of proteins of the tuber, for sulphuric and nitric acids disappear parallel with the increase in glutathione and in approximately the right proportion to account for the sulphur and nitrogen in the glutathione. A fall in acidity accompanies the rise in glutathione. This is accounted for by the use of citric acid in respiration and sulphuric and nitric acids in the synthesis of glutathione. A rise in the reducing power of the juice also accompanies the rise in glutathione. This is accounted for in part, especially in iodine reduction, by the increase in sulphydryl groups in the synthesized glutathione.

Many chemical changes are caused in plant organs by bud-forcing chemicals, as well as by chemicals that are not bud forcers, and there are no doubt many still to be found. The very multiplicity of changes, together with the fact that none of the changes brought about by the several bud-forcing and other chemicals is parallel with the bud-forcing action, means that the

metabolic changes do not throw much light on the mechanism of the bud-forcing action.

These chemically induced metabolic changes are, however, of great interest because they show in how many ways and to what degree the chemical composition of plants and plant organs can be changed by the introduction of foreign chemicals.

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CHAPTER 8

Plant Cell Membranes

WANDA K. FARR

The studies of the formation and structure of plant cell membranes, which were carried out at Boyce Thompson Institute for Plant Research, Inc. over a ten-year period (1930–1940), had their origin in the earlier work of Clifford H. Farr and Wanda K. Farr on the cell divisions of pollen mother cells,[7, 8] and the growth of root hairs in solutions.[6, 9] In the consideration of these two aspects of growth, *i.e., cell division* and *cell enlargement*, the problems relating to the elaboration of cell membrane materials in the living protoplasm, as well as those relating to the methods of formation and microscopic structure of mature membranes, were constantly in evidence. In 1929 the Division of Cotton Marketing, United States Department of Agriculture, provided the facilities for an intensive study of the third major aspect of growth, *cell differentiation*. The project outlined dealt specifically with the cotton fiber, and particular emphasis was placed upon the study of the formation and structure of its cell wall. The thin, colloidal cell membrane of root hairs, the more or less general absence of cellulose, and the lack of pronounced microscopic structural differentiation in the walls of many of them had rendered them of limited value for the study of the so-called "cellulose" fibers of industrial importance. The cotton fiber membrane, with its large accumulations of doubly refractive cellulose and microscopically visible fibrillar structure, promised greater advantages.

For more than a year the work was carried out in Washington, D. C. and at Clemson College, S. C. Late in 1930 the headquarters for this research was transferred from Washington to Boyce Thompson Institute. Laboratory space and facilities were provided by the Institute for Mrs. Farr and one assistant. Continued cooperation with Clemson College afforded field-grown cotton fibers of various stages of development. Methods of growing the cotton plants to maturity in the Institute's greenhouses were soon worked out; they provided one of the most important steps in the detailed studies of cotton fiber development which followed. At this same time a cooperative arrangement was made with the x-ray laboratory of the Department of Chemistry, University of Illinois, in order that this more recently developed technique might be added to the microscopic and chemical techniques already in use.

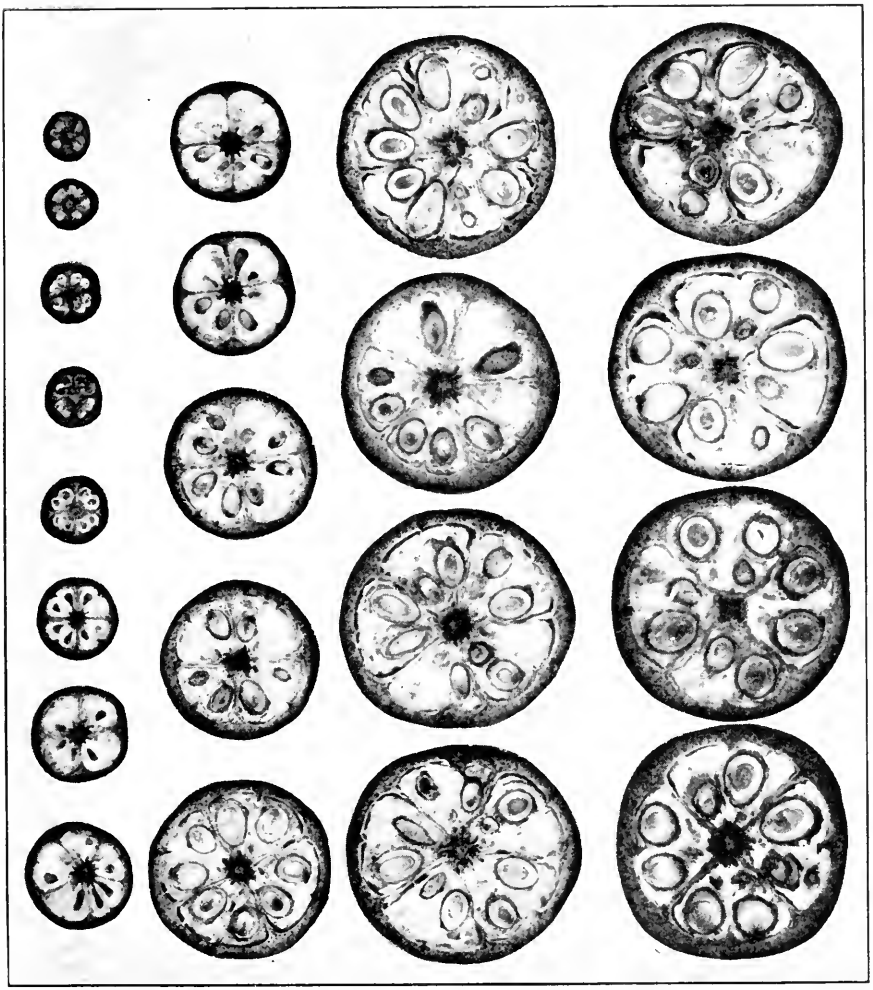

FIGURE 97. Median cross-sections of bolls of *Gossypium hirsutum* L. showing daily increase in size from the date of flowering to the twenty-first day of development ($1\frac{1}{8}\times$).

General Studies of Cotton Fiber Growth

The results of the earliest work were of a more general nature, dealing with the origin and early stages of elongation of the cotton (*Gossypium hirsutum* L.) fiber [10] (Fig. 97); cell divisions in the epidermal layer of the ovule subsequent to fertilization [11] (Fig. 98); structural features of the wall suggested by x-ray diffraction analyses and observations in ordinary and plane-polarized light [19] (Fig. 99); and fiber abnormalities as related to varietal differences and to the density of the fiber mass within the boll.[12]

Large numbers of dividing cells in the epidermal layer from the date of flowering to the twelfth day following showed that cell enlargement is not alone responsible for the tangential extension of the epidermal layer of the

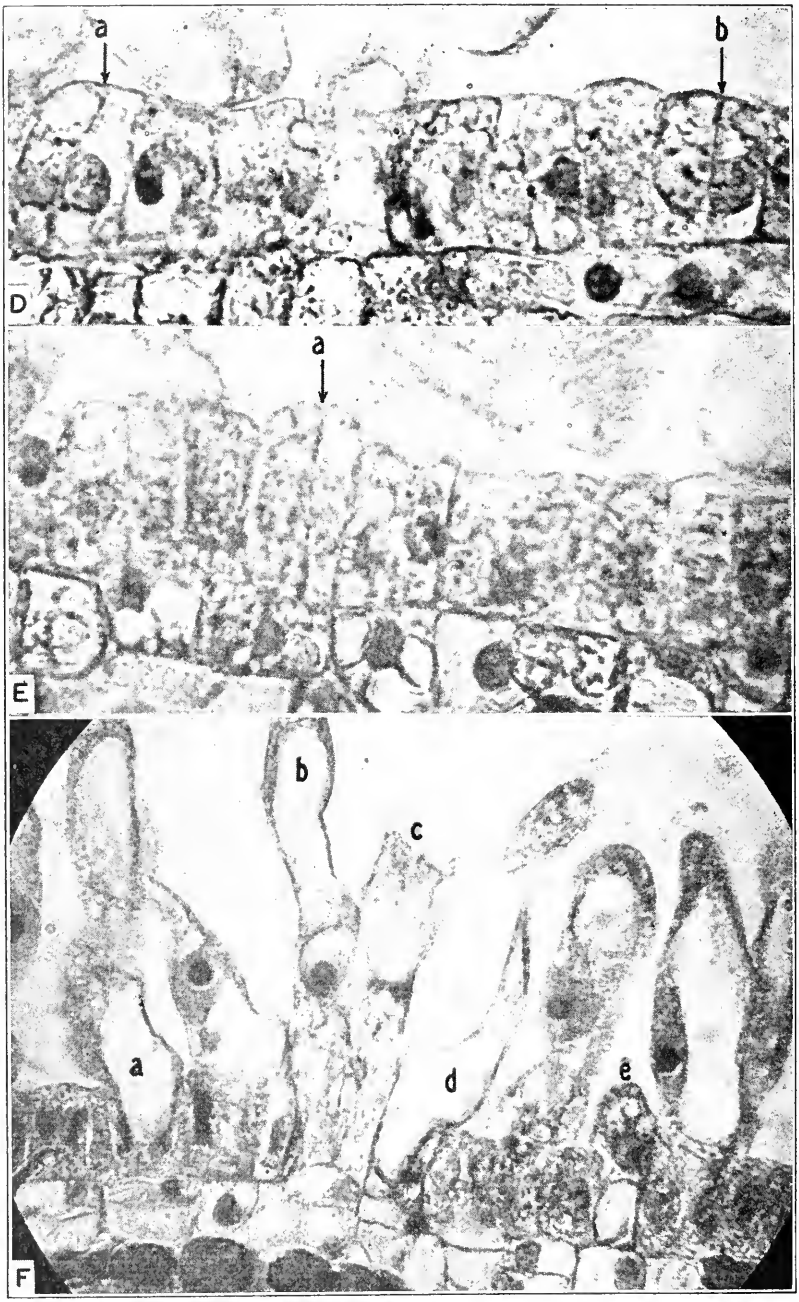

FIGURE 98. Late stages in cell division and certain appearances in early stages of fiber formation. D, *a*, late stage of nuclear reorganization; D, *b*, early stages of nuclear reorganization; E, *a*, early stage of fiber formation in two adjacent cells whose nuclei appear to be incompletely reorganized; F, *a*, *b*, *c*, and *d*, lateral wall relationships in the basal portions of fiber-forming cells; F, *e*, young fiber showing dense nucleus and basal vacuole. (D and E, 1170 ×; F, 660 ×.)

seed coat. Early stages of fiber elongation from epidermal cells which were apparently daughter cells of these recent divisions furnished direct evidence that fibers may originate from cells which are not yet formed upon the date of flowering.

The absence of liquid substance in the boll cavity which had been commonly designated "boll sap" removed the possibility of nutrition of the developing fiber through such a medium, and indicated that the materials for its growth are transported through its basal connection with the seed.

The study of the formation of abnormalities in developing fibers showed that while cell enlargement and the formation of a thick cell membrane are two of the most conspicuous phases of cotton fiber growth, the tendency to enlarge in an approximately linear direction is inherent. If obstructed in one region of the boll cavity, however, the portion of the fiber concerned appropriates any available space in the immediate vicinity for enlargement. This may result in change in diameter, change in direction of growth, or any one of many types of abnormality. In the three varieties of cotton, Pima, Super Seven, and Acala, the measured densities of the fiber masses were found to increase in the order named. Fiber abnormalities were observed to increase in the same direction and a definite relationship was suggested between these two factors.

Broadening of the Experimental Approach

During the progress of these studies it became evident that the advantage of the cotton fiber, with respect to the large quantity of cell membrane material, was not furnishing, under the experimental conditions employed, additional information concerning the fine structure of the cell membrane and the formation of membrane-building materials in the living protoplasm. From the viewpoint of plant cells in general, the cotton fiber is one of the most highly differentiated. Even among those cell membranes which contain cellulose — and many do not — it would be classed as a specialized and not a primitive type. The failure to observe finer details of membrane formation and structure indicated a possible need for a broader attack upon the problem, involving cells of various types throughout the plant kingdom. Facts gleaned from such sources might then be used in attempting to understand the development and structural problems of the cotton fiber.

This task was undertaken by Mrs. Farr and Dr. Sophia H. Eckerson of the Institute staff. Their first studies were made with the cellulose-forming bacterium, *Acetobacter xylinus*. All the microscopic observations were made without the use of the usual bacteriological methods of staining; however, certain microchemical reactions and polarized light were used to assist in the examination of individual organisms and chains of organisms (Fig. 100). The cellulose layer surrounding the protoplast and the noncellulosic exterior layer of the bacterial organism were thus differentiated. These procedures represented a more direct approach to the physical and

chemical properties of a bacterial membrane and one less likely to affect its natural properties than many of the staining techniques in current use. They necessitated some changes in microscopic technique, however, the most important of which consisted in the readjustments of the illuminating system. This involved mainly the lowering of the intensity of the artificial light by suitable rheostat control, so that the structural differentiations in the diminutive organisms would not be obscured by a flood of bright light.

The results of these bacterial studies were of interest in the light of current attempts to "synthesize" cellulose membranes by growing *A. xylinus* in glucose solutions.[25] In these contemporary Canadian experiments the process of cellulose formation was considered to be *intercellular*, the cellulose molecules having been synthesized directly from the sugar molecules in the nutrient medium and deposited in long, well-oriented chains of cellulose unit-cells. At the request of and from cultures furnished by Dr. H. L. Hibbert, the entire "membrane" was found by Farr and Eckerson [20] to consist of bacterial organisms with no true intercellular substance. The single bacterium is composed of a protoplast surrounded by a cellulose membrane which, in turn, is covered with a layer of gelatinous material which reacts positively with the ruthenium red test for pectic substance and negatively to the H_2SO_4 and I_2KI test for cellulose. Studies in both ordinary and polarized light at low and high magnifications produced no evidence of continuous, long chains of cellulose in the membranes. They did indicate, however, extreme regularity in directional arrangement of the strands of the aerobic organisms as they grew in thin layers upon the surface of the nutrient media.

The development of microscopic techniques by means of which such bacterial membranes could be analyzed was of even greater importance. The optical systems, thus illuminated, were then available for use in examining other types of plant cells. One of the first of these which was studied was the developing conidiophore of *Aspergillus niger*. Dr. Charles Thom had called to Mrs. Farr's attention structures in the wall of the mature stalk of a certain strain of this fungus which closely resembled the spiral fibrils in the wall of the cotton fiber. In the protoplasm of the very young stalks tiny granules were found which gave the characteristic cellulose reaction with sulphuric acid and iodine. As the stalks developed they were carefully mounted for observation in both ordinary and polarized light. It was thus found that, within the limits of one sporangiophore, the successive

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FIGURE 99. Hand-colored photomicrographs of portions of fibers in plane-polarized light: *a* to *r* from Upland cotton, *s* and *t* from Jungle cotton; long axes of portions oriented at 45° with reference to the plane of vibration of the light; *d*, *n*, *o*, and *r* slightly swollen in a solution of ammonium thiocyanate; *a*, *b*, *c*, *l*, *m*, *s*, and *t* photographed with the analyzer; *d*, *n*, *o*, and *r* without the analyzer; *c* and *m* colored with the selenite plate (red of the first order); *a*, *b*, *d*, *l*, *n*, *o*, *r*, *s*, and *t* without the selenite plate; *a*, *b*, *c*, *l*, and *m*, 690 ×; *d* and *n*, 810 ×; *o*, *r*, *s*, and *t*, 1200 ×. (*Coloring of photomicrographs was done by Miss Flora White.*)

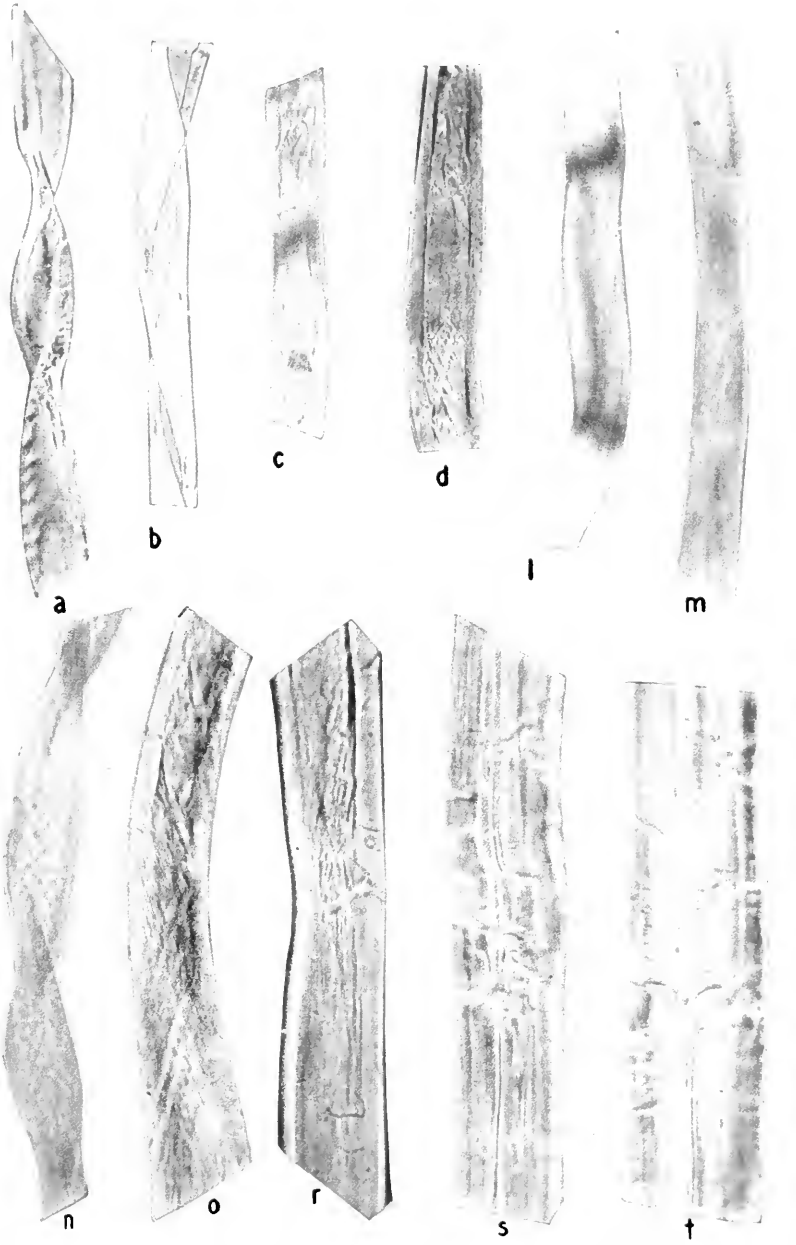

FIGURE 99. Portions of fibers in plane-polarized light. (*For description see legend on page 264.*)

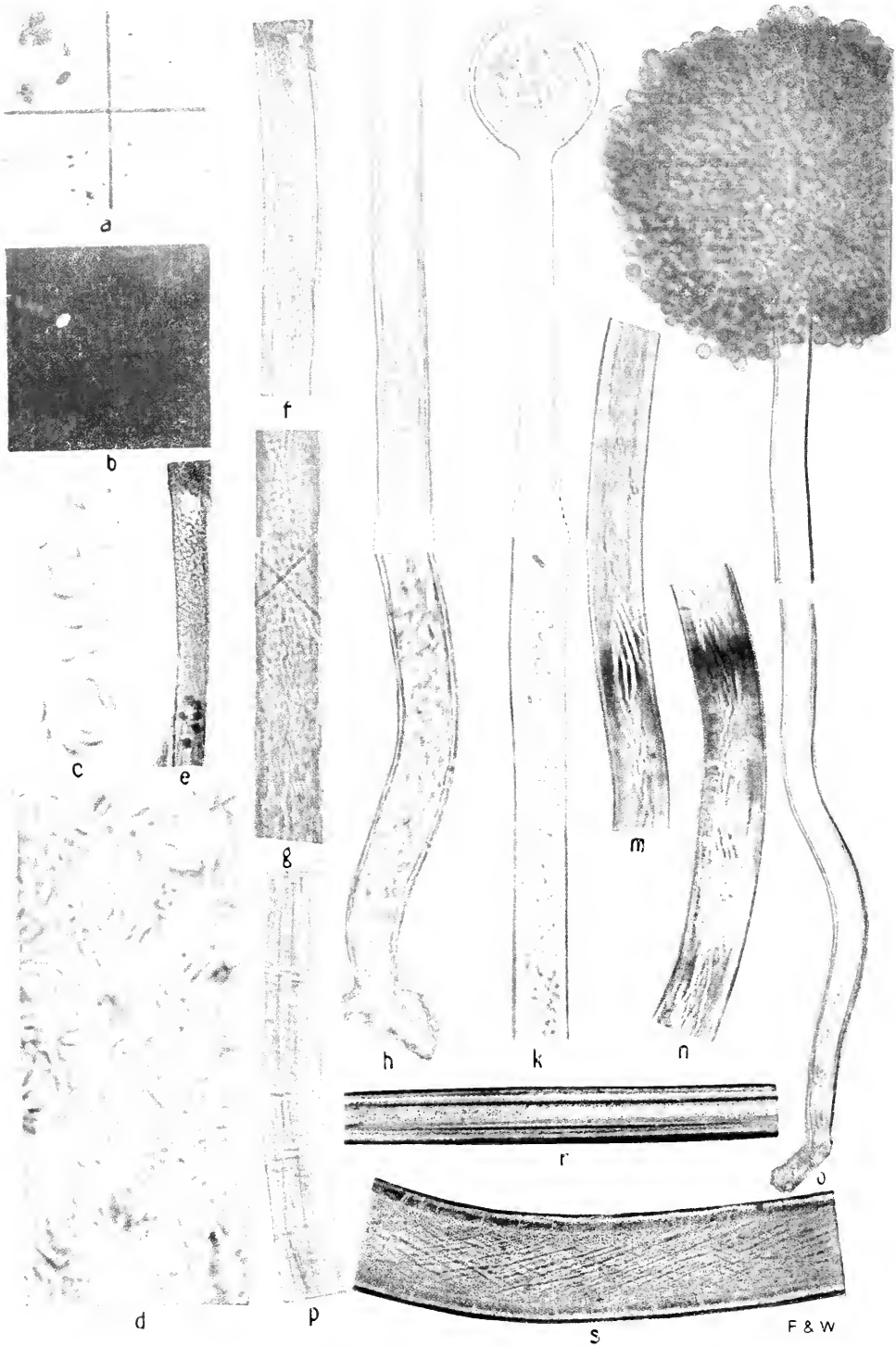

FIGURE 100. (*For description see legend on page 265.*)

stages of development of the single fibrils could be observed. Each fibril was formed by the arrangement, in single rows, of the diminutive cellulose particles. In the mature conidiophore these fibrils were in spiral arrangement in the secondary lamellae. The primary wall in this strain of *Aspergillus* was found to be comparatively thin, non-doubly refractive, and largely pectic in composition, as indicated (Fig. 100) by its staining with ruthenium red. By similar reactions to ruthenium red the individual fibrils and even the individual cellulose particles were found to be coated with a non-cellulosic substance, one component of which is pectic. The deep yellow coloration of this colloidal coating with iodine solution explained, at the time, Strasburger's earlier observations of protein "microsomes" which behave similarly in building up the cell wall lamellae. The cellulose "microsome" is coated with a non-crystalline film containing both protein and pectic material. However, the developmental importance of this characteristic protein reaction was not recognized until later when, in the course of studies of cellulose formation, it was found to come into existence in a protoplasmic matrix rich in protein.

With these observations of bacteria and fungi as a basis, a comparison of young cotton fibers in various stages of development showed the presence of similar cellulose particles and similar stages of fibril formation. In the cotton fiber, however, the quantity of colloidal material is greater than in the conidiophore of *A. niger* and had previously obscured the brightness of the cellulose particles in polarized light. The larger amounts of colloidal material were associated in the cotton fiber with strength and flexibility; the smaller amounts in the conidiophore with weakness and brittleness.

These results were published by Farr and Eckerson under the title "Formation of cellulose membranes by microscopic particles of uniform size in linear arrangement." [20] The broader botanical attack upon cell-membrane

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FIGURE 100. *a*, Single bacteria from cultures of *Acetobacter xylinus* in positions of extinction and brightness in polarized light with selenite screen (2700 ×). *b*, Mount shown in *a* without selenite screen (2700 ×). *c*, Sulphuric acid-iodine reaction in *A. xylinus* (1950 ×). *d*, Pectic coating upon the surface of *A. xylinus* stained with ruthenium red (1950 ×). *e*, Cellulose particles separate and in chains in young sporangiophore of *Aspergillus niger*. Polarized light (700 ×). *f*, Portion of young sporangiophore of *A. niger* showing original pectic membrane and pectic coating upon the individual cellulose particles stained with ruthenium red (1150 X). *g*, Cellulose particles in the process of fibril formation in sporangiophore of *A. niger* (1900 ×). *h*, Tip and base of young sporangiophore of *A. niger* showing earliest cellulose membrane formation near base of stalk. Polarized light (1700 ×). *k*, Developing sporangiophore of *A. niger* showing increasing thickness of cellulose membrane in lower portion. Polarized light (1700 ×). *m*, Portion of stalk of mature sporangiophore of *A. niger* showing crossed spiral arrangement and reversal area of cellulose fibrils. Polarized light (1150 ×). *n*, Another portion of a mature sporangiophore of *A. niger* showing more frequent areas of reversal. Polarized light (1150 ×). *o*, Mature sporangiophore of *A. niger* showing thick cellulose membrane throughout its entire length. Polarized light (500 ×). *p*, Portion of fiber of *Hibiscus spathecus* stained with ruthenium red to indicate its outer pectic layer (700 ×). *r*, Portion of intact fiber of *H. spathecus* showing longitudinal areas of coloration in polarized light (200 ×). *s*, Base of *H. spathecus* fiber slightly crushed to bring out crossed spiral arrangement of fibrils (550 ×).

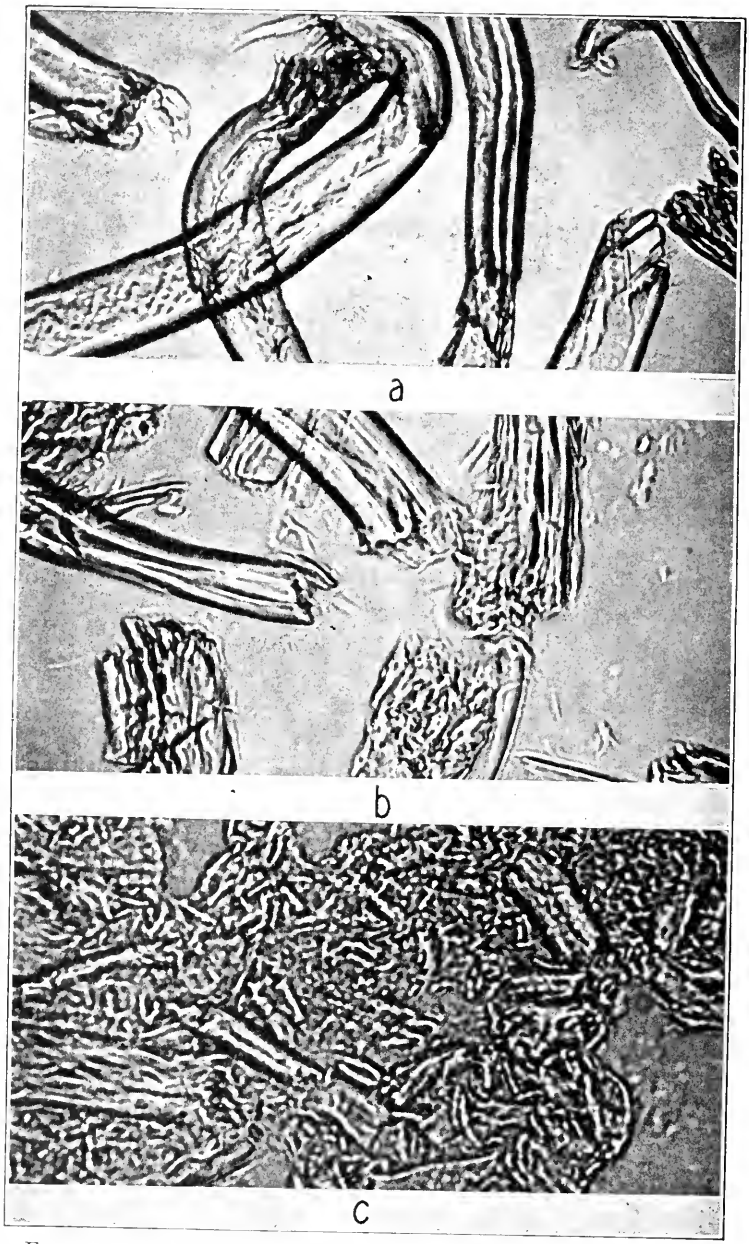

FIGURE 101. *a*, Portions of fibers from the residues after treatment for 2½ hours with HCl. The fibril and particle structure of the membranes are shown as well as the cross-sectional rupturing of the fiber (350 ×). *b*, More advanced degree of fiber dissociation after 18 hours' treatment with HCl (350 ×). *c*, Extreme degree of fiber dissociation after 5 days' treatment with HCl (350 ×).

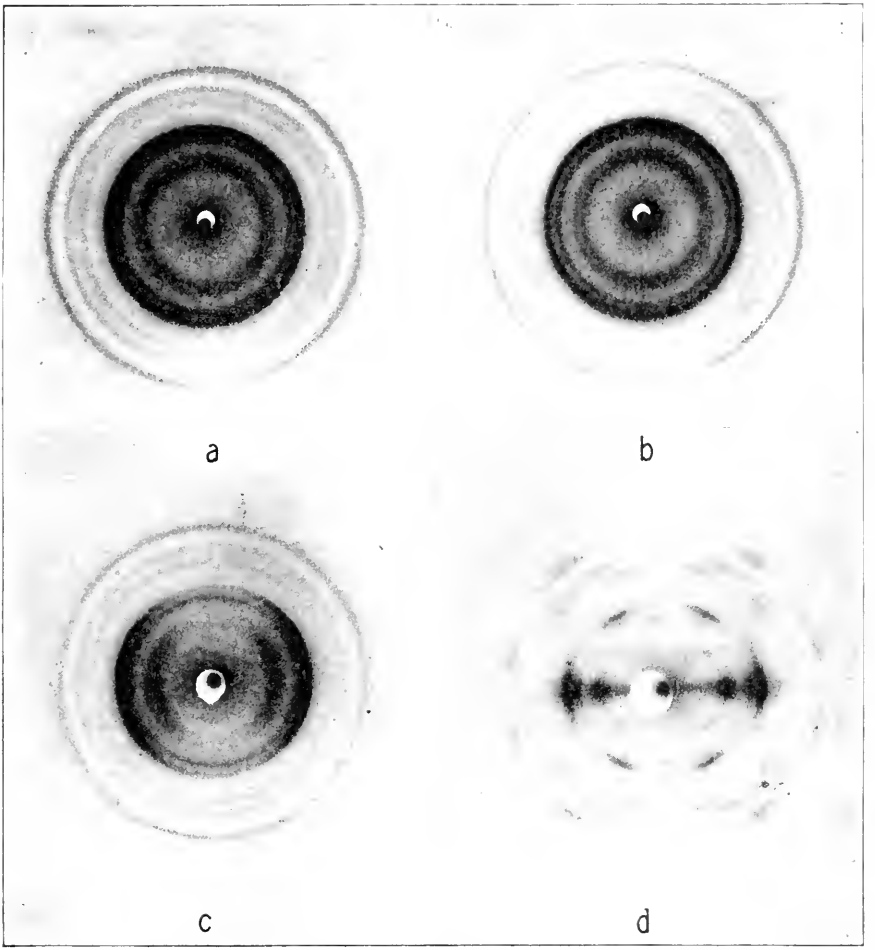

FIGURE 102. X-ray diffraction patterns of cellulose. *a*, Cellulose particles from cotton fibers separated by 18-hour treatment with HCl; *b*, pulverized cotton fibers; *c*, paralleled cotton fibers; *d*, paralleled ramie fibers.

formation and structure would seem to have been justified by the fact that the particulate structure of the cellulose fibril was first observed in a fungus as a result of optical improvements which had been made to meet the microscopic requirements of unstained bacteria.

These observations of fibril formation recalled the earlier reports of similar phenomena by Strasburger [35] and Wiesner.[36] In addition to reporting the existence of "microsomes" or "dermatosomes" in the living protoplasm and their behavior in building up the cell membranes, Wiesner had shown that the granular dermatosomes maintain their identity in the fibrils of the mature membrane and had demonstrated the fact by disintegrating the membrane of the cotton fiber into lamellae, the lamellae in turn into fibrils, and the fibrils into dermatosomes by treatment with hydrochloric

acid. Farr and Eckerson [21] published a similar result in 1934 under the title "Separation of cellulose particles in membranes of cotton fibers by treatment with hydrochloric acid" (Fig. 101). X-ray diagrams of these preparations (Fig. 102) were published by Farr and Sisson [22] during the same year. The controlled treatment with hydrochloric acid had removed

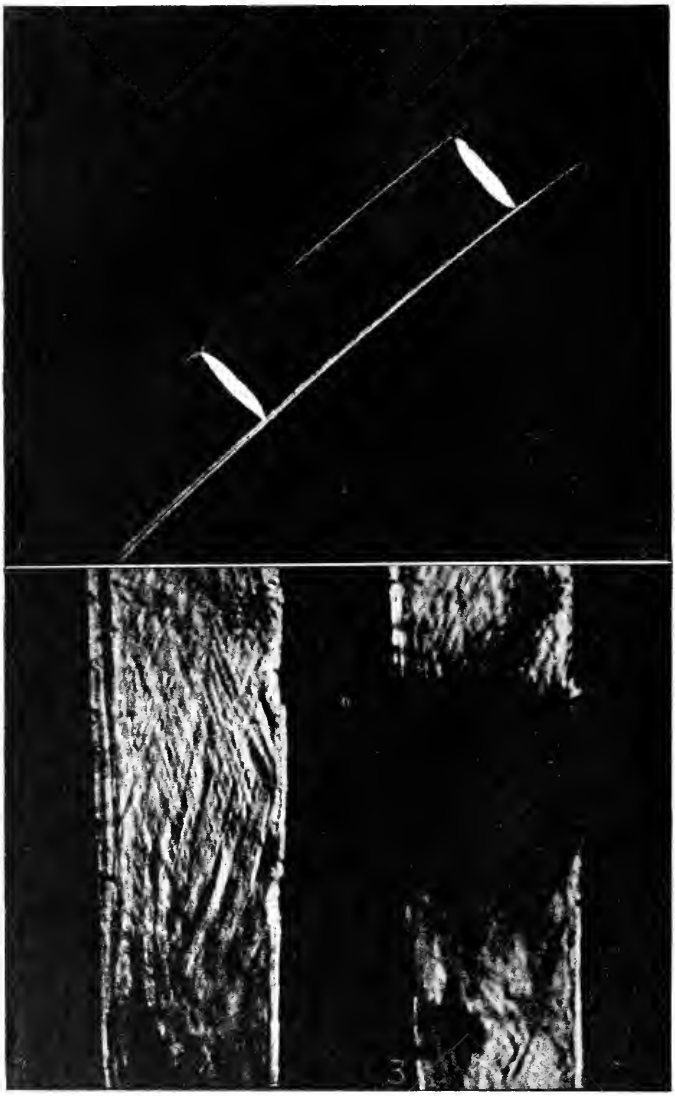

FIGURE 103. 1. Cell membranes of *Spirogyra* sp. show the presence of crystalline material through their double refraction in polarized light (480 ×). 2. Spiral fibrils in the cell membrane of a cotton fiber (*Gossypium hirsutum*) in polarized light (775 ×). 3. Fibrils in the cell membrane of the cotton fiber are arranged parallel to the axis at intervals throughout its length and produce "extinction areas" in polarized light (775 ×).

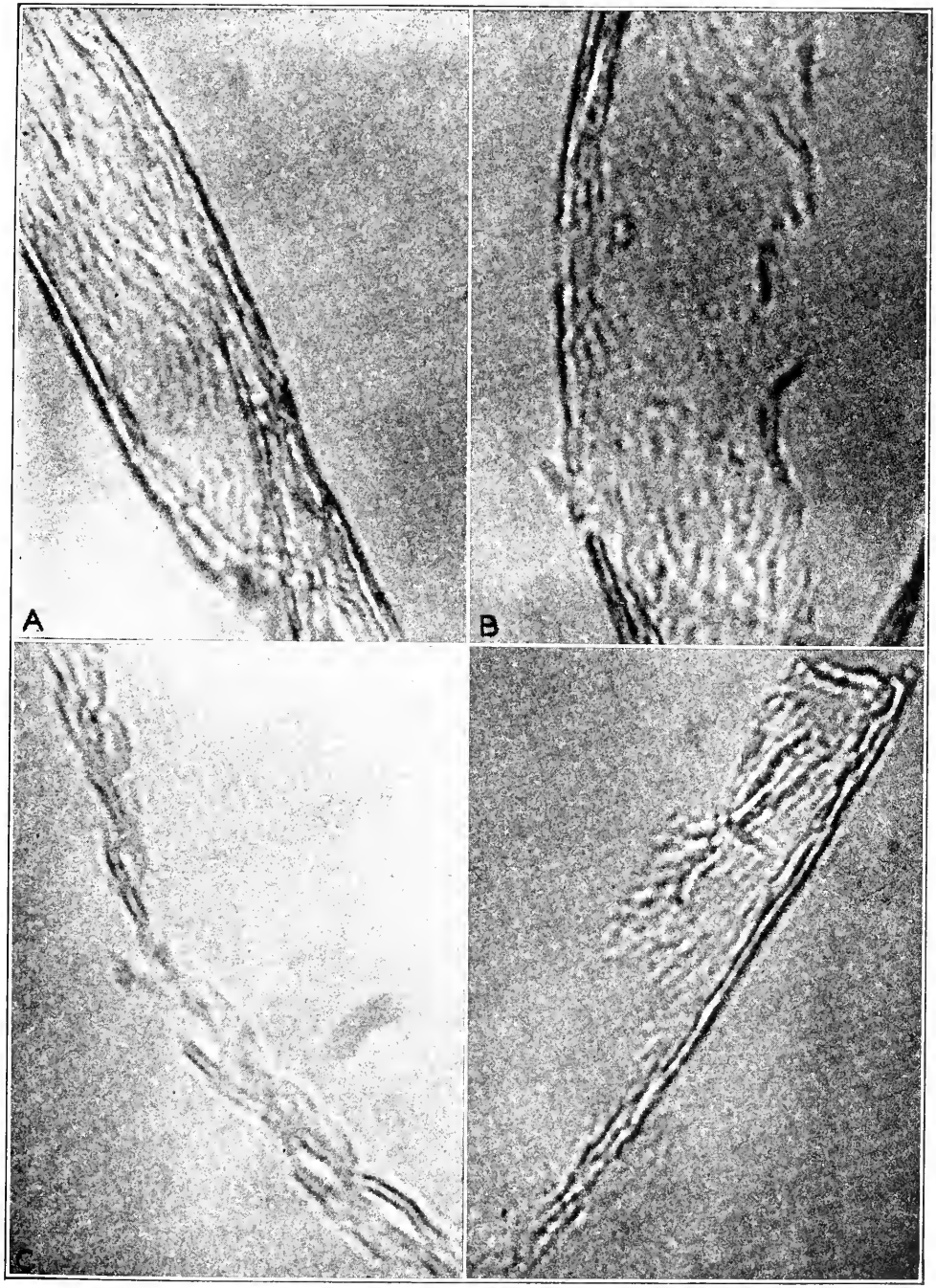

FIGURE 104. Cotton fibers, partially disintegrated by bacteria. A, From 24-day culture (675 ×, enlarged to 1000). B, From 8-week culture (900 ×, enlarged to 1350). C, From 9-week culture (900 ×, enlarged to 1350). D, From 24-day culture (675 ×, enlarged to 1000).

the greater part of the relatively small amounts of non-cellulosic material from the fiber membrane, had reduced the physical state of the fibers to a fine white powder, but had not changed the native cellulose pattern. Three years later Sisson,[28] by means of x-ray diffraction analysis, corroborated the earlier work of Farr and Eckerson [20] on the presence of crystalline cellulose in young cotton fibers.

These botanical studies were of sufficient chemical interest to bring a contribution from The Chemical Foundation, Inc. in 1936 for the increase in staff and facilities for plant cell-membrane research. The work was continued at Boyce Thompson Institute. Drs. Florence L. Barrows, Jack Compton, Stanton A. Harris, Florence E. Hooper, Richard E. Reeves, and Wayne A. Sisson were added to the research staff and the numbers of laboratory assistants increased correspondingly.

Studies of various types which were undertaken resulted in the publication of papers dealing with certain colloidal reactions of cell membranes to treatment with sulphuric, hydrochloric, and phosphoric acids; [14] the isolation of pectic acid from the cotton fiber; [24] the effect of certain non-cellulosic constituents upon the x-ray diagram of cellulose; [27] orientation in young cotton fibers, as indicated by x-ray diffraction analysis; [30] a consideration of the microscopic structure of plant cell membranes (Fig. 103) from various parts of the plant kingdom in relation to the micellar hypothesis of Nägeli; [16] x-ray diffraction behavior of cellulose derivatives; [34] the disintegration of the cell membrane (Fig. 104) of the cotton fiber by a pure culture of bacteria; [26] microscopic analyses of additional cell membranes from various parts of the plant kingdom; [2] x-ray analyses of textile fibers; [29] x-ray diffraction analysis and its application to the study of plant constituents (Fig. 105); [31] the behavior of the cell membrane of the cotton fiber in cuprammonium hydroxide solutions with particular reference to their dispersion, electrokinetic, and coagulation behavior; [4, 15, 32] the lamellate structure (Fig. 106) of certain plant cell membranes; [3] and the structural relationship of rayon to natural cellulosic fiber materials, as shown through a study of the viscose process.[5] The studies of the behavior of cell membrane materials in cuprammonium hydroxide and in the carbon disulphide used in the viscose process showed that in both instances the cellulose particles are dispersed, not dissolved, in the medium, and that the non-cellulosic materials present in small quantities in the cotton fiber membrane play a part in bringing about this dispersion and in producing the final viscosities of the mixtures. These observations are in keeping with the fact that highly purified cell membranes of cotton fibers lose their viscosity-producing power in many reagents, although the nature of their cellulosic component remains relatively unchanged as observed microscopically and examined by means of x-ray diffraction (Fig. 107).

Observations on the membranes of epidermal cells of the *Avena* coleoptile revealed another different inter-particle relationship.[23] In the cotton fiber and wood fiber, where fibrils constitute a unit of structure, the end-to-

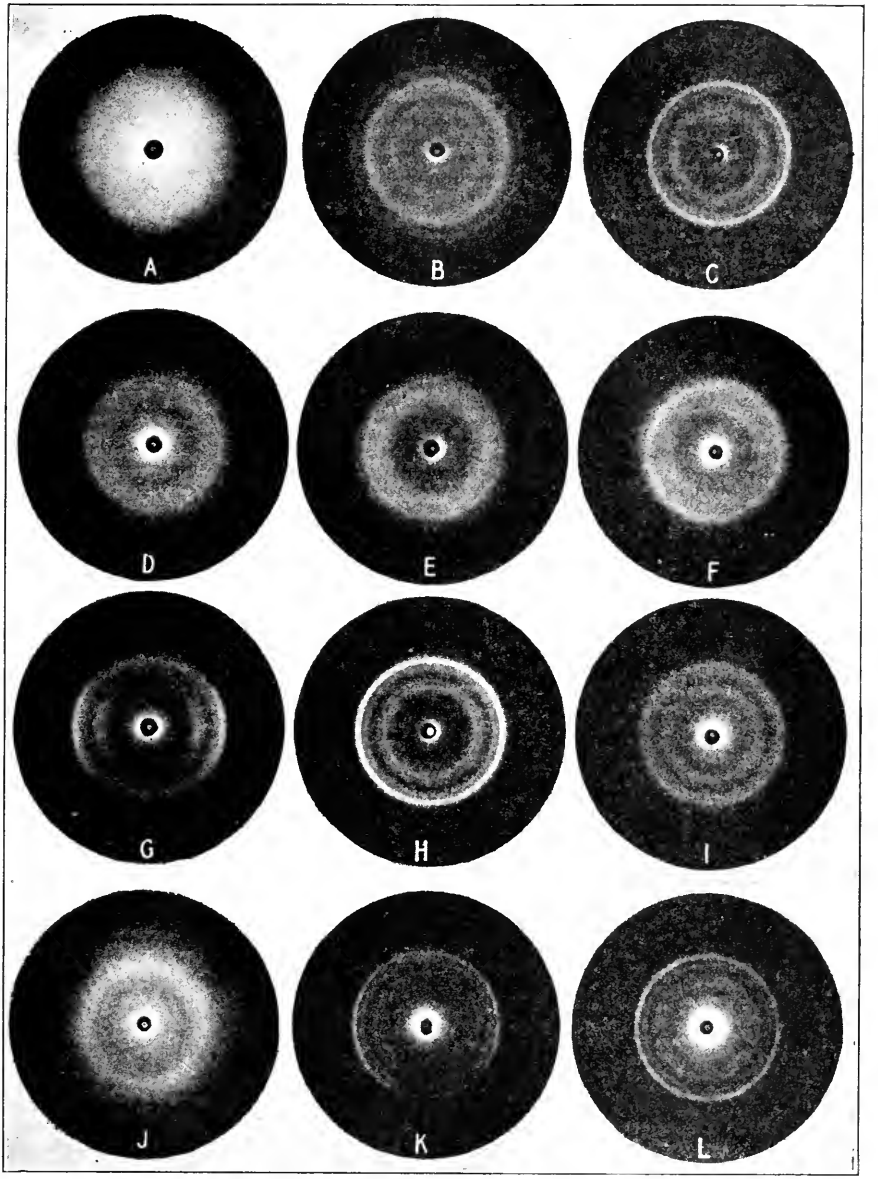

FIGURE 105. Detection and estimation of plant constituents: presence of cellulose in lignin, A, lignin; B, cellulose from lignin; C, cotton cellulose; detection of cellulose in young cotton fibers, D, 10; E, 25; F, 30; G, 35; H, 50-day-old fibers; I, same as D after extraction; presence of cellulose in *Valonia* cytoplasm, J, original cytoplasm; K, cellulose obtained from cytoplasm by extraction; L, cellulose from cell wall.

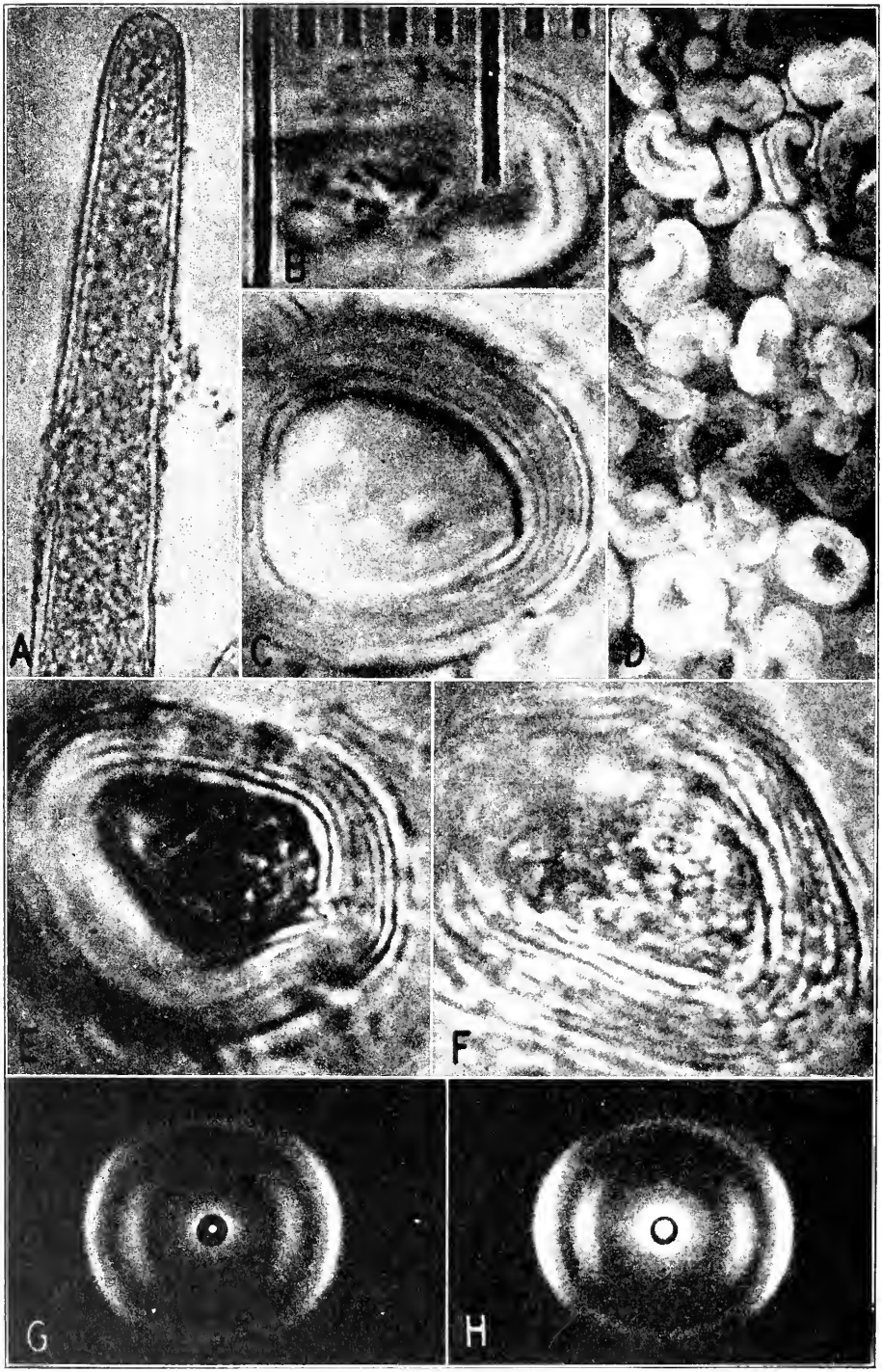

FIGURE 106. (*See legend on opposite page.*)

end bondage of the cellulose particles is strong. In the cells of the *Avena* coleoptile, where rapid cell elongation takes place, these end-to-end bondages are weak enough to be broken during this growth process; in the mature, elongated membrane, the single rows of particles are arranged side by side along the long axis of the cell in "barrel-hoop" fashion. In this connection it may be pointed out that in all the cells studied to date, the burden of swelling, plasticity, and elasticity has rested upon the intercrystalline material. When extreme plasticity and absorptive properties are desired, and strength is not at a premium, as in the root hair, the cellulose is often excluded entirely from the construction of the cell membrane. When strength as well as flexibility is desired, the colloidal materials, such as pectin and protein, are reduced in quantity; in some instances they are rendered more impervious to swelling agents by compound formation (*e.g.*, calcium or sodium pectates), and are intimately blended with cellulose particles to produce the desired physical and chemical combination.

Formation of the Cellulose Particles

The findings up to this point had furnished no clue to the method of elaboration of cell membrane materials in the living protoplasm. Small cells, such as the cotton fibers, required that the observer look *through* the membrane in order to see protoplasm during its period of synthetic activity. Protoplasm expressed from such cells tends to produce artifacts by coagulation and other physicochemical changes; optical sectioning of carefully mounted fibers revealed nothing definite in these cells as long as an entirely unknown process was involved. The large, one-celled alga, *Valonia ventricosa*, had been examined and reported upon at one of the meetings of the American Chemical Society.[13] Earlier workers had been attracted to the study of *Valonia* because of its highly crystalline, well-oriented membrane.[1] We were particularly interested in studying it because of the ease with which its protoplasm could be removed and maintained in good condition for microscopic examination. Arrangements were made, through the courtesy of the Carnegie Institution of Washington, to obtain fresh samples of *Valonia ventricosa*, *V. macrophysa*, and a closely related form, *Halicystis osterhoutii* from their Dry Tortugas laboratory and the Bermuda Biological Station for Research, Inc. Preserved samples of *Halicystis ovalis* were furnished from the beaches of Pacific Grove, California, by Dr. George J. Hollenberg.

The mature membrane of *Halicystis* was found to contain a high proportion of non-cellulosic material. Through this colloidal matrix the cellulose

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Figure 106. Cotton fibers grown in constant light. Lamellae about 1 μ wide. A, 15 days, 1 lamella, 2nd forming (920 $\times$). B, 49-day cross section; 6 cellulose particles with micrometer scale (1 space equals 2.35 μ measured from center to center of adjacent lines). C, E, F, 49-day cross sections at middle. D, 60-day dry, cut in cork (460 $\times$, enlarged to 890). G, X-ray diffraction pattern of fibers matured in constant light. H, In daylight.

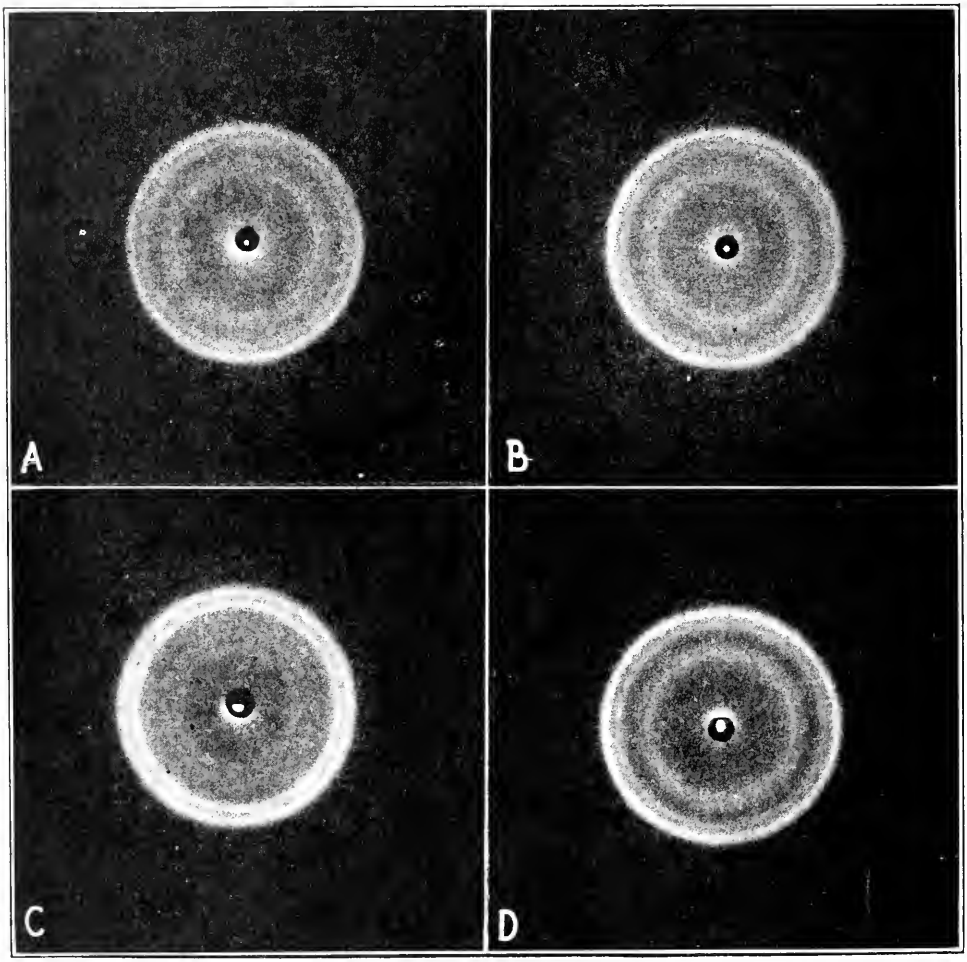

FIGURE 107. A, X-ray diffraction pattern of cellulose particles from cotton fibers after treatment for 48 hours with the standard solution (*American Chemical Society*) of cuprammonium hydroxide. B, A similar sample after three months' treatment with the same reagent. C, 3 g of cellulose particles separated by treatment with HCl (sp gr 1.19) produce a mixed mercerized and native cellulose x-ray diffraction pattern after treatment for 18 hours in cuprammonium hydroxide. D, 7 g of cellulose particles similar to those used in C produce a native cellulose x-ray diffraction pattern after treatment for 18 hours in cuprammonium hydroxide.

particles are distributed at random, with no tendency toward fibril formation. The x-ray diagram of the cellulose in this membrane (Fig. 108) shows that it is in a "mercerized" state, comparable to that produced in the cellulose of a cotton fiber when it is treated with strong (17 to 18 per cent NaOH) alkali.[33]

In the protoplasmic mounts of *Halicystis* were found the structures which had long been the object for which we had searched — either the

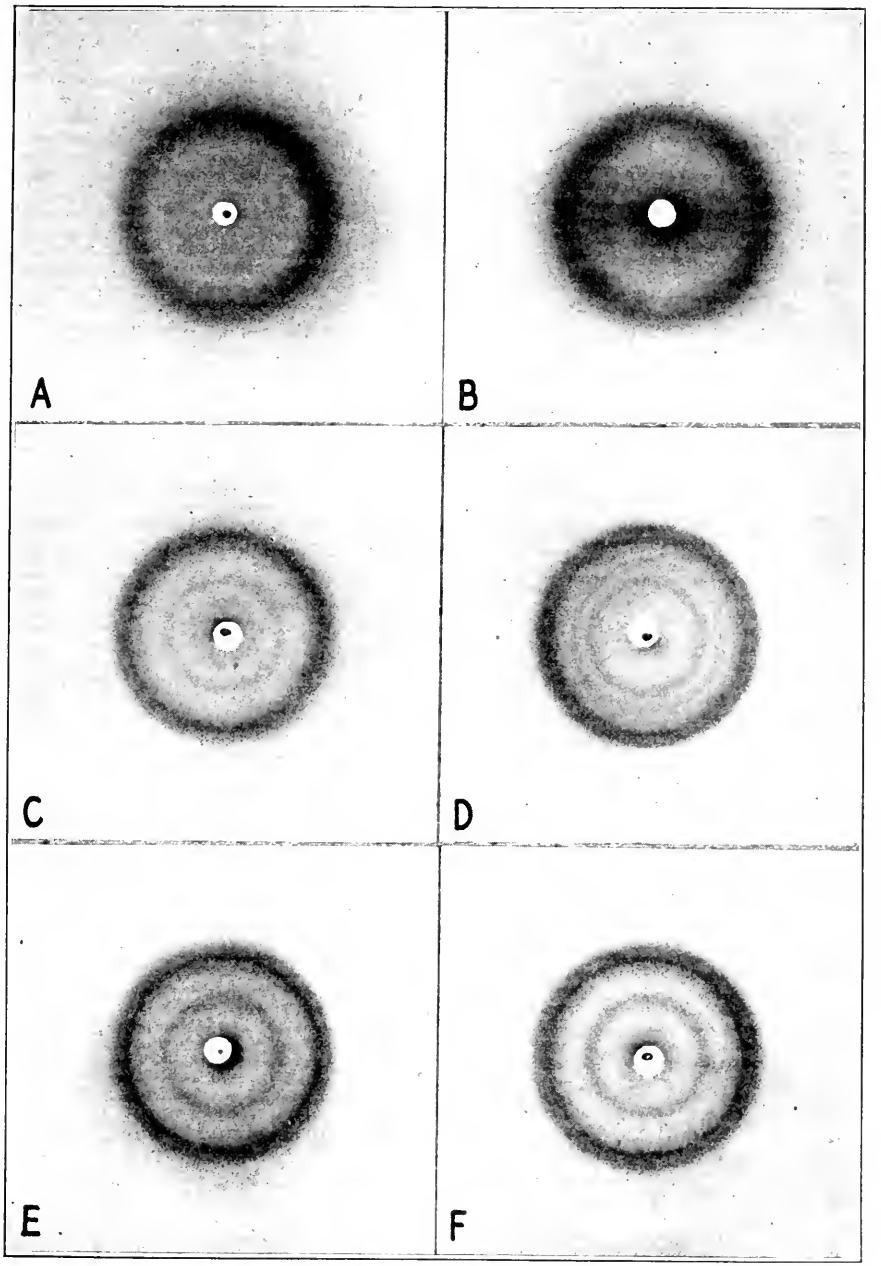

FIGURE 108. X-ray diffraction diagrams of *Halicystis*. A, untreated; B, stretched 75 per cent (fiber axis vertical); C, after purification treatments; D, after treatment with hot glycerin; E, after treatment with hot 0.1 N hydrochloric acid and 1 per cent sodium hydroxide; F, for comparison, the x-ray diagram of mercerized cotton treated as in E.

cellular organs or regions in the protoplasm which elaborate cellulose to be used in the construction of the cell membrane. These proved to be cellulose-forming plastids. The mechanism of formation of cellulose in the chloroplasts of *Halicystis* has no apparent points in common with the mechanisms of starch formation as it has been observed in many types of plant cells. It operates by the successive formation of cellulose rings of uniform thickness and increasing diameter, as the plastid enlarges. These rings, at first in a liquid state, then gel-like, and then solid, finally fragment to form mercerized cellulose particles. When the period of cellulose synthesis comes to a close, the plastid membrane disintegrates and the cellulose particles, with their coating of colloidal plastid plasma, are set free in the outer regions of the cytoplasm of the cell. They are later deposited directly, with their associated materials of plastid and cytoplasmic origin, to form a new lamella of the cell membrane. The newly deposited lamellae are green due to the presence of the chlorophyll of the plastid plasma (see Fig. 109).

After observing this phenomenon of cellulose formation in the chloroplasts of *Halicystis*, a careful study of the protoplasm of the cotton fiber was resumed, with the result that colorless plastids, which were performing a similar function of cellulose formation by a similar process of ring formation and fragmentation, were found. These plastids had been seen and photographed previously in many living cotton fibers. Their function in the cell had been obscured by the slight differences in refractive indices of the plastid plasma and the cell plasma which surrounds them (Fig. 110). They had been observed and considered to be vacuoles in the young fibers. Once removed from the fiber, however, the stages of cellulose ring formation and fragmentation (Fig. 111) were clearly visible.[17]

The chloroplasts of *Valonia* were found to be engaged in the elaboration of cellulose by a different process from that observed in the chloroplasts of *Halicystis* and in the colorless plastids of the cotton fiber. Following the formation of a single cellulose ring in the very young plastid, a cellulose fibril begins to form in the plastid plasma. These fibrils attain great length and, in the mature plastid, are coiled tightly within the plastid membrane. When the membrane of the mature plastid disintegrates, the coiled cellulose fibril is freed and straightens. At this stage it can be disintegrated into cellulose particles with slight pressure. Continued study of the early stages of cell membrane formation has indicated, however, that in the living cell the fibril is deposited directly in the lamella of the membrane after it has uncoiled from the plastid. Observations show that it is deposited in close

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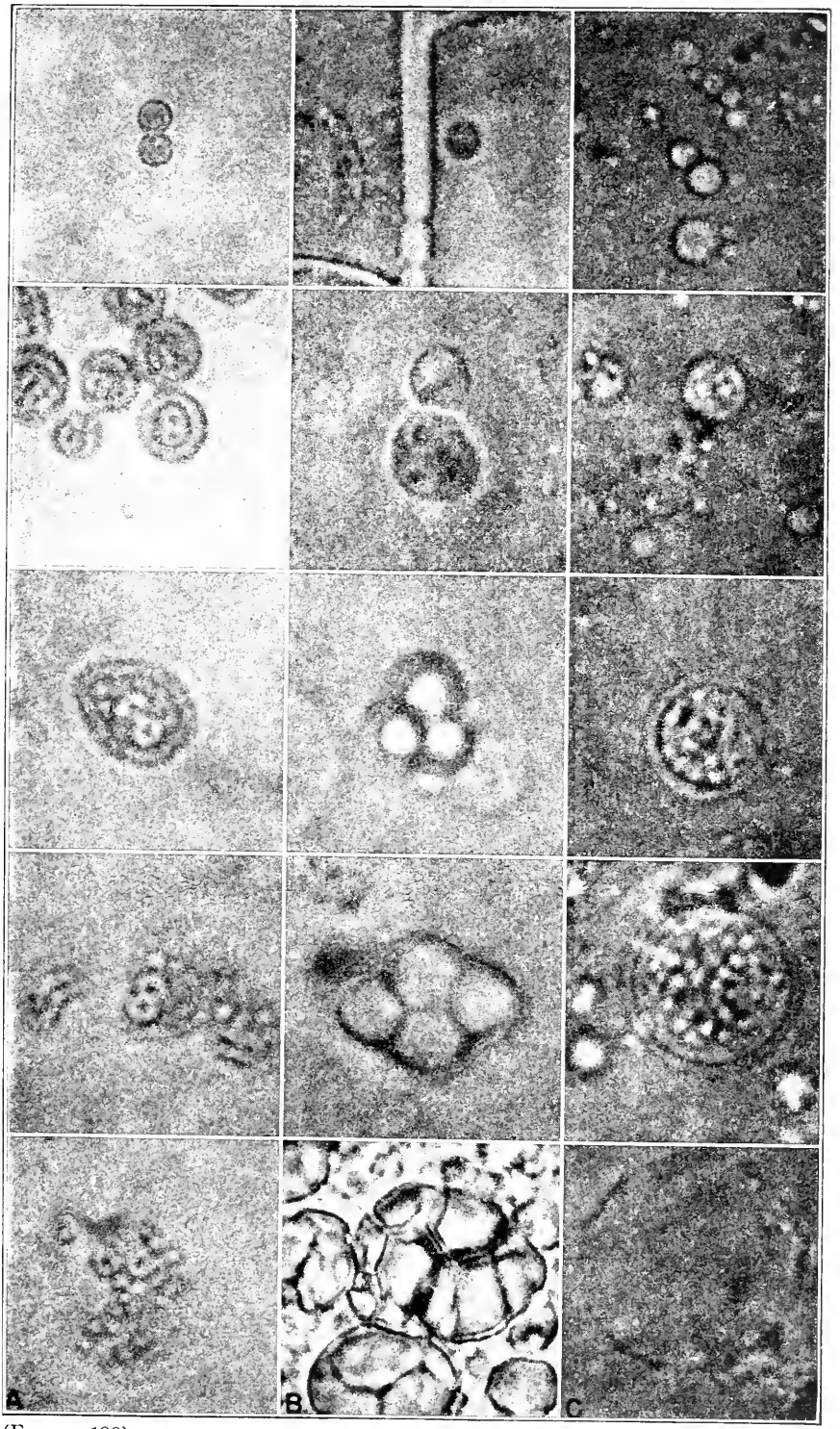
FIGURE 109. *Column* A, Stages in development of the chloroplast of *Halicystis* showing cellulose ring and cellulose particle formation (1540 ×). *Column* B, Stages in starch formation in the chloroplasts of the cotton plant (1540 ×). *Column* C, Cellulose particle formation takes place in the colorless plastids of the cotton fiber by a process of successive ring formation and fragmentation essentially similar to the mechanism of cellulose formation in *Halicystis* (1540 ×).



(Figure 109)

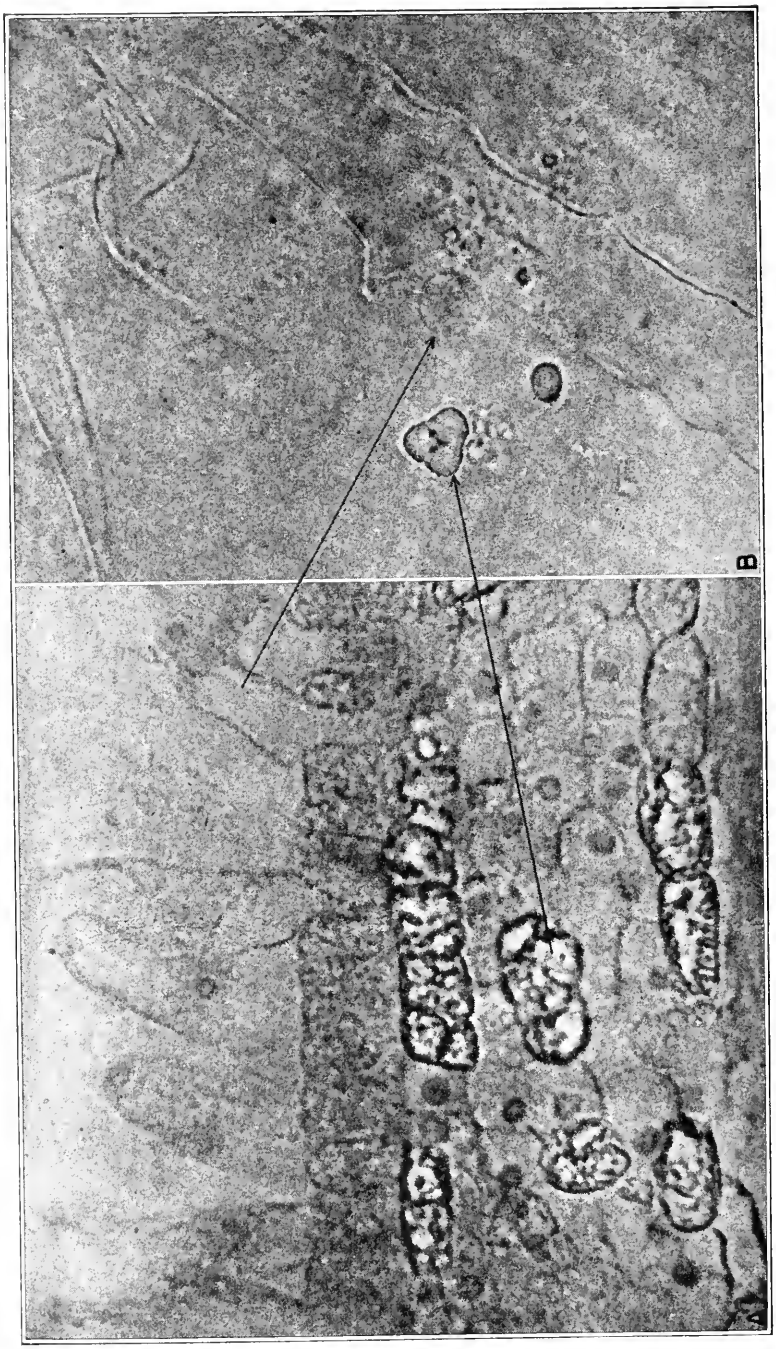


FIGURE 110. A, Cross section of a young cotton seed showing the cells of the epidermal layer in the process of elongation to form cotton fibers and certain cells of the outer integument of the seed filled with starch (420 ×). B, Starch-forming plastid from an integument cell and a cellulose-forming plastid from a cotton fiber photographed together to show their comparative visibility (1100 ×).

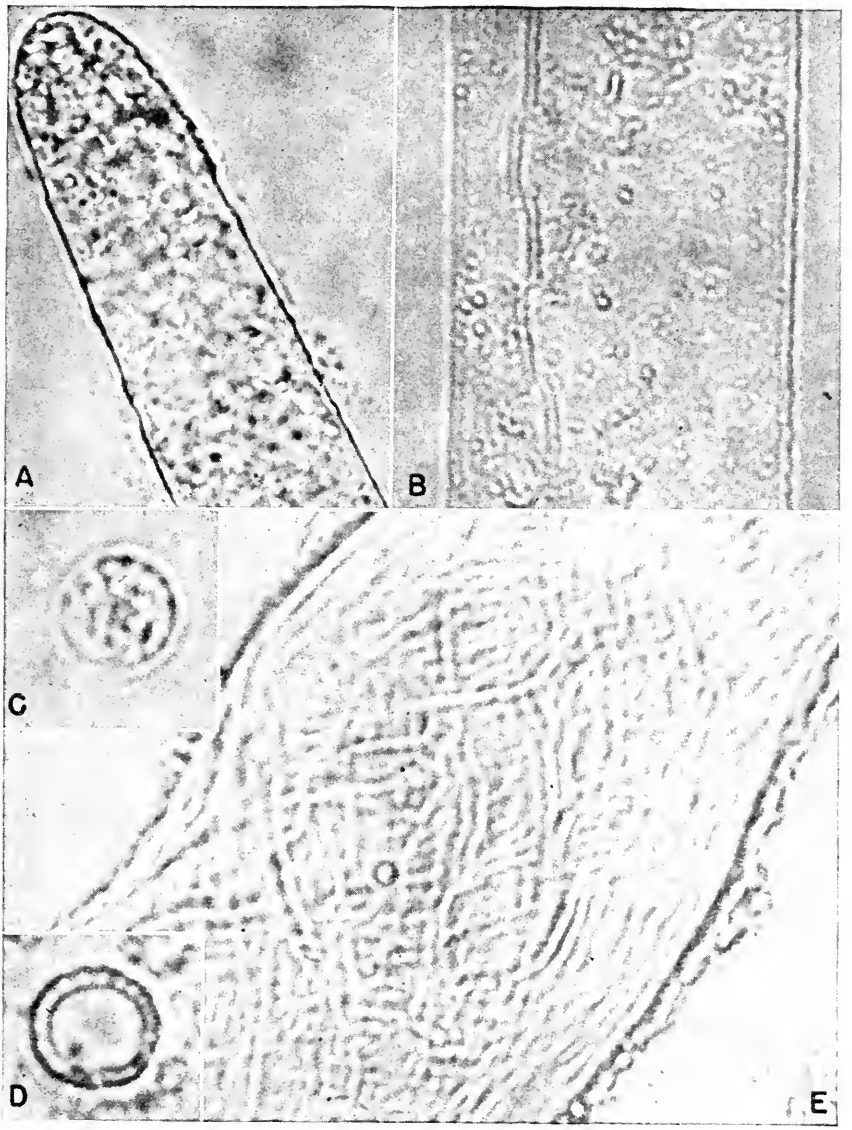


FIGURE 111. A, In the protoplasm of young cotton fibers, disc-shaped cellulose-forming plastids, varying over a wide range in diameter, are barely visible. The relative refractive indices of the plastids and of the cytoplasm in which they are floating obscure their contents and cause them to resemble vacuoles (1000 ×). B, Early stage of fibril formation in the young cotton fiber (1320 ×). C, Cellulose particles and a cellulose ring in a plastid removed from a young cotton fiber (1650 ×). D, Fragmentation of a cellulose ring, taken from a plastid, to form the cellulose particles (1650 ×). E, Cell membrane of the cotton fiber after the deposition of cellulose fibrils in spiral arrangements (1254 ×).

proximity to and in precise alignment with the adjacent fibrils, thus excluding, in large measure, the colloidal, non-cellulosic materials from the cell membrane. This procedure will account for the high degree of crystallinity and the regular orientation of the mature *Valonia* membrane.[18] Detailed accounts of the visible aspects of cellulose and cell membrane formation in both *Halicystis* and *Valonia* (Fig. 112) are being prepared for publication in *Contributions from Boyce Thompson Institute.*

These studies of bacteria, fungi, algae, and other types of cells from various parts of the plant kingdom, represent the experimental procedures in the attempt to understand the chemical and physical variations involved in the formation and structure of plant cell membranes.

When the cell membrane studies at the Institute terminated in 1940, sufficient evidence had been accumulated to indicate the chemical heterogeneity and physical complexity of plant cell membranes in general; the particulate state of the cellulose in many membranes from various parts of the plant kingdom; the importance of non-cellulosic materials in membrane formation and function; the intimate colloidal associations of the membrane components, which render their identification extremely difficult; the importance of using fresh (undried) material, whenever possible, for experimental purposes; and the need to develop more precise methods of fractionation and identification in order to determine the roles played by the various membrane components in both native and processed materials.

The physical aspects of cellulose synthesis in the living plastid, as well as the behavior of membrane-forming materials during their period of organization in the outer layers of the protoplasm, suggest a new field of experimental approach to the study of the formation and structure of plant cell membranes. The forces involved are frequently neither of a simple molecular nor of a gross physical type. They fall rather into the colloidal field of molecular aggregates of various dimensions and degrees of purity. Surface coatings sometimes mask the chemical identity of such molecular aggregates and determine their behavior in an electrical field. As data accumulate, it becomes more evident that the properties of both living and processed plant cell membranes must be interpreted in terms of the colloidal systems which they represent, and not in terms of the molecular behavior of any one component.

The skill with which nature synthesizes and manipulates the plant cell membrane materials will become more and more impressive as a clearer understanding of the chemical and physical forces involved is obtained. Until the accumulated information is more extensive than what we have at present, we shall not be able to understand fully, *e.g.*, the observed similarities of properties of membranes of cells of very different types and the differences in the make-up of membranes of closely related cells. To future research is left the task of finding and establishing basic principles of structure and composition common to all types of plant cell membranes,

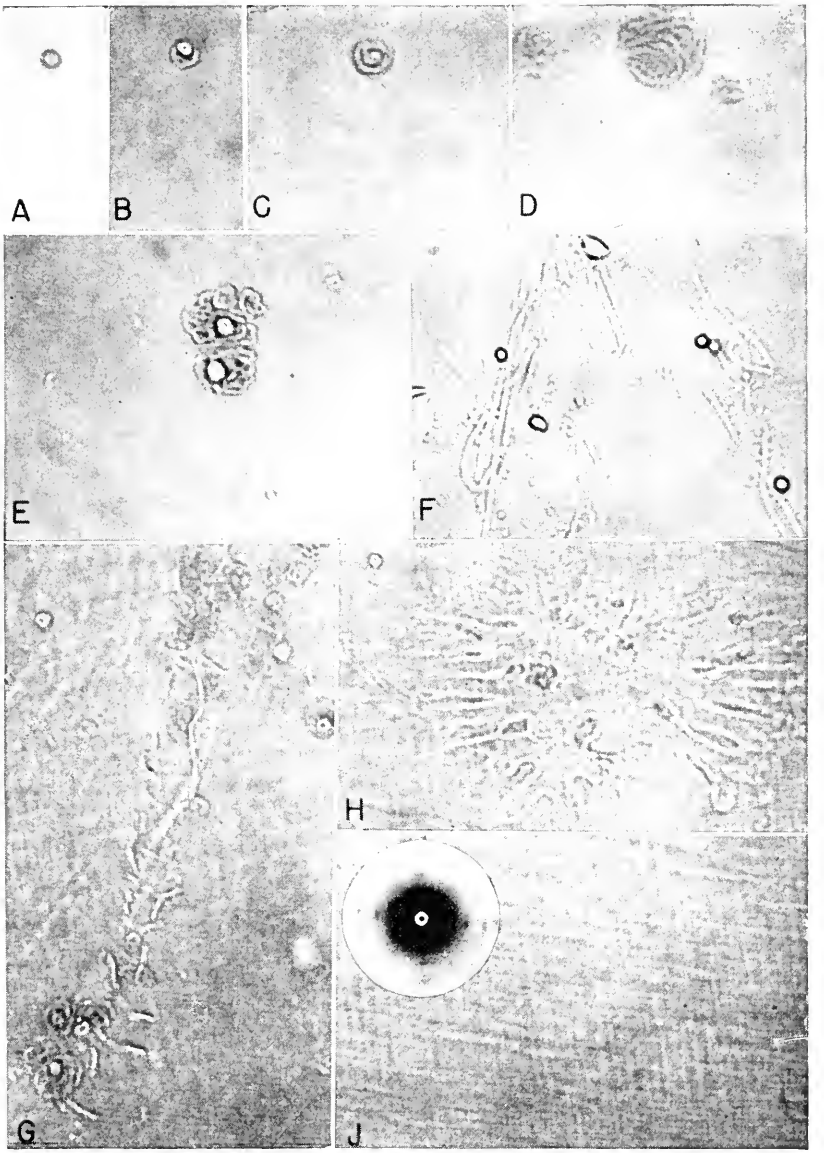


FIGURE 112. A–D, Cellulose ring and cellulose fibril formation in the developing chloroplasts of *Valonia ventricosa* (500 ×). E, Removal of plastid membranes from two plastids and washing away green plastid plasma makes rings and fibrils more clearly visible (625 ×). F, After removal of plastid membranes mature fibrils straighten and readily break into cellulose particles (700 ×). G, H, Fibrils uncoil from plastids on inner surface of membrane and align themselves with fibrils in developing membrane (G, 625 ×; H, 700 ×). J, Orientation of fibrils in mature membrane as shown by microscope and X-ray diffraction (700 ×).

and of explaining the procedures by means of which every given type of plant cell produces the membrane suited to its vital needs.

Summary

(1) Problems relating to the formation and structure of plant cell membranes have been approached, in the Boyce Thompson Institute for Plant Research, through microscopic, chemical, and x-ray diffraction analyses of cell membranes from various parts of the plant kingdom.

(2) The first experiments dealt exclusively with the phenomenon of *cell enlargement* in root hairs, in the membranes of which cellulose is either absent or only sparingly present. Their plastic properties are determined by the colloidal mixtures of non-cellulosic materials.

(3) Cotton fiber studies began with the more general aspects of their development from epidermal cells of the seed coat and resulted in additional information concerning their origin from cells which had divided subsequent to fertilization; their nutrition through the basal connection with the seed and not from "boll sap"; and the spiral fibrillar structure of their secondary lamellae, as shown in polarized light and by means of x-ray diffraction. The latter studies were made possible by the presence of crystalline cellulose in the fiber membranes in addition to colloidal non-cellulosic materials.

(4) Failure to obtain information concerning the fine structure of the fibrils of the secondary lamellae led to studies of less highly differentiated cellulose-forming cells including bacteria, fungi, and algae.

(5) As a result of improved microscopic techniques, designed to observe the cellulosic and non-cellulosic portions of *Acetobacter xylinus*, crystalline cellulose was identified for the first time in the protoplasm of young cotton fibers. It was in the form of ellipsoids, approximately $1.0 \times 1.5\ \mu$ in size, which were named "cellulose particles" and were identified with Strasburger's "microsomes" and Wiesner's "dermatosomes."

(6) Single rows of these cellulose particles are arranged end to end to form the fibrils of the cotton fiber. The process takes place in the outer regions of the living protoplasm and is followed by the deposition of spirally arranged fibrils in a matrix of non-cellulosic colloidal material, to form the secondary lamellae. Both pectic material and protein are present in this matrix. Although the lamellate structure of the secondary wall indicates the periodic deposition of wall materials, evidence was obtained to indicate that this is not a daily periodicity; hence the lamellae do not represent "daily growth rings."

(7) In cells such as the green alga (*Halicystis*) fibrils are not formed and cellulose particles in the successive lamellae are in more or less random arrangement. In the *Avena* coleoptile, end-to-end bondage of cellulose particles is not strong enough to prevent separation during cell elongation. The stretching of the non-cellulosic membrane materials leaves the cellulose in ring-like bands along the extended membrane.

(8) The process of cellulose formation in living protoplasm was first observed in *Halicystis*. The mechanism involved is one of successive cellulose ring formation in the green chloroplasts, followed by ring fragmentation to form the cellulose particles.

(9) Cellulose formation by a similar method was later observed in colorless plastids of the cotton fiber and in cells of the leaf and stem tissues of the cotton plant. In the latter cells starch and cellulose are formed simultaneously in chloroplasts and colorless plastids respectively.

(10) A conspicuous variation in the process of cellulose formation in the chloroplast of *Valonia* consists in the formation of one closed cellulose ring followed by the formation of a coiled cellulose fibril.

(11) All mature cell membranes containing cellulose particles in various proportions and types of arrangement were found to contain non-cellulosic materials of various types and in different proportions. These combinations of crystalline and non-crystalline components in intimate colloidal association constitute a basis for understanding the relative properties of the membranes of highly differentiated fibrous cells, such as cotton and ramie, as well as those of the more primitive bladder-like cells, *Halicystis* and *Valonia*. Considerations of the function of non-cellulosic materials in controlling the phenomena of plasticity and deformability during the period of *cell enlargement*, and the function of cellulose in establishing strength and rigidity in the process of cell differentiation, constitute a new analytical approach to the study of these two aspects of plant growth.

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CHAPTER 9

Plants Grown under Controlled Environmental Conditions

Just previous to the planning and building of the Institute there were several new developments in plant and animal physiology that indicated the desirability of equipment for growing plants on a fairly large scale and under a wide range of conditions as to light (quality, intensity and daily duration), concentration of carbon dioxide, temperature, humidity, nitrate supply, etc. The following may be mentioned as the more prominent of these researches. In 1920 Garner and Allard [17] had published their monumental discovery that day length initiated flowering in various kinds of plants. Kraus and Kraybill [20] gave a new slant to the problem of reproduction in plants when they published their paper on the importance of the proper balance between carbohydrate and nitrogenous substance in plants as a determiner of fruit set and development. This paper focussed much attention on the significance of the C/N ratio in plants. This ratio is of course determined by the relative amount of photosynthesis on one hand, and the amount of nitrogen compounds absorbed on the other; therefore, all factors that affect these two processes in plants need consideration.

Schanz [30, 31] found evidence that ultraviolet rays may cause cataract by coagulating the proteins of the crystalline lens of the eye, and that these rays are of great importance in determining the form of plants. The injurious action [40] of ultraviolet was receiving much consideration. Before the Institute was formally opened, Steenbock,[35] and Hess and Weinstock,[19] had found simultaneously that ultraviolet rays impart antirachitic action to various vegetable and animal materials. It turned out later that the action of the ultraviolet rays was on one of the sterols, *i.e.*, ergosterol.

It has long been known that the carbon dioxide content of the atmosphere is too low to give maximum photosynthesis when other conditions are at or near the optimum for this process. The experiments of H. Fischer, Riedel, Jesse and others,[24, p.121] just previous to 1920, indicated that crop yields could be increased markedly in greenhouses and even outside by CO_2 enrichment of the air; this of course increases the amount of photosynthesis, as does longer daily illumination. Would it induce the flowering of long-day plants as does the long day in accordance with the C/N ratio conception, or is flower induction by day length due to a specific effect of light quite independent of photosynthesis?

Three rather large installations [26] for modifying and controlling growth conditions for plants were built as a part of the Institute: (A) the constant-condition dark and light rooms where plants could be grown under artificial light exclusively with day length, temperature, humidity, and carbon dioxide concentration of the air regulated; (B) the gantry crane house where plants were grown during the day in daylight and 6 or 12 hours during the night under light from tungsten lamps attached to a gantry crane; (C) the spectral glass houses in which plants were grown under different regions of the solar spectrum, as determined by the solar transmission of the glasses on the several houses. Later an insulated greenhouse was built which was lighted by sunlight through a southern exposure of glass during the day and which was further illuminated by tungsten lights attached to a thermostat. These lights were the sole heat source of the house, and at the same time they supplemented the sunlight, largely at night or on cloudy days, for when the sun was shining little additional heat was required. Besides these larger pieces of equipment, much other apparatus was purchased or made at the Institute for regulating, measuring, and recording various growth conditions. The several pieces of equipment will be briefly described later in connection with the experiments carried out with them.

Plants Grown Entirely in Artificial Light

To attempt to grow plants under artificial light in competition with sunlight is very expensive. Arthur [5] figured the value of the radiant energy falling on an acre of land at Yonkers, New York, during 1936 under the assumption that it could be converted quantitatively to electricity and the electricity sold at two cents a kilowatt hour. The figures in Table 29 are calculated from New York meteorological data.

On this basis the annual value of sunlight falling on an acre at Yonkers is about $106,000. This makes it very clear why artificial light cannot compete with sunlight in growing plants, and why men like Abbott of the Smithsonian Institution spend their time working up apparatus for trapping sunlight as a source of energy. Who ever heard of a crop being worth a considerable fraction of $106,000 per acre year?

Constant-condition rooms. The two constant-condition rooms [5, 6] (Fig. 113) were built in the basement under the greenhouses, each having a floor space of 11 × 11 feet, or 121 square feet. Both rooms were attached to the same air-conditioning and carbon dioxide-enriching systems so that during any given period both had the same temperature, humidity, and CO_2 concentration in the air. One room was dark and gave the plants their night; the other was illuminated by 25 1000- or 1500-watt Mazda lights. The lighting made it difficult to air-condition the room. This was accomplished by placing a plate-glass ceiling between the lights and growing room, over which a weir-regulated layer of water flowed. A large fan also aided in cool-

Table 29. With Electricity at 2¢ per Kilowatt Hour, What Was Sunlight Worth per Acre for Each Average Day During the Year 1936?

(*The figures are calculated from New York Meteorological data*)

Month	Total gram calories/sq cm/mo	Average kw hours/acre/day	Average net worth/acre/day of sunlight
May	16,935	25,689	$514.00
July	15,824	23,995	480.00
June	13,510	21,173	423.00
August	12,672	19,243	385.00
April	10,752	16,844	337.00
September	9,479	14,868	297.00
March	8,520	12,939	259.00
October	7,135	10,822	216.00
February	5,659	9,175	184.00
November	4,968	7,810	156.00
January	4,030	6,117	122.00
December	3,771	5,740	115.00
Average for year	9,438	14,534	$291.00

ing by giving a rapid exchange of air about the lamps. While Mazda lights have about 90 per cent of their energy in the infrared, this cooling reduced the heat ray to about 50 per cent, about the proportion of heat to light ray existing in sunlight. The light reaching the plants, however, was dominantly red-yellow, so mercury-vapor lights in glass tubes were burned along the walls of the growing chamber to increase the blue-violet rays. Even with this enrichment, the light in this room was dominantly red-yellow as compared with daylight. With all 25 1500-watt lights burning, the intensity of the light in the growing room was about 900 foot-candles. This is a low intensity compared with the maximum of sunlight at Yonkers at noon in June, which is about 10,000 foot candles. The light in the room, however, was constant for 24 hours of the day, whereas that in nature is markedly variable during the day, and is non-existent at night. When 25 1000-watt lamps were used or when only a portion of the 25 lamps were on, the intensity of the light was lower. The same was true after the lamps began to age. To avoid the reduction in intensity due to aging of the lights, they were changed after 40 to 45 days of constant burning, or the experiments were limited to this period. During some of the experiments the intensity of the light in this room was as low as 350 foot-candles.

In most of the experiments the temperature of the room was held at 78° F (26° C), but runs were also made at 68° F (20° C). This proved fortunate in the study of the potato, as we shall see later. The CO_2 in these rooms was run at ten times normal, or 0.3 per cent* and the relative

* The CO_2 enrichment of the air in the constant-condition rooms was always accomplished by use of tanks of liquid CO_2. Mostly the same was true of the greenhouses. For two years the source of CO_2 for one of the greenhouses was scrubbed flue gas from the Institute boilers. To free the flue gases of all toxic or tarry materials, and at the same

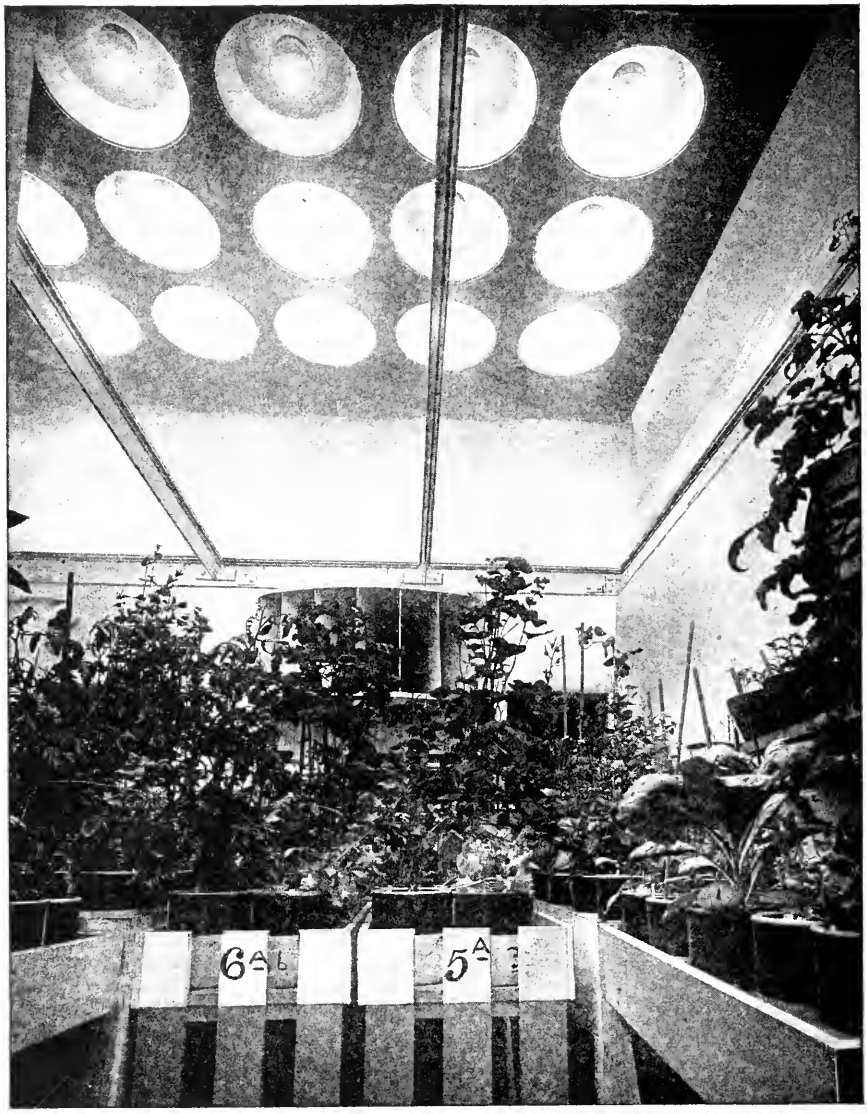

FIGURE 113. Constant condition light room.

humidity largely at 80 per cent. All plants except those grown under continuous illumination were kept on wheeled benches so they could be rolled from the light to the dark room and *vice versa* to get the light day and night desired. The plants were grown under 5, 7, 12, 17, 19, and 24 hour daily

time leave the CO_2 in the gas, required a complex system of scrubbers. Planning and building the scrubbers and determining the chemicals and other materials to be used in the several units was a research problem in itself. Because of lack of space the reader is referred to the original article for details.[6]

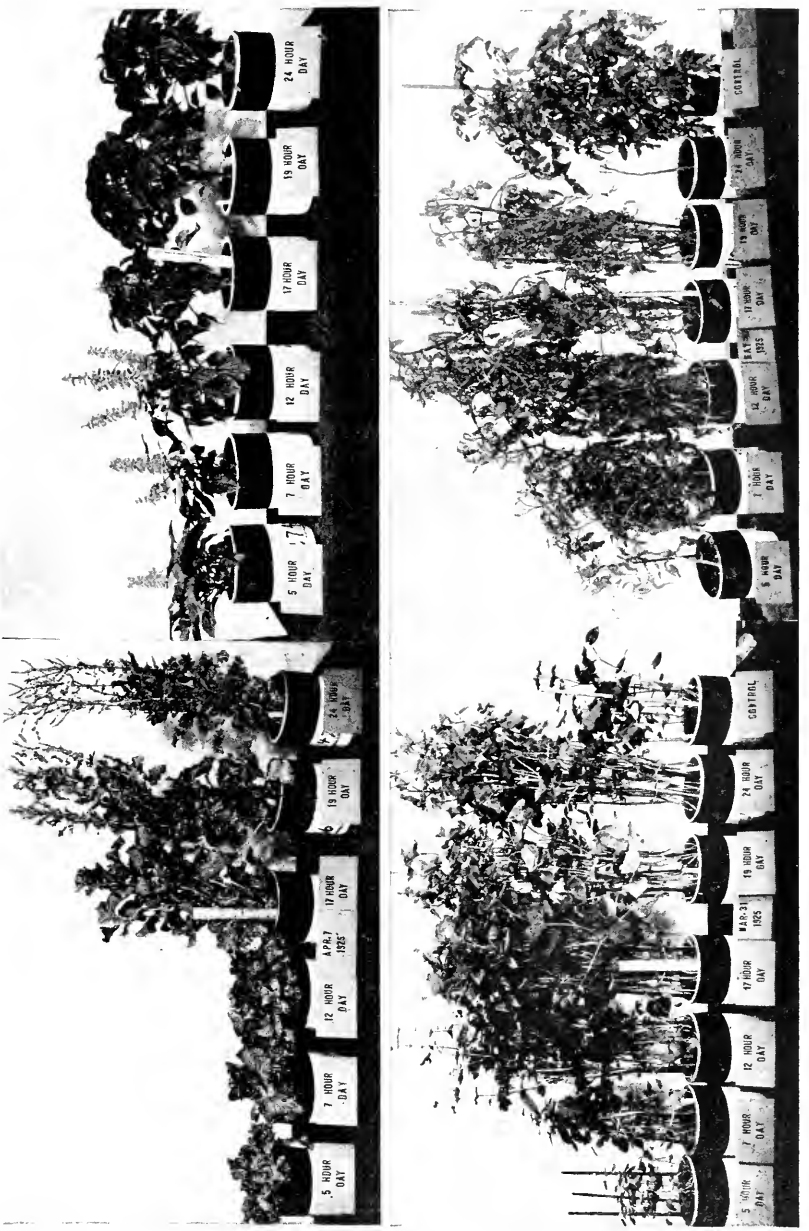

FIGURE 114. Lettuce, salvia, buckwheat, and tomato grown on 5, 7, 12, 17, 19, and 24-hour days in the constant light room.

illumination. This kept all space in the light room occupied all the time. When the 5-hour-day plants were getting their night, the 19-hour-day plants were occupying their space in the light room. The same was true of the 7-hour- and 17-hour-day plants, and two shifts were run in the 12-hour day. This economical use of light was desirable even in an experiment, for the cost of lighting this room was great. With the maximum illumination used, the current for the lights alone at two cents a kilowatt hour cost $18 a day. The cost for air-conditioning, CO_2 enrichment, etc. must of course be added to this. To illuminate an acre of land with the same light system and intensity would cost $6480 a day. These costs put in doubt the feasibility of economically growing plants exclusively in artificial light, even if a much more economical light than the Mazda is found.

Now let us look at the plants grown in these rooms. Fig. 114 shows lettuce (upper left), salvia (upper right), buckwheat (lower left), and tomatoes (lower right) grown in these rooms for 32 days, each with six different daily periods of exposure to light (5, 7, 12, 17, 19, 24 hours). Lettuce did not flower with 5, 7, or 12 hours of daily illumination, but it did so with 17, 19, and 24 hours. It is a long-day plant. The cereal grains also flower earlier on long days, as shown in Fig. 115 for barley.

FIGURE 115. Barley grown during the period February 28 to March 24, 1926, at 68° F (20° C), in the constant light room on 5, 7, 12, 17, 19, and 24-hour days. The plants marked control at right were grown under ordinary greenhouse conditions.

In this plant the amount of growth increases as the day increases from 5 to 17 hours. Heading has already occurred in the 17-hour day and has occurred still earlier on the 24-hour day. Note in this figure that the control in the ordinary greenhouse during February and March showed a little less growth than the plants in the constant-condition room on the 12-hour day but more than the plants in the shorter days. In contrast to lettuce,

salvia flowered with 5, 7, and 12 hours of daily illumination, showed only incipient flowering with 17 hours, and no flowers at all with 19 and 24 hours. It is a short-day plant. Buckwheat flowered on all day lengths and produced more dry weight as the day lengthened up to a given day length. The plant grew much more on the longer days with artificial light than it did in the greenhouse when the run was made during March. This is a day-length indifferent plant. The tomato also flowered on all day lengths in which it would continue to grow, but it was soon killed under continuous artificial illumination here used. When given 12 hours a day of artificial illumination and 12 hours a day of sunlight it did better. The sunlight seemed to balance the injurious effect of artificial light to a degree, but not completely.

The tomato is especially sensitive to continuous artificial illumination, and geraniums and coleus are likewise much injured by it. Even plants that showed no visible injury in continuous artificial illumination gave indications of incipient injury. Cabbage * increased in weight and carbohydrate content and fell in nitrogen per cent as the day length increased up to 17 or 19 hours. With continuous illumination, weight and carbohydrate content fell again and nitrogen percentage increased. Spring wheat and barley showed similar day-length curves in artificial light, and radish showed a continuous increase in dry weight and carbohydrate percentage up to 17 or 19 hours, but a proportional increase was not maintained for the 24-hour illumination. Apparently artificial light alone shows the same day-length effect as daylight or a combination of daylight and artificial light; radishes flowered on long days but not on short days, and in the spring cereals flowering was hastened by long days. The potato showed good tuberization even in continual artificial illumination at 68° F (20° C) but poor under continuous illumination at 78° F (26° C). Tuberization seems to be favored by the joint action of low temperature and long day. The supplemented daylight in the gantry crane house showed similar effects on tuberization. The radish seems to respond readily to increased nitrogen content with abundance of nitrates in the soil and to increased carbohydrate content with conditions that favor photosynthesis, long day and increased CO_2 content of air. With 12-hour illumination and 12-hour daily darkness it has the same composition as on 24-hour alternate illumination and 24-hour darkness; it does not flower in the first case but does in the second. We shall return to a fuller consideration of the chemical composition of plants grown in these rooms after describing the experiments in the gantry crane house and in other special growing conditions, at which time there will be evidence for more general conclusions.

* Cabbage leaves wrinkled and cracked under artificial illumination and the injury increased with day length. This reaction to artificial light was partly corrected by daylight. It was also less marked in artificial light of lower intensity, *i.e.*, 350 foot-candles. The authors suggest that the constant direction of the artificial light rather than the quality or daily duration may cause the wrinkling and cracking.

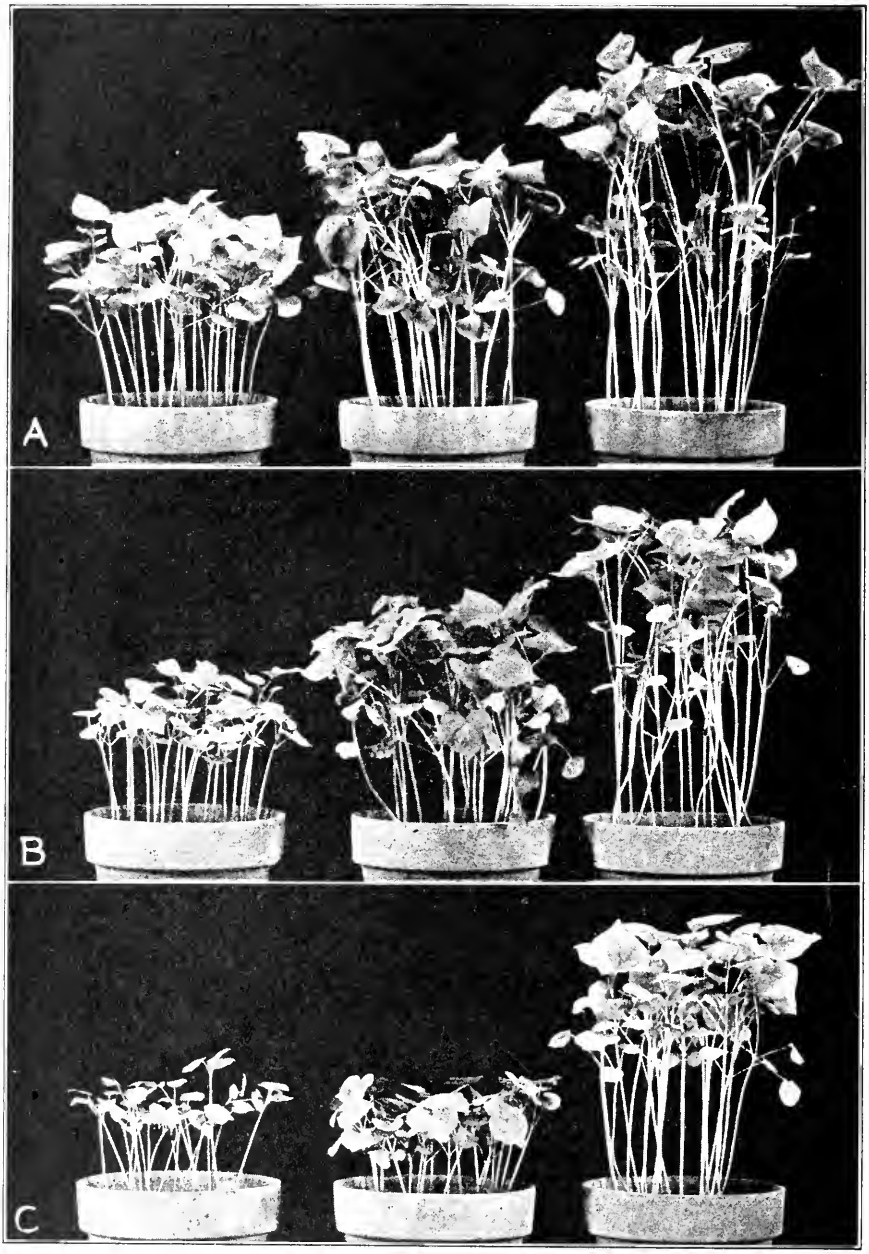

FIGURE 116. Buckwheat plants. *Left to right:* A, greenhouse, neon lamp, Mazda lamp. B, greenhouse, sodium vapor lamp, and Mazda lamp. C, greenhouse, mercury vapor lamp, and Mazda lamp.

Relative effectiveness of Mazda, neon, sodium vapor, and mercury vapor lamps. Buckwheat seedlings [3, 14] were grown for 8 to 11 days under continuous illumination from Mazda, neon, mercury vapor, and sodium vapor lamps of equal intensity as determined by the Weston photronic cell, which measures largely visible rays and has a light sensitivity similar to that of the human eye. The temperature was about 77° F (25° C), the humidity 70 per cent, and the light intensity was 700 to 800 foot-candles, as against a maximum of 900 in the constant-condition room. The lights were adjusted as the plants grew, to keep the intensity at the tip of the plants constant. Fig. 116A shows growth of buckwheat plants under neon lamps with greenhouse-grown check on left and Mazda light-grown check on right; B shows the same for the sodium vapor lamp, and C for the mercury vapor lamp. The artificial illumination was of course continuous 24 hours a day in all cases, while the greenhouse check had less — 12 hours' daily illumination. One would judge from the relative heights of the plants in this picture that Mazda lamps gave much more growth than the other artificial lights. When the growth is measured by increased dry weight (Table 30) the difference is less, or in case of the neon light the production is actually greater than for the Mazda light.

Table 30. Ratios of Dry Weight Produced under Each Lamp as Compared with the Mazda = 1.00; Calculated from Table I [14]; Weights in Grams per Plant

	Ratio leaf areas per plant	Stems	Leaves	Whole plant	Weight cal'd to equal energy basis in visible region
Mazda	1.00	1.00	1.00	1.00	1.00
Sodium vapor	1.12	0.70	1.12	0.90	1.41
Neon	1.00	0.92	1.35	1.10	1.20
Mercury vapor	0.73	0.43	0.96	0.66	0.62

The figures in the last column of Table 30 represent the dry weight which might be obtained if equal energy value in the visible had been used. The Weston photronic cell registers 98 per cent of the visible energy of the sodium vapor light, 65.4 per cent of that of the neon, 62.4 per cent of that of the Mazda, and 58.1 per cent of that of the mercury vapor. These figures are taken into consideration in the calculation. For details on the calculation the original article should be examined.[14] Figuring on the efficiency of the energy in only the visible portion of the spectrum of sodium vapor and Mazda lamps in increasing the dry weight of plants, the former is 1.41 to 1. If the calculation for the relative efficiency of the two lamps were made on the basis of the total radiant energy (including the infrared) the sodium vapor would rank much higher, because the radiant energy of sodium vapor is largely in the visible and that of Mazda lamp largely in the infrared. In the constant-light room, as we have seen, with Mazda lamps supplemented by mercury vapor in glass, a fairly good growing light was obtained

FIGURE 117. An arrangement of four 10,000-lumen sodium vapor lamps designed especially for plant work.

at a cost of 15 cents a square foot day. As we shall see later, sodium vapor lights supplemented with mercury vapor or Mazda lights give a good growing light for plants at 3 cents per square foot day. This study indicated the efficiency of the sodium vapor lamp and led to the experiments reported in the next section.

The authors make the following statement concerning these four lights and the absorption of their rays by chlorophyll: [14, p.130] "A consideration of the absorption spectrum of chlorophyll shows no relation between the emission bands of the various lamps, the absorption bands of the chlorophyll pigments, and the efficiency of the lamp in producing dry weight of plant tissue. The sodium lamp was found most efficient, with the main output of energy at wave lengths 588 and 589 mμ, a point at which chlorophyll absorption is near the minimum. The neon lamp was second in efficiency with the main output band near the maximum of chlorophyll absorption in the red-orange region. The mercury vapor lamp was least efficient, yet has much of the energy output in the blue-violet region where chlorophyll absorption is maximum. The sodium lamp has an efficiency of 45 lumens per watt and a remarkably low power loss in the auxiliary transformer unit of only 25 watts, as compared with a current consumption of 200 watts in the arc itself." This is understandable when it is realized, as is well known, that photosynthesis makes very inefficient use of light at the best. It should be emphasized that the plants in this work were grown under the lights for only 8 to 11 days. Plants grown continuously for a long period under sodium vapor lamps unaccompanied by any other illumination are injured. The three gaseous discharge lamps produced greener leaves and a lower ratio of stems to leaves than the Mazda lamp.

Supplemental sodium vapor lamps as constant light source. Since sodium vapor lamps had proved a good and cheap light source for growing seedlings over a short period, they were tried [12] for growing plants over a long period under continuous illumination. Plants do well for a short time under this light but after two months of continuous exposure only a few yellow leaves remained at the tip of the plants. This is especially true at temperatures somewhat above 70° F (21° C) and far less marked at 63° F (17° C). These plants recover when still under continuous illumination from the sodium vapor lamp if they are illuminated two hours daily with 85-watt capillary mercury vapor lamps with the temperature at 63° F (17° C). The final set-up for these experiments is shown in Fig. 117.[7] Four 10,000-lumen sodium vapor lamps are mounted in the form of a square two feet on a side and a small 85-watt capillary mercury arc lamp, type H–3, is mounted in the center of the square, all with reflectors as shown. The sodium vapor lights burn continuously and the mercury vapor lamp is turned on two hours each day. Several kinds of plants (begonia, gardenia, cotton, geranium, and buckwheat) did well under this illumination. It did not prove satisfactory for the tomato, which does not do well under any constant illumination yet tested. Fig. 118 shows several kinds of plants grown

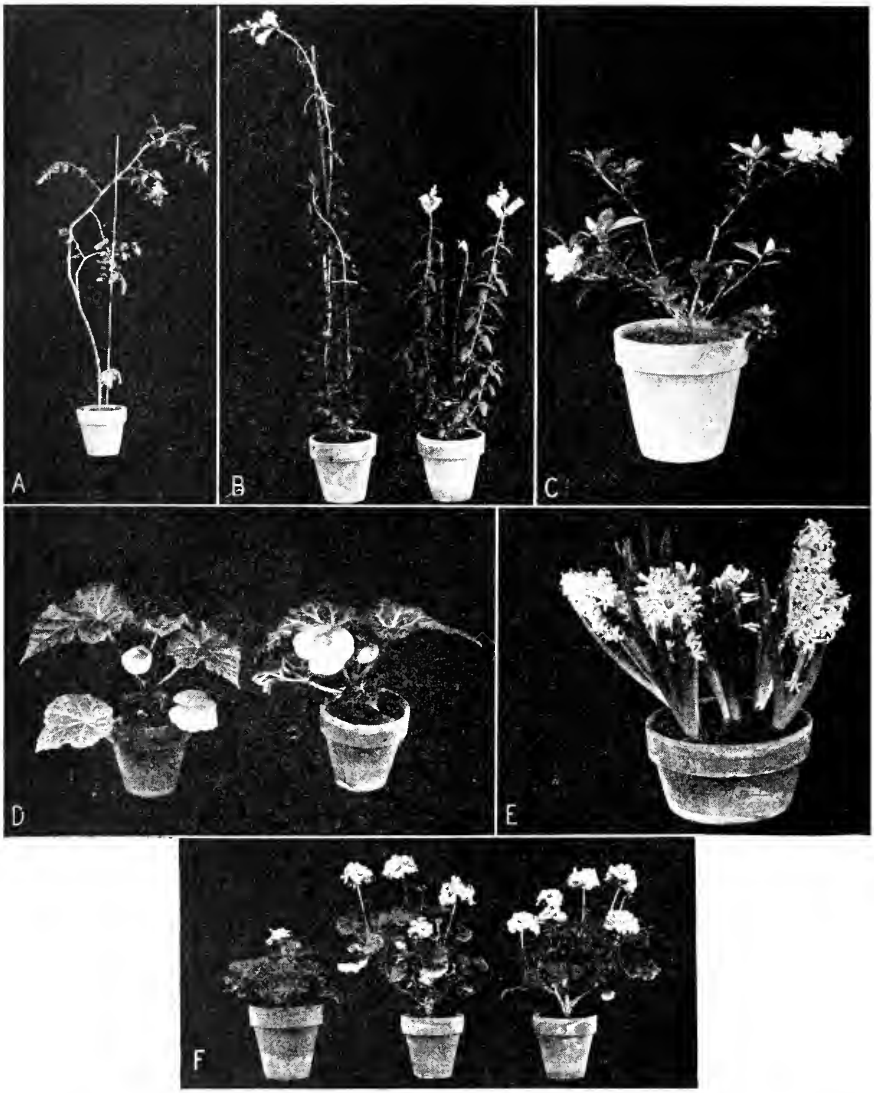

FIGURE 118. Various plants grown under continuous sodium vapor light supplemented by mercury vapor two hours per day. A, Tomato. B, Snapdragon. *Left:* artificial light; *right:* greenhouse control. C, Gardenia. D, Tuberous rooted begonia. *Left:* greenhouse; *right:* artificial light. E, Hyacinth. F, Geranium. *Left:* greenhouse control; *center:* grown under continuous sodium vapor lamps supplemented by Mazda lamps, intermittent illumination, 10 minutes on alternated with 20 minutes off; *right:* grown under continuous 400-watt high intensity capillary mercury arc lamp.

under this system of lights. The geranium that was injured by the continuous illumination in the constant-light room did well under this combination of lights. Later experiments have shown that a 150-watt Mazda lamp burned intermittently can be substituted for the mercury vapor lamp.

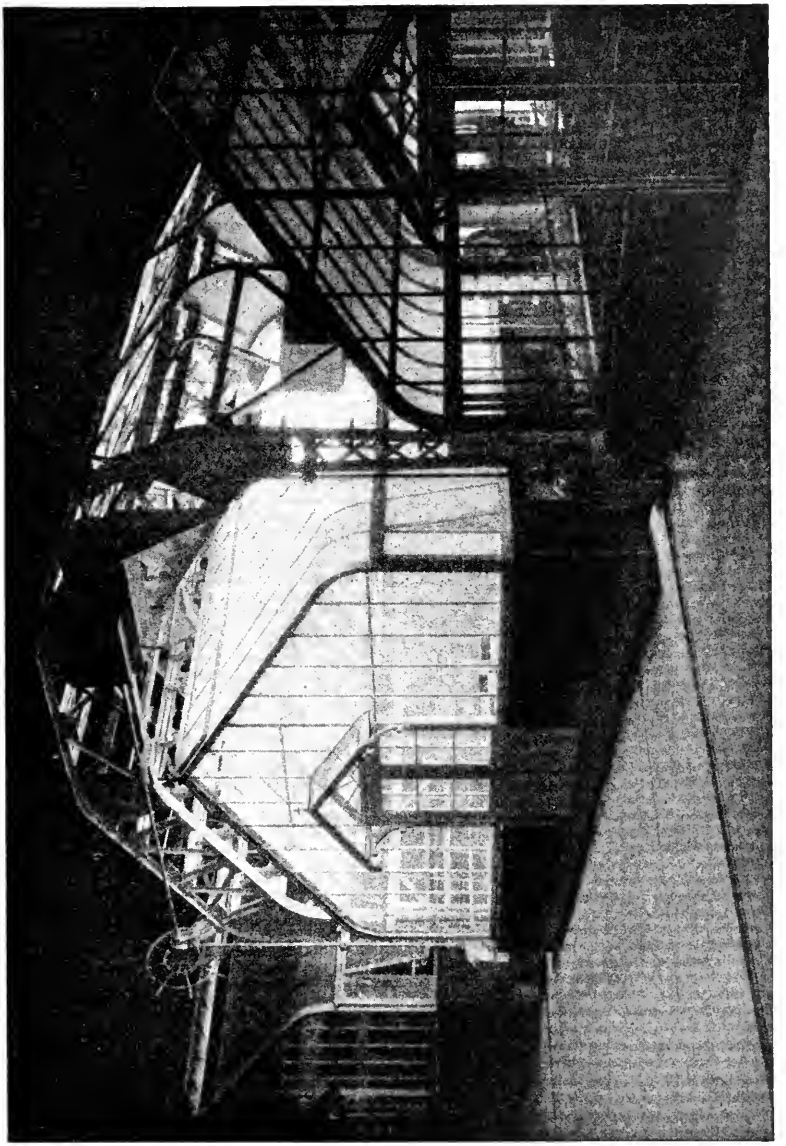

FIGURE 119. Greenhouse illuminated at night.

FIGURE 120. A, Red clover. The two pots of plants *at the left* were grown in the control greenhouse, *the two center* with 6 hours of artificial light supplementing daylight and with about ten times the normal concentration of CO_2 (gantry crane greenhouse). *The two at right* received the same illumination but no gas. Age from seed, 40 days. B, Three of the same pots of clover shown in A with the 24-hour day plant added. The latter was grown in the constant light room with artificial light only. Age from seed, 69 days.

The arrangement shown in Fig. 117 illuminates about 16 square feet of bench surface at 3 cents a square foot per day. The mercury vapor lamp used in this experiment differs from most mercury arcs in that it has a continuous spectrum similar to sunlight over which is superimposed a relatively strong bright line spectrum of mercury. The Mazda lamp, which seems to balance the sodium vapor lamp about as well as the mercury arc does, has dominant energy in the infrared and red-yellow, the blue-violet being rather weak. These experiments indicate a generalization that we shall have occasion to emphasize in connection with experiments to be reported later. Green plants will not grow normally under monochromatic

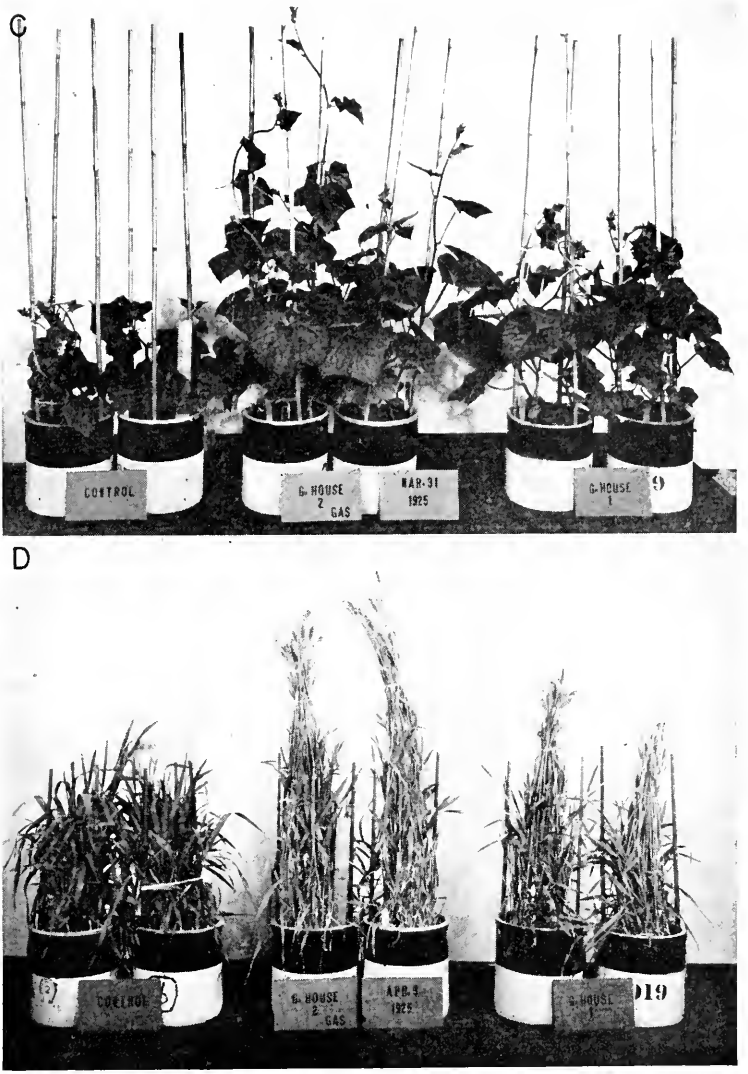

FIGURE 120 (*continued*). C, Cucumber. Two pots (*at left*) grown in control greenhouse. Two (*at center*) in gantry crane house with daylight plus 6 hours of artificial light each night plus ten times the normal CO_2. Two (*at right*) same as *center* except no extra gas. Plants are one month old from seed. D, Barley grown at a high temperature, 78° F (26° C). The two pots of plants *at left* were grown in the control greenhouse, two *at center* in the gantry crane greenhouse with daylight plus 6 hours supplementary lighting plus a higher concentration of CO_2, the two *right* same as *center* except no extra gas. Age from seed, 40 days.

light. Indeed, it is a question whether green plants will grow normally during their entire life period under any narrow band of the visible spectrum. If they will, it is probably under bands in the blue or violet. This region has as yet not been produced in sufficient intensity to give good growth.

Growing Plants under a Combination of Sunlight and Artificial Light

Gantry crane greenhouses. Two of the greenhouses (Fig. 119), each approximately 20 × 25 feet, were equipped for supplementing daylight with artificial light at night. The equipment consisted of a large gantry crane electrically driven so it could be easily moved over the greenhouse at night and be removed in the daytime. The crane carried 48 1000-watt lights, so spaced as to give uniform intensity over the center area of the greenhouses. This enabled the investigator to lengthen the daily period of natural illumination at will, with an intensity of light amounting to 400 or 500 foot-candles. In most of the experiments one of the houses was illuminated from 6 P.M. to midnight and the other one from midnight to 6 A.M. and the experiments were run during the winter months. One of the houses, G–2, was piped for CO_2 enrichment and CO_2 was run at 0.3 per cent when needed ventilation did not interfere. The other house, G–1, was run with normal CO_2 concentration.

From Fig. 120A and B, it is evident that red clover grows much faster when the daylight in winter is supplemented by 6 hours of artificial illumination at night; if this is accompanied by ten times the normal concentration of CO_2 there is another great addition in growth. The plants with extra light, and with extra light and CO_2, are in full bloom after 40 days of growth from seeds, while those without extra light are not in bloom at the end of the experiment. Under continuous artificial illumination red clover shows fine growth and flowering. In Fig. 120C the cucumber shows considerably more growth with 6 hours' extra illumination and very marked response to increased CO_2. Barley (D) responded as do the summer cereals generally by early heading on the long day. The extra CO_2 gave great additional growth over the 6 hours' extra illumination alone.

Fig. 121 shows that Clydesdale oats grown at 78° F (26° C) are headed after 40 days of growth in 6 hours extra light both with and without extra CO_2, and that CO_2 does not increase the growth at this temperature. The same oats grown under a 12-hour day are not headed and are taller than the plants with 6 hours' extra illumination. At 68° F (20° C) the 18-hour-day plants are headed whether with or without extra CO_2. The CO_2 under this condition gave much bigger plants. In this case plants were grown with extra CO_2 but no extra illumination. Extra CO_2 alone does not induce earlier heading. Blue Stem, a spring wheat, grown at 78° F (26° C) is headed after 47 days from seed when grown with 6 hours' extra illumination both with and without extra CO_2; extra CO_2 gives noticeable increase in growth. The plants grown under the 12-hour day are not headed. A similar condition holds for Blue Stem wheat grown at 68° F (20° C). Here again extra CO_2 with a 12-hour day does not cause heading. This wheat shows heading and great growth in height under a 24-hour day of artificial light.

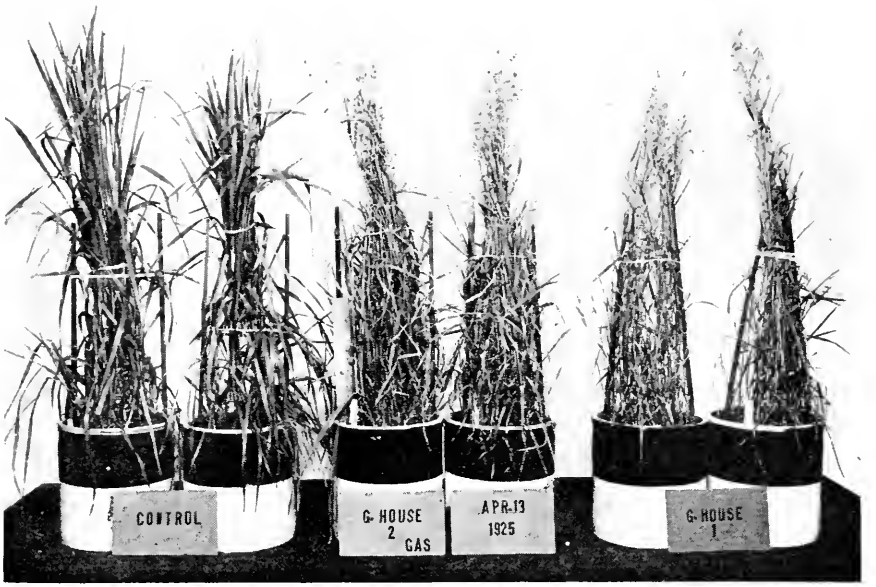

FIGURE 121. Clydesdale oats grown at 78° F (26° C). *Beginning at left:* control; 6 hours extra light and 0.3 per cent CO_2; 6 hours extra light, normal CO_2. Control house enriched with scrubbed flue gas. All plants 45 days old from seed.

As is well known, winter wheat is greatly delayed in heading and finally heads only sparsely if the imbibed grains or the plants in the rosette stage are not subjected to a low temperature for a considerable period. Fig. 122 shows a few heads starting on Hybrid 128 after 66 days' growth from seed in the 6 hours' extra illumination and extra CO_2 and under continuous illumination. Turkey Red wheat shows no heads when grown under like conditions for the same period. The ragweed is a short-day plant as shown in this figure. The plants grown in the control house during the winter months are in flower, while those grown with 6 hours' extra illumination have not flowered, although the 6 hours' extra illumination gave a marked increase in volume of growth. The extra CO_2 in addition to the 6 hours' extra illumination again adds markedly to the volume of growth. This makes clear why people in the latitude of New York begin suffering from ragweed hayfever in August; the day length at that time is right for inducing flowering of the weed. An 18-hour day for the entire growing season would largely eliminate flowering and ultimately destroy the plant, for it is an annual. We have already mentioned that the tobacco plant is injured by continuous illumination in the constant-condition room. This is illustrated in Fig. 122. In this figure also it is evident that the tobacco plant shows great additional growth in the greenhouse in winter when the sunlight is supplemented with 6 hours' artificial illumination at night. Extra CO_2 in the air together with 6 hours' extra illumination does not give additional growth.

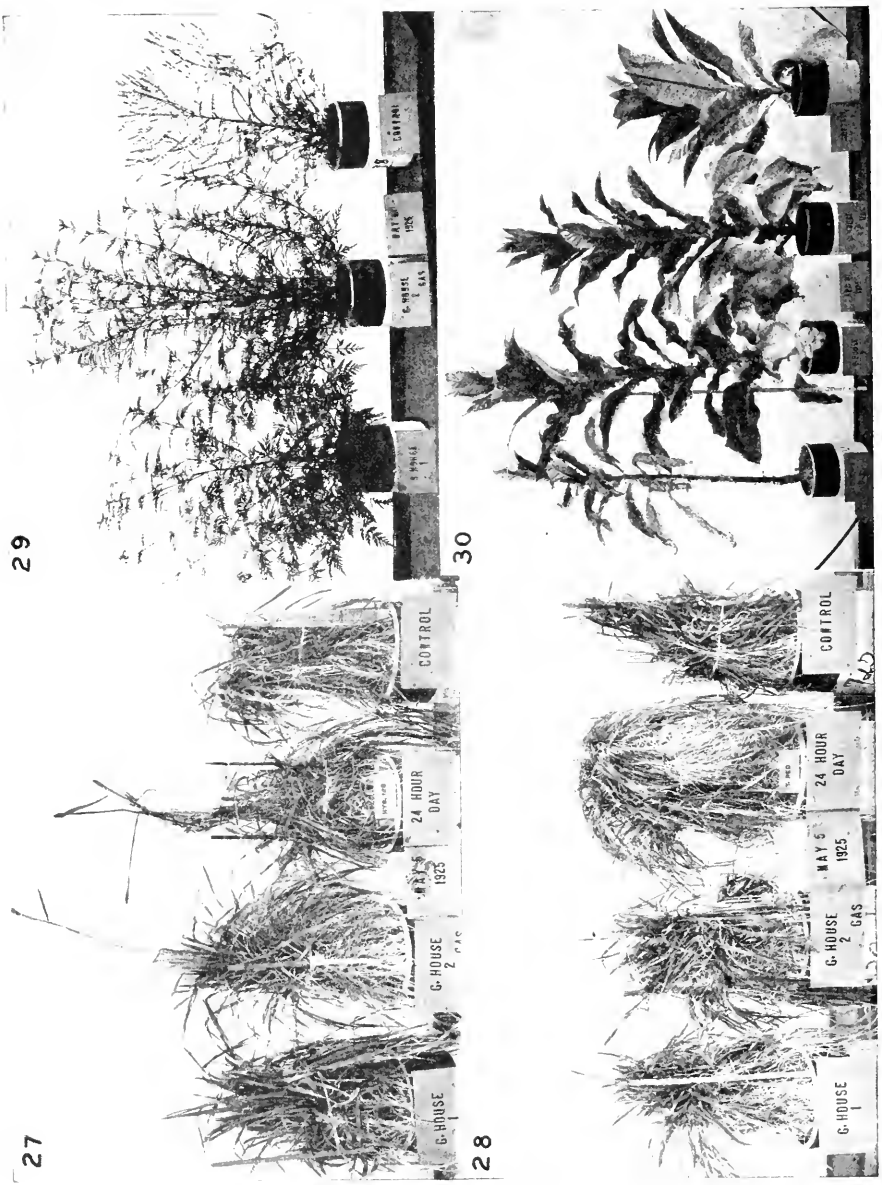

FIGURE 122. (27) Hybrid 128 wheat, (28) Turkey Red wheat (*left to right*): 18 hr. day; 18 hr. day + CO_2; 24 hr. day; control. (29) Ragweed (*l. to r.*): 18 hr. day; 18 hr. day + CO_2; control. (30) Havana tobacco (*l. to r.*): 24 hr. day; 18 hr. day; 18 hr. day + CO_2; control.

FIGURE 123. (31) Premier and Hoosier Beauty roses grown in gantry crane house with additional light and CO_2 showing the effect in forcing clusters of three sturdy roses at one time. New canes with flowers were produced from the root-stock in about the same time required to produce a single flower bud from existing canes in the control plants. (32) Nasturtium plants 70 days old from seed. The one *in the center* receiving both additional light and gas is flowering profusely. The plants from Greenhouse 1 receiving additional light only are little better than the controls (*at right*) on the normal length of day. (33) Geranium showing the flowering with supplementary light in Greenhouses 1 and 2 and the injury of continuous illumination (24-hour day). The control plant is *at the right*. (34) Variegated coleus, showing the increased growth with both additional light and gas (Greenhouse 2) as compared with additional light only (Greenhouse 1) *at left* and control greenhouse *at right*. The 24-hour day plant shows considerable light injury. (35) Eggplant showing the additional growth and fruiting with additional light only (*left*) and with both light and gas (*center*). The control *at right* did not fruit during the experiments.

It is seen in Fig. 123 that a ten-fold increase in CO_2 in the air with 6 hours' extra illumination gives a marked increase in the growth and flowering of rose, geranium, nasturtium, and eggplant, and also in the growth of varie-

gated coleus. The response of nasturtium to extra CO_2, both as to growth and flowering, is especially striking, exceeding in this respect the response of any other plant tested. There was relatively little response to the 6 hours' extra illumination without extra CO_2 except in the eggplant. This figure shows the injury caused in geranium and coleus by continuous illumination in the constant-light room.

Experiments were also run in the gantry crane greenhouses using 12 hours of artificial light and 12 hours of daylight. Even this proved injurious to tomato plants. In fact, tomato plants receiving 12 hours of artificial illumination at night are injured by daylight of more than 6 hours daily. The injury was lessened by reducing the intensity of the light. The authors suggest that it would be interesting to grow tomatoes well within the Arctic Circle in greenhouses in summer so that growing temperatures could be maintained and the plants subjected to continuous sunlight. We have already seen that certain plants (geranium, variegated coleus, etc.) that are injured by continuous illumination in the constant-light room thrive under continuous illumination from sodium vapor lamps supplemented by mercury vapor or tungsten lamps. A proper balance in the light spectrum seems to be demanded. It may be that daylight has a properly balanced spectrum for continuous illumination of even the tomato without injury.

Insulated greenhouse and intermittent light effect. Impressed as they were by the inefficient use of heat by conventional greenhouses, Arthur and Porter [12] designed an insulated greenhouse (8 ft. × 19 ft. inside) that makes efficient use of the sunlight in mid-winter and at the same time can be heated economically by use of 10 500-watt tungsten lamps attached to a single thermostat.

Fig. 124 gives a general idea of the structure, but for a detailed description the reader is referred to the article cited above. During the winter months the heat requirements for the house kept the lights on about 3.4 hours per day and this always at night except on cloudy days. With bright sunlight in mid-day even in zero weather one of the windows had to be opened slightly to prevent overheating. Since the house was so successful in preventing loss of heat, it also prevented the entrance of CO_2 from the outside air. Photosynthesis by the plants soon depleted the air of CO_2. As we shall see later, this deficiency was supplied in various ways. The average cost of heating the house with lights during December, January, and February was about 36 cents per day. The three to four hours of supplemental artificial light during the winter months produced growth in plants equivalent to that in an ordinary greenhouse in March and April.

The artificial light furnished in the insulated greenhouse was of course on and off intermittently during the night as the heat requirements demanded. Plants in nature receive intermittent light, but on longer day and night periods. A further study [8, 9] of the effect of short intermittent periods of light at night was made. To further this study and add more

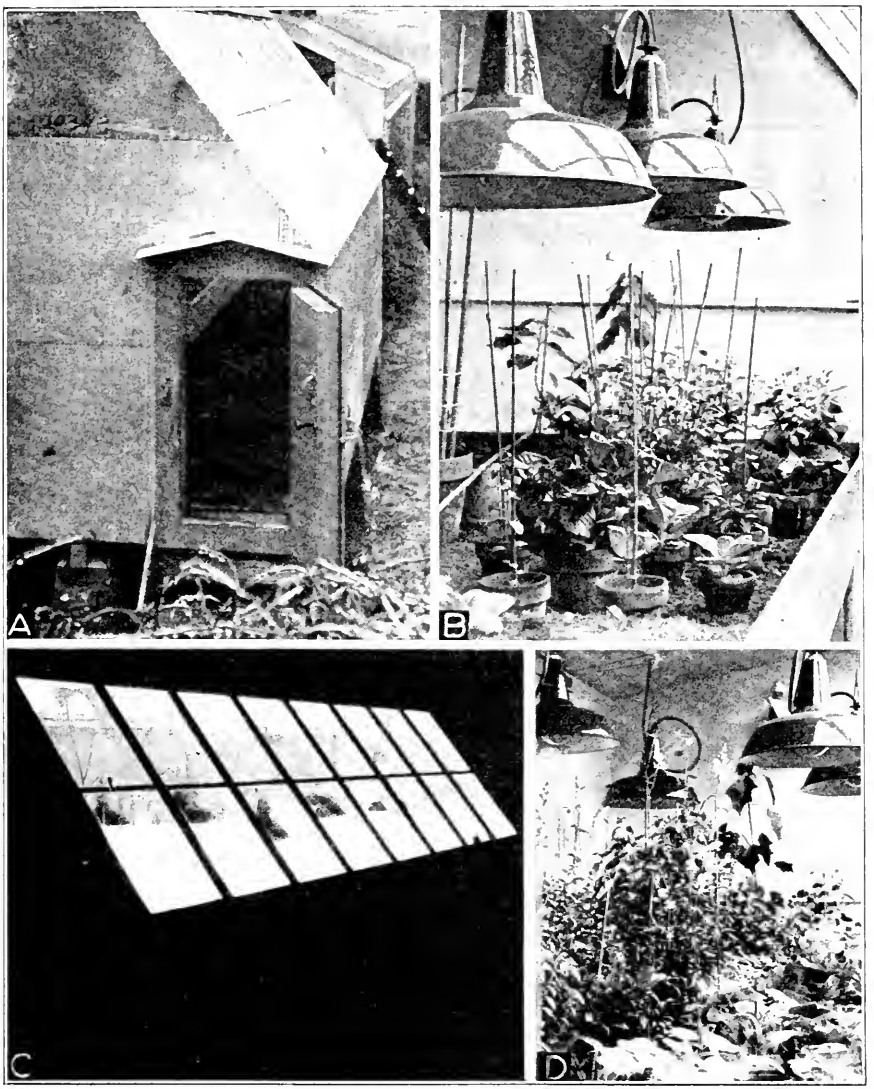

FIGURE 124. A, End view photograph of the new type greenhouse showing the refrigerator type of door and the method of supporting the house on cement pillars. C, Night view through the storm sash. B, Plants inside the house on December 29. D, Same plants taken February 9, or 42 days later.

available space, a portion of an ordinary greenhouse was equipped with lamps to correspond to the insulated house, and these lights were turned on and off by the insulated house thermostat. Dry weight production and flowering were greatly increased in both lighted houses as compared with the controls without additional light, but dry weight production was less in the heated insulated house than in the ordinary greenhouse with the

same amount of light. This was found to be due in part to the low CO_2 supply as a result of low rate of air exchange in the insulated house. Three sources of CO_2 were used for increasing the concentration of this gas in greenhouse air: lumps of solid CO_2, steel cylinders of liquid CO_2, and the CO_2 respired by fowls. When the last source was used it was found necessary to place the fowls in an outside chamber and to filter the respired air through water to remove ammonia which arose from fermentation of the droppings.

Other mechanisms were used to turn on 500-watt Mazda lamps over plants in greenhouses at night. An electric clock mechanism proved especially valuable for it could be adjusted to any alternate periods of illumination and darkness desired.

Table 31. Dry Weights of Buckwheat Plants Produced in Each Type of Greenhouse during Periods Indicated. Season of 1935–1936

	No. of plants	Av. dry weight per plant (grams)
A. Grown from Nov. 7 to Dec. 11		
Control greenhouse	44	0.22
Greenhouse with 500-watt lamps	38	0.27
Insulated greenhouse with 500-watt lamps	47	0.24
B. Grown from Dec. 11 to Jan. 14		
Control greenhouse	40	0.14
Greenhouse with 500-watt lamps	30	0.52
" " 300-watt "	30	0.34
Insulated greenhouse with 500-watt lamps	64	0.40
C. Grown from Jan. 14 to March 4		
Control greenhouse, sample (1)	20	1.10
" " " (2)	34	0.90
Greenhouse with 500-watt lamps, sample (1)	27	3.06
" " " " " (2)	18	2.72
Insulated greenhouse with 500-watt lamps, sample (1)	20	1.33
" " " " " " (2)	18	1.40
D. Grown from March 4 to April 14		
Control greenhouse, sample (1)	20	1.32
" " " (2)	20	1.43
Greenhouse with 500-watt lamps, sample (1)	32	1.92
" " " " " (2)	26	1.68
Insulated greenhouse with 500-watt lamps, sample (1)	16	0.56
" " " " " " (2)	20	0.53

Table 31 shows the increase in dry weight in buckwheat plants grown in the control greenhouse, in an ordinary greenhouse with lights turned on intermittently during the night, and for the insulated greenhouse with the

same illumination at night. The great advantage of the night-illuminated houses over the control greenhouse shows up in the periods December 11 to January 14 and January 14 to March 4, and is far less marked in the early fall and late spring periods. The reasons for this are simple: in fall and spring the lights for heating the insulated house were on for much less time during the night, and the daylight was brighter and of greater duration in fall and spring. This table also shows that the dry weight increase was less in the insulated house than in the artificially lighted ordinary house. This is due in the main to CO_2 deficiency in the insulated house. This was later cared for by CO_2 enrichment of the air, as mentioned above. Probably the daylight is not quite as intense in the insulated house because of the distribution of windows.

Table 32. Dry Weight Production of Buckwheat in Greenhouse with Intermittent Light

Lighting interval	No. of plants	Total dry weight (grams)	Dry weight per plant (grams)
Sunlight plus 7.06 hours of light each night at intervals of 6 min, 8 sec on, alternated with 4 min, 24 sec off	51	21.506	0.422
Sunlight plus 7.14 hours of light each night at intervals of 2 hr, 20 min on, alternated with 1 hr, 31 min off	47	19.704	0.419
Sunlight only. Same temperature as above, 55° to 60° F (13° to 16° C) at night	45	11.416	0.254
Sunlight only. Temp. 75° F (24° C) at night	47	10.032	0.213

Table 32 shows the effect of clock-regulated intermittent illumination of buckwheat plants at night in ordinary greenhouses. The intensity of the artificial illumination was the same as that used for the data shown in Table 31. Both the 7.06 hours of illumination at night in 6-minute and 8-second periods and the 7.14 hours of nightly illumination in 2-hour and 20-minute periods give great increases in dry weight as compared with daylight alone. Night temperatures of 55° to 60° F (13° to 16° C) in the control houses gave greater increases in dry weight than 75° F (24° C) night temperatures. Lower respiration at the lower temperature at night is in part the explanation of this effect. Low-temperature periods are of great significance to plants, as we have already seen with seeds and as we shall see in a later section of this chapter dealing with yarovization and with the necessity of a low-temperature period to prepare the rosette of *Digitalis* and other plants for flowering. In general, shorter periods of intermittent illumination at night are better than longer periods because of the rise in temperature caused by the long periods. The reader is referred to the original [8, p.40–42] for a discussion of the relative efficiency of alternate

periods of lighting of various lengths. This involves the effect of the lighting on the opening of stomates as well as the latent period between the beginning of illumination and the beginning of photosynthesis.

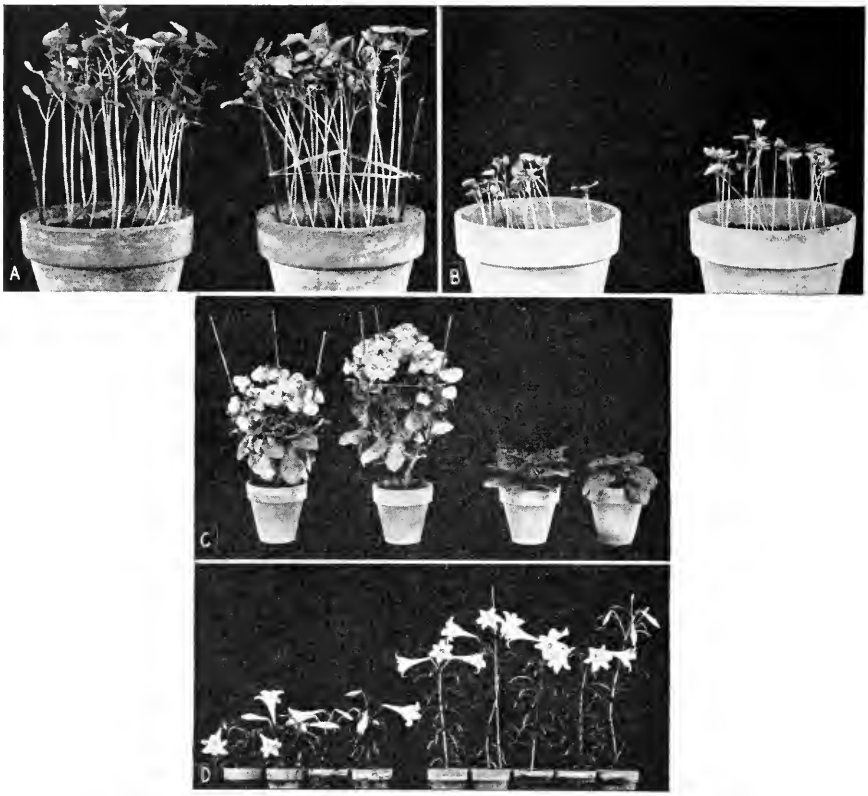

FIGURE 125. A, Buckwheat plants grown under artificial light at 68° to 72° F (20° to 22° C). *Left:* continuous illumination; *right:* intermittent illumination, 5 seconds on, alternated with 5 seconds off. B, Same as A except at a temperature of 95° to 100° F (35° to 38° C). C, *Calceolaria*, two *at left* grown with intermittent light each night, two *at right* control greenhouse without additional light. D, Lily (*L. harrisii*) planted September 17, photographed December 24; four *at left* grown at 55° to 60° F (13° to 16° C) control greenhouse, five *at right* same temperature but given intermittent light each night.

Fig. 125 shows the growth of buckwheat under continuous illumination with artificial light and half-time intermittent (5 seconds on and 5 seconds off) illumination with the same light intensity. From the height of the plants at 68° to 72° F (20° to 22° C) one might think that they grew as well when illuminated half the time as continuously. At the high temperature of 95° to 100° F (35° to 38° C), they seem to do better. Continuous illumination at this temperature added to the injurious effect of the high temperature. Table 33 shows the increases in dry weight of the plants grown under continuous illumination and half-time illumination. At

68° to 72° F (20° to 22° C) the half-time illumination gives very little more than one-half the increased dry weight of full-time illumination. At 85° to 90° F (29° to 32° C) the half-time illumination gives less than half the increased dry weight produced by continuous illumination. At 95° to 100° F (35° to 38° C), because of the injurious effect of the high temperature, the half-time illumination gives 80 per cent as great increased dry weight as continuous illumination. The 68° to 72° F (20° to 22° C) temperature is better than either of the higher temperatures.

Table 33. Dry Weight Production of Buckwheat Plants under Artificial Light

Temperature	Intermittent *		Continuous	
	No. of plants	Av. wt. per plant (gram)	No. of plants	Av. wt. per plant (gram)
68°–72° F (20°–22° C)	100	0.077	87	0.140
85°–90° F (29°–32° C)	81	0.046	69	0.112
95°–100° F (35°–38° C)	71	0.041	61	0.050

* Five seconds on, alternated with 5 seconds off.

Supplementing greenhouse light in the winter with intermittent artificial light at night hastened the growth and flowering of many plants such as *Calceolaria*, lily, gladiolus, and carnation, as is shown by Figs. 125 and 126. The beneficial effect on flowering was especially great on long-day plants or plants that flower sooner on the long day. Several varieties of large flowering gladiolus could be brought to flower by late January from bulbs planted in early October when grown at low temperature with intermittent light for an average of 3.4 hours each night. At 2 cents a kilowatt hour the cost for this supplemented light amounted to about 5 cents per stalk of gladiolus. This includes all the heating cost in the insulated house and part of that in the ordinary greenhouse.

Garner [16] mentions that, except for the onion, bulb or tuber formation occurs on short days. Zimmerman and Hitchcock [41] find that dahlia produces fibrous roots on long days and tuberous roots on short days. The same investigators [42] confirm the earlier work of Garner and Allard that the Jerusalem artichoke produces underground stems on long days and tubers on short days, and the work of Knott that the growing stem tip is the region of light perception. As already stated in this chapter, Arthur and coworkers have found that potatoes tuberize on a long day and even under continuous illumination if the temperature is sufficiently low. Gladiolus forms corms on short and on long days if flowering has ceased or is prevented.

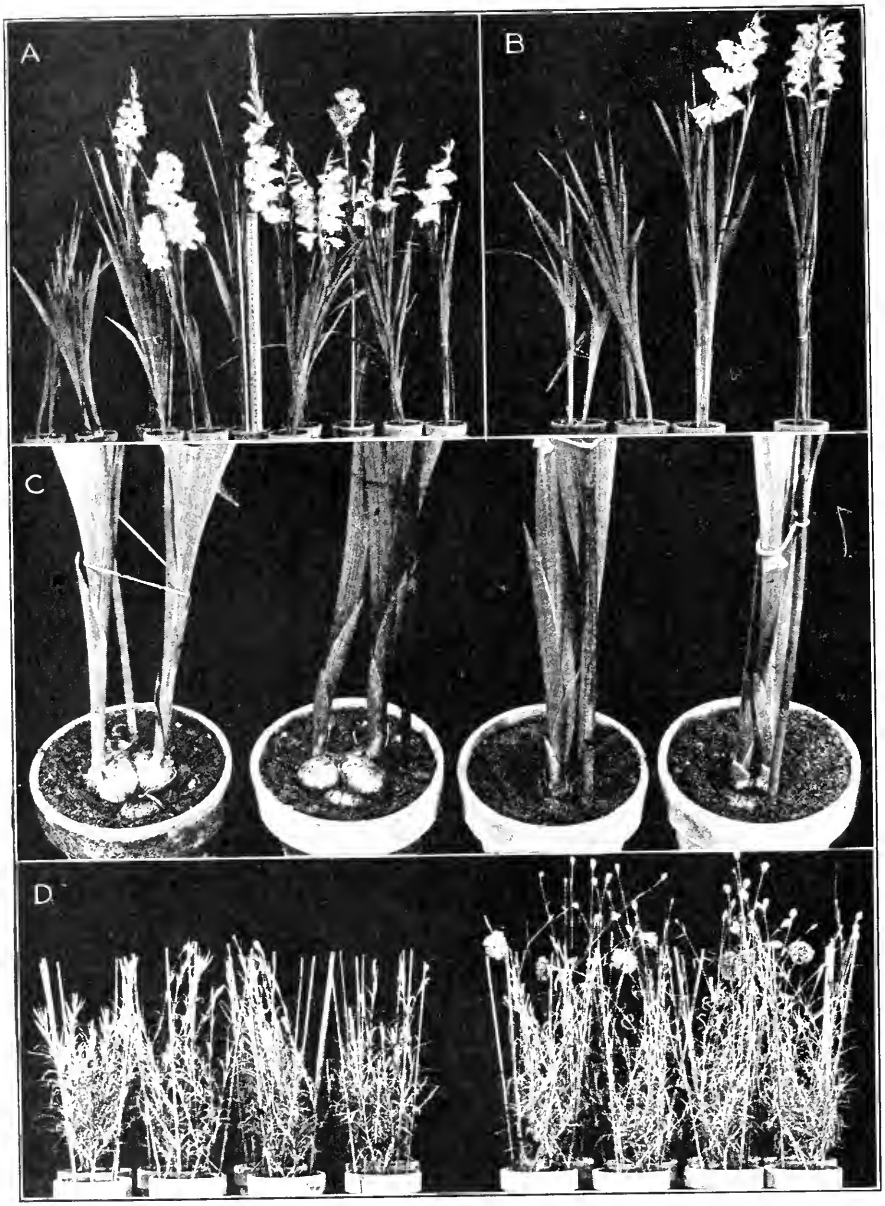

FIGURE 126. A, Gladiolus plants flowering under intermittent light. The two plants *at left* are from the control greenhouse without light. B, Picardy and Giant Nymph gladiolus. *Left:* two plants grown in control greenhouse; *right:* two plants grown with intermittent light. C, Same plants as B showing new corms forming only on those plants in short day or control greenhouse which do not flower. D, Carnations on January 28. *Left:* control greenhouse; *right:* greenhouse with intermittent light.

The Carbohydrate Nitrogen Ratio in Plants

By wide variations in growth conditions [6] the ratio of easily hydrolyzable carbohydrates (fiber not included) to total nitrogen content of plants can be varied widely in some plants. In other plants it can be varied only moderately. The carbohydrate per cent of course increases with rise in conditions that favor photosynthesis — increase in light intensity, day length, and CO_2 concentration of the air up to the optimum for each. The nitrogen content rises with the per cent of available nitrogen in the soil or nutrient solution. In some plants the increase in the nitrogen fraction occurs over a wide range of nitrogen supply, while with others the plants themselves limit nitrogen absorption under a high available supply.

Kraus and Kraybill [20] had concluded that the tomato, which is indifferent to day length as to fruiting, fruited only when there was a proper carbohydrate/nitrogen (C/N) ratio in the tissue. Arthur and associates grew the tomato under a wide range of conditions which gave also a wide range in the C/N ratio. The tomato plant did not fruit in the 5-hour day, but did fruit in any length of day from 7 to 19 hours. It did not fruit in a 24-hour day where extreme foliar injury occurred. In the range of 7- to 19-hour day the C/N ratio rose as the day length increased. The gantry crane greenhouse with 6 hours of artificial light at night and 0.3 per cent CO_2 also gave fruiting plants with very high C/N ratio; these showed a variation in C/N ratio from 7.5 to 39. Arthur and associates suggest that the failure of the tomato to fruit is not caused by an improper C/N ratio but by a deficiency in carbohydrates or nitrogen compounds, just as the deficiency in other nutrient factors such as phosphorus or potassium might lead to failure to set fruit. Several other plants were also studied as to possible significance of the C/N ratio as a fruiting factor.

Radish plants showed a great variation in C/N ratio with variation in growth conditions. The total carbohydrate, dry weight basis, varied from 7.47 to 34.73 per cent among the plants that were flowering or showed flowering response. There was a range of 8.95 to 21.23 per cent among those that did not flower. Total nitrogen varied from 1.51 to 5.92 per cent in the first group and from 2.77 to 7.27 per cent in the second. The radish flowered on a long day and failed to flower on a short day, regardless of the C/N ratio in either case. Radish plants growing in the greenhouse during the winter flowered when illuminated 6 hours at night with 170 foot-candles of artificial light, although this extra light gave no appreciable increase in the carbohydrate content; they also flowered with 6 hours of 700 foot-candles of artificial light at night which increased the carbohydrate content considerably. Radish plants grown alternately in 24 hours of light and 24 hours of darkness flowered, whereas those grown in 12 hours of daylight and 12 hours of darkness did not flower, although the C/N ratio in both was similar. The variety of lettuce studied was a long-day plant like the radish. In this also somewhat less extensive chemical analyses indicated

that the C/N ratio varied broadly, both in short days where no flowering occurred and in long days where flowering always occurred.

Salvia plants were grown in a great range of day lengths, light intensity, CO_2 supply in the air, and nitrogen supply in the soil with little variation in the C/N ratio in the plants. The carbohydrate content varied from 22.87 to 28.19 per cent and the nitrogen from 2.04 to 3.24 per cent. Increasing the nitrates in the soil from a very low level to a concentration that injured the plants gave little change in the percentage of nitrogen in the plant. Salvia seems to regulate nitrogen absorption regardless of the available supply, as has been found by Woo [6] for *Amaranthus retroflexus* and by Hooker and Bradford [6] for apple. Salvia is a short-day plant flowering in a 5- to 15-hour day but rarely in a 17-hour day. While C/N ratio varies relatively little in this plant, it is the day length and not the C/N ratio that determines flowering.

Buckwheat, which flowered on all lengths of day from 5 to 24 hours, showed a great variation in the carbohydrate and nitrogen percentage in the plants grown under various conditions. On the dry weight basis, plants grown in a control greenhouse for 64 days in the winter of 1927 contained 7.94 per cent carbohydrates, while a plant grown in a 24-hour day for 33 days contained 39.41 per cent. Plants grown in a 24-hour day for 65 days contained 0.46 per cent nitrogen, while those grown in a 5-hour day for 69 days contained 3.44 per cent nitrogen, and those grown in a control greenhouse during the winter for 40 days contained 3.72 per cent nitrogen, all on the dry weight basis. It is quite evident from the results stated in this paragraph that buckwheat, a plant that flowers on all lengths of days tested, shows no relation between flowering and the C/N ratio of the tissue.

Chemical analyses were made of a number of other plants (cabbage, red clover, soybean, cucumber, potato, ragweed, tobacco, several small grains, and corn) grown under a wide range of conditions from very favorable to unfavorable for high photosynthesis and nitrogen absorption. In general, increasing the light intensity, day length, and CO_2 content of the air increased the dry weight, the size, and the carbohydrate percentage of the plants. The increases held generally for increase of day length only up to a 17- or 19-hour day, but did not hold for increase in day length from a 17- or 19-hour day up to a 24-hour day. The failures to increase up to the 24-hour day may be due to the injurious effects of the artificial light used; or it is possible that plants need some rest from photosynthesis even under sunlight. Most of these plants showed an increase in the nitrogen fraction with increase in nitrogen content of the soil; but the small grains and corn seem to regulate nitrate absorption, as does salvia mentioned above. The day length rather than chemical composition, or C/N ratio, determines the flowering of all photoperiodic plants. Plants indifferent to day length as regards flowering, flowered over a wide range of C/N ratio, as did the photoperiodic plants.

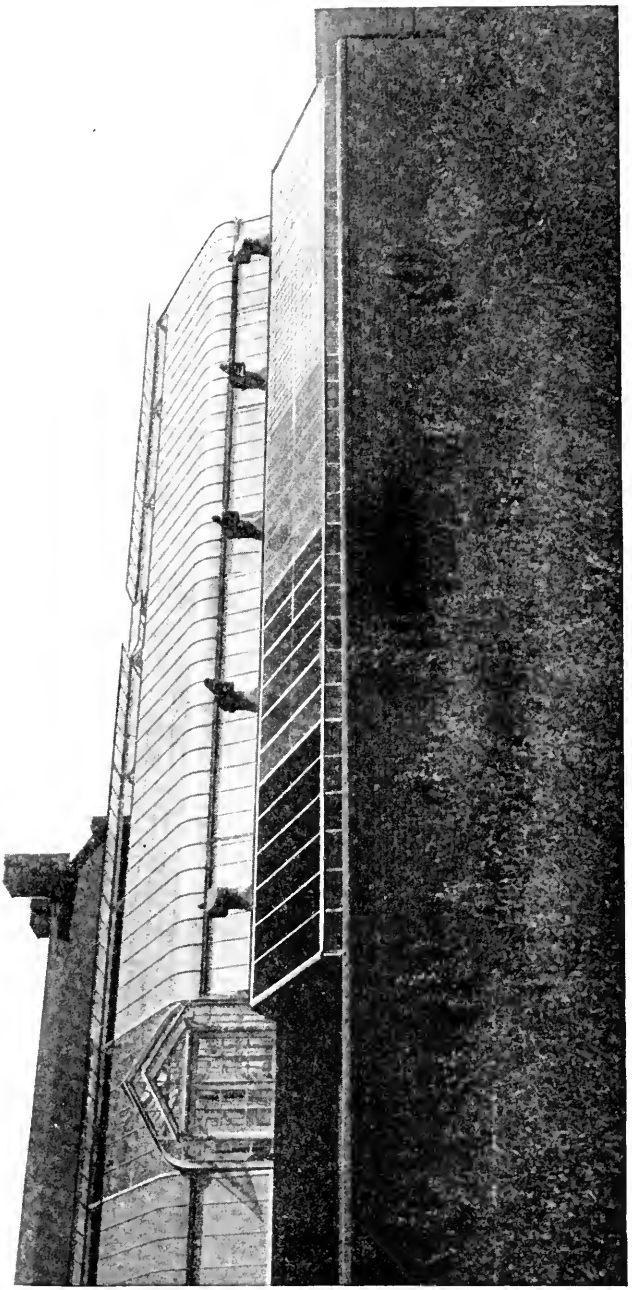

FIGURE 127. Spectral glass greenhouses.

Spectral Greenhouses

Fig. 127 shows the five spectral greenhouses used by Popp [29] in his investigations of the effect of spectral range of light on the growth of plants. These houses were numbered successively from right to left. The light transmission curves of the several houses are shown in Fig. 128. House 1

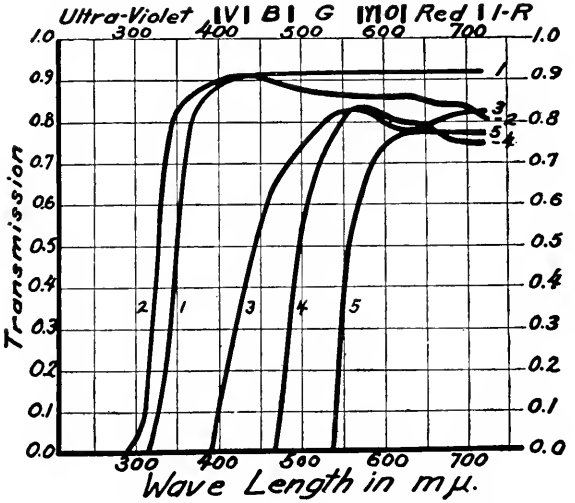

Figure 128. Transmission curves of glasses in the visible and ultraviolet. Figures on curves represent house numbers: 1, is ordinary greenhouse glass (house 1); 2, is Corning glass G86B (house 2); 3, is Corning's Noviol O (house 3); 4, is Corning's Noviol C (house 4); 5, is Corning glass G34 (house 5).

is window glass and cuts out all ultraviolet shorter than 312 mμ; house 2 is an ultraviolet-transmitting glass which cuts out ultraviolet shorter than 296 mμ; house 3 cuts out practically all the ultraviolet rays 389 mμ and shorter; house 4 cuts out all ultraviolet, violet, and part of blue up to 472 mμ; while house 5 cuts out all the ultraviolet, violet, blue, and the shorter green up to 529 mμ. Table 34 shows the percentage of total solar energy transmitted by the glasses on each of the five houses. For details

Table 34. Relative Transmission of Total Intensity of Light by Glasses in Different Houses

House number	Approximate transmission (%)	Actual intensity 10:00–11:00 A.M. Oct. 2, 1924 (foot-candles)
Outside	100.0	5833
1	80.0	4615
2	46.6	2795
3	66.1	3756
4	56.7	3372
5	37.0	2199

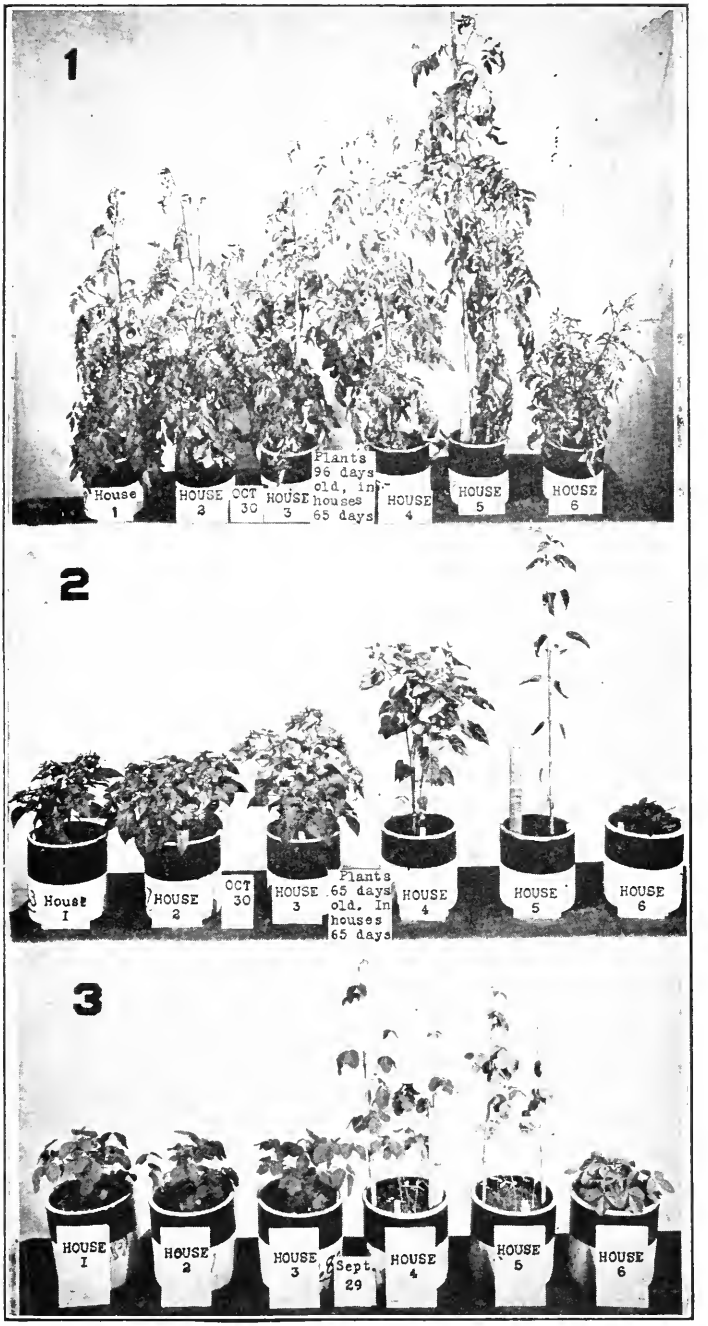

FIGURE 129. Plants grown in spectral greenhouse. 1, Tomato. 2, Four o'clock. 3, Soybean.

of methods of culture the reader is referred to the original article. The plants numbered 6, in Fig. 129, were grown outside. The experiment was started August 26 but in some cases the plants had been growing for some time before they were placed in the spectral houses. The tomatoes were 31 days old when they were placed in the greenhouses on August 26. Fig. 129 shows the growth of tomato, four o'clock, and soybean grown in the five spectral houses and outside (6). The growth in 6 was during September for soybean and during September and October for four o'clock and tomato. This means, of course, that the environmental conditions for 6 were very different from those of the spectral greenhouses. All three of these plants showed normal sturdy growth in houses 1, 2, and 3; that is, normal growth and growth of about equal stature occurred in all houses where the full range of the visible spectrum was transmitted in spite of there being considerable variation in the percentage transmitted in the different regions of the spectrum, as shown in Fig. 128 and Table 34. On the other hand, all three of these plants show spindly growth in houses 4 and 5. This is especially marked in house 5, where all the violet, blue, and much of the shorter green are removed and less marked for 4, except in the case of soybean, where the violet and the shorter blue are removed. The soybean takes on the character of a twiner in both 4 and 5.

Fig. 130 shows Sudan grass and sunflower (*Helianthus cucumerifolius*) growing in the five spectral greenhouses for 76 days, and carrot for 139 days. All the plants show normal development in houses 1 to 3 in which a great portion of all the visible rays are transmitted. Also in all these houses Sudan grass and the sunflower were setting flowers in 76 days. In houses 4 and 5 the Sudan grass showed much less growth than in houses 1 to 3 but the growth was equal in houses 4 and 5. The sunflower showed much less growth in 4 than in 1 to 3, and very little growth in 5. Neither Sudan grass nor sunflower flowered in houses 4 and 5. The carrot shows least growth in house 5, somewhat more in 4, and decidedly more in houses 1 to 3.

The author [29] summarizes his results on the effect of different portions of the solar spectrum as follows, with minor modifications. When plants were grown in daylight from which all wave lengths shorter than 529 mμ were eliminated, they developed the following characteristics as compared with plants grown in the entire spectrum of daylight. (*a*) An increased rate of elongation of the stem of all species during the first two or three weeks' growth; a greater final height in soybeans, tomatoes, four o'clocks, and coleus, but a decided decrease in height in sunflowers, petunia, buckwheat, and Sudan grass. (*b*) A considerable decrease in thickness of stems. (*c*) A reduction in the number of branches or side shoots. (*d*) A general curling or rolling of leaves. (*e*) Good development of chlorophyll, but a reduction in anthocyanin of leaves and flowers. (*f*) Less differentiation of stem and leaf tissues, less compact and thinner-walled cells, and a reduction in strengthening tissues. (*g*) Considerable delay in time of flowering and a reduction in the number of flowers produced. (*h*) Very weak development of seeds,

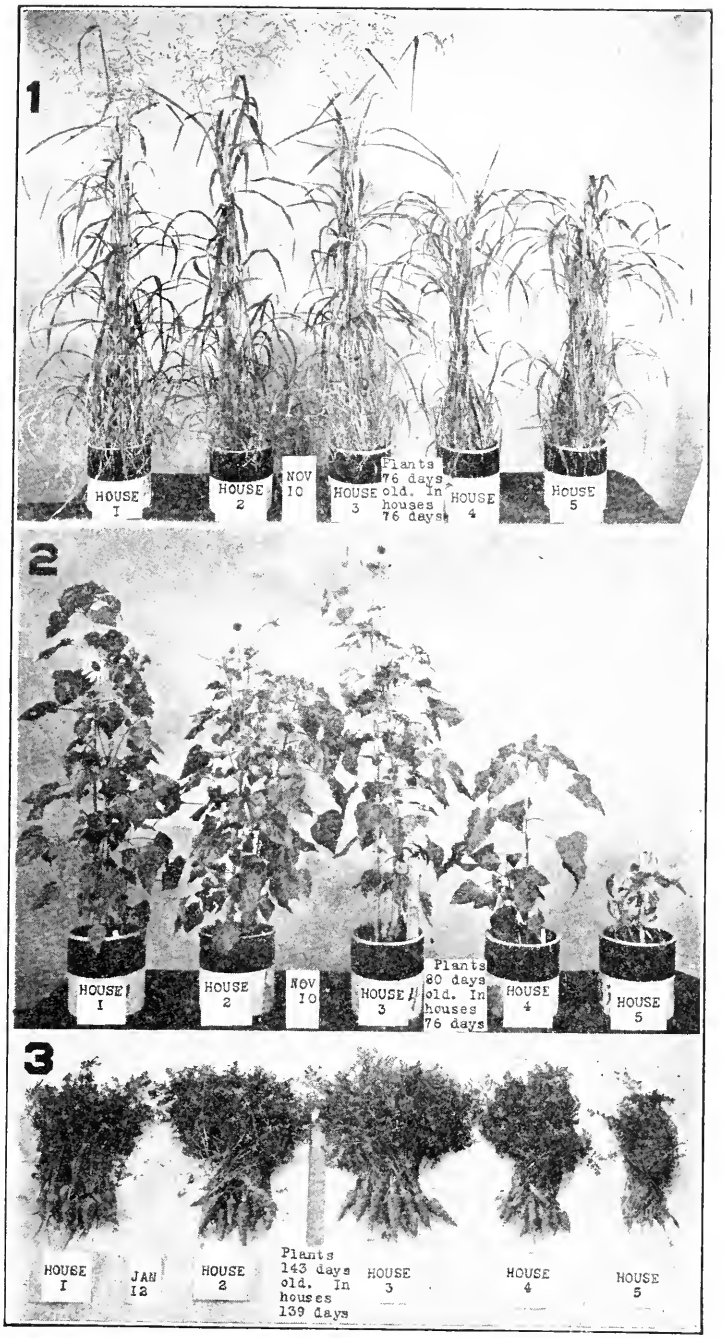

FIGURE 130. 1, Sudan grass from houses 1 to 5, 76 days from time of planting, when plants had been in houses 76 days. 2, Sunflowers from houses 1 to 5, 80 days from time of planting, when plants had been in houses 76 days. 3, Carrots from houses 1 to 5, 143 days from time of planting, when plants had been in houses 139 days.

fruits, and general storage organs. (*i*) Decrease in fresh weight and dry weight and an increase in percentage of moisture. (*j*) Considerable decrease in starch and total carbohydrates, and generally an increase in total nitrogen; often an increase in soluble nitrogen compounds.

The degree to which these different effects were produced varied with different species, but all species, aside from the abundance of chlorophyll, had an etiolated appearance.

When all wave lengths shorter than 472 mμ were removed, the same effects were produced as listed above, but to a somewhat lesser degree.

When only ultraviolet rays were eliminated, none of the foregoing results were obtained with any of the plants used, although there was a small increase in length of stems in all species except buckwheat, as compared with plants receiving these rays. Tomatoes, petunias, Sudan grass, and sunflowers bloomed somewhat earlier than they did under any other conditions. In general, there was very little difference between plants that received all the rays of the spectrum of daylight and those from which only ultraviolet rays were eliminated.

The results obtained with plants from which all wave lengths shorter than 529 or 472 mμ were eliminated are somewhat similar to those obtained when plants are grown under greatly reduced light intensity. That light intensity was not an important factor in the present experiment is proved by the fact that normal, vigorous growth was obtained when the plants received the full spectrum of daylight at an intensity which was at all times lower than that of the house in which all wave lengths shorter than 472 mμ were removed, and only slightly greater than that of the house in which wave lengths shorter than 529 mμ were eliminated.

The results as a whole indicate that, while ultraviolet rays are not indispensable, the blue-violet end of the spectrum is necessary for normal, vigorous growth of plants.

In a later study [1] the glass on spectral house 3, the ultraviolet-excluding glass, was replaced by a glass that cut out the red rays and transmitted rays between 585 and 348 mμ. This glass gives low transmission of solar energy, about 11 per cent in contrast to 37 per cent for house 5, the weakest transmission of the original spectral houses. The transmission of this glass is also mainly in the blue-violet. Fig. 131 shows (top) petunias grown in greenhouses 2, 3, and 5 and (bottom) four o'clocks grown in greenhouses 2, 3, and 5 and outside in shade and in full sunlight. The four o'clock and petunia plants grown in house 5 are spindling, as was the case with four o'clock and soybean in Fig. 129. Both the plants grown in house 3 with blue-violet light are short and sturdy but show little growth. It is possible that sufficient intensity of light in the blue and violet will give normal plants and good growth.

The window glass in spectral house 1 was later replaced by Aklo with very low transmission in the infrared. The infrared transmission of this glass is described by Arthur,[2, p.7] but no results on its use for growing plants

FIGURE 131. (*Above*) Petunias grown in spectral houses 2, 3, and 5. This shows the effect of growing a plant without the blue region (house 5) and without red (house 3). (*Below*) Four-o'clock grown in spectral houses 2, 3, and 5. The "shade" plant was grown outdoors under shading cloth at an intensity slightly higher than house 5. Roof plants were grown in open sunlight. House 2 transmits the visible and ultraviolet regions, house 3 glass transmits no red, and house 5 glass transmits no blue.

have been published as yet. The south side of one of our regular greenhouses was later covered with Sunlit glass. This is a thin clear glass with high transmission in all ranges of the solar spectrum. It transmits some ultraviolet at 255 mμ and has 50 per cent transmission at 290 mμ. The plants grown in this greenhouse are not strikingly different from those grown in an ordinary glass house.

From Popp's results it is evident that the ultraviolet of sunlight has little part in determining the stature, form, and flowering of plants, whereas the blue-violet is very important in determining form. Arthur [1] says there is no marked difference in form, dry weight, or time and amount of flowering whether plants receive only the ultraviolet transmitted by window glass or receive in addition the shorter ultraviolet of sunlight. Shirley [32] speaks of the entire spectrum, visible and ultraviolet, as more important for the growth of plants than any portion of the spectrum, and of the blue region of the spectrum as more important than the red. Whatever the significance of ultraviolet on the form, stature, and flowering of plants, it has a number of other interesting effects, as we shall see in a subsequent section of this chapter. For the later results artificial sources of ultraviolet were used with filters, to give control of both range of wave length and intensity.

Some Effects of Ultraviolet Rays on Plants

The formation of anthocyanins in plants may take place in darkness, as is the case with beet roots; it may be hastened by light, as in cranberry and Abundance plum; or it may be formed only in light and only in the cells actually exposed to the light, as in McIntosh and other red apples. Arthur [2] made a critical study of the effect of light of various wave lengths, of temperature, and other factors on the development of the red pigment in the McIntosh apple and other fruits. This work did much to clarify previous disagreements in the literature on this subject. Fig. 132 shows some of the more important findings. *A* of this figure shows that short ultraviolet rays of a mercury vapor lamp in quartz kill the apple tissue within 30 minutes and render the tissue incapable of later developing the red pigment. *B* of this figure shows that the apple is injured by radiation with light that is strong in infrared rays. An apple has a small specific surface when compared with a leaf and it is highly cutinized, which means that it loses heat slowly either by radiation or transpiration. *W* of this figure shows that the ultraviolet transmitted by window glass has low effectiveness in developing the red pigment. *P* and *C* show that the shorter ultraviolet rays transmitted by Corex or Pyrex glass gave good color development within 43 hours in green McIntosh apples picked on August 25. The author concludes that the highly effective region of the spectrum lies between 312 and 290 mμ, or beyond the ultraviolet transmitted by window glass. The visible portion of the spectrum from 600 mμ to the ultraviolet will induce pigment development but it is very slow in action. The author finds that 15° C (59° F) is the most effective temperature for pigment development.

Anthocyanins appear in many plant cells, mainly in early spring and in autumn at times of low temperatures; under these conditions soluble sugars are also abundant in plant organs. This has led many workers to conclude that high sugar favors anthocyanin development. Arthur [4] finds that low

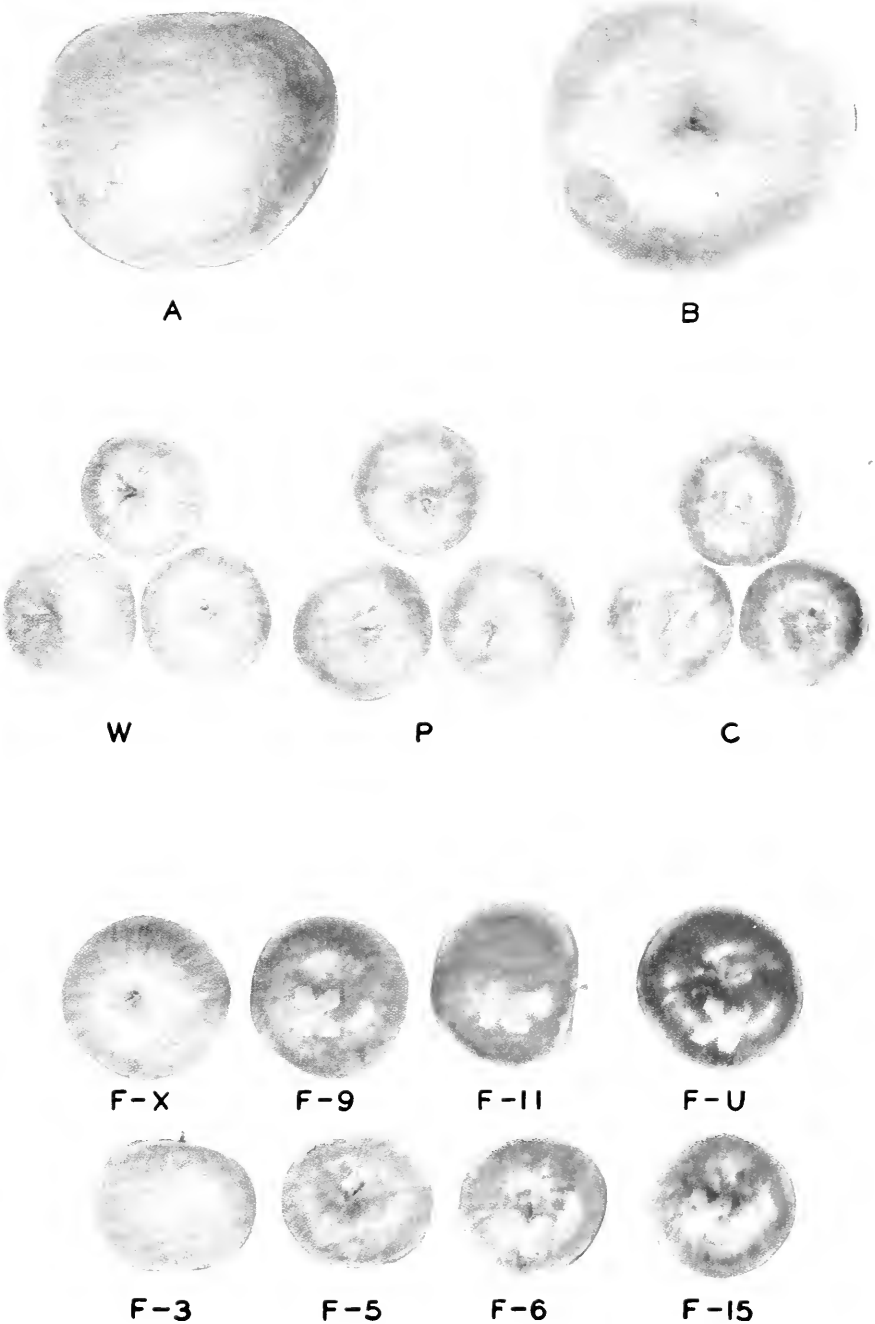

FIGURE 132. Apples colored by artificial light. A, B, W, P, and C *described in text.* F–X through F–15: Apples exposed for 5 days ending September 14th to mercury vapor arc in Uviol using various filters. In all cases the filters increased in effectiveness as their transmission in band 312 to 290 mμ increased.

temperature favors development of anthocyanin in the apple without a change in sugar content. He also points out that the small amounts of this pigment found in cells call for relatively little sugar as a building material and concludes that low temperature probably acts directly rather than through sugar accumulation.

While fruits picked on August 25 showed rapid reaction to the optimum light and temperature, the rate of coloring slowed down after the 25th of August, whether the fruit remained on the tree or was picked. Living skin taken from the apple and floated on water developed pigment as readily as skin on the apple, but dead skin did not develop pigment. Arthur believes the reason apples kept in storage until early November lose the power to develop pigment is because the epidermal cells gradually die during a month or more of storage. The cranberry and Abundance plum capable of developing pigment in darkness developed it much more rapidly if exposed to the most effective band of ultraviolet at 15° C (59° F).

A State of Washington orchardist stated that if this process could be developed for commercial application he would sell his orchards, because high coloring is one of the big selling points of Washington apples. He is probably in no danger, for it is expensive to expose all the surface of each apple to the proper ultraviolet rays at 15° C (59° F) for about two days.

Lojkin [22] exposed several kinds of green plants, both growing in soil and detached from their roots, to several sources of ultraviolet rays to determine the rate of production of vitamin D within the plants. She also exposed rats directly to the same ultraviolet sources to determine the length of exposure necessary for complete antirachitic protection.

Rats fed on the Sherman-Pappenheimer limited diet 84 needed 30 minutes' daily exposure to midday sunlight passing through an ultraviolet-transmitting filter, designated as *N*, in order to give complete antirachitic protection. During summer months 15 minutes of daily exposure is required. When this diet is supplemented by 5 grams of green lettuce per rat per day, both the rate of growth and the needed exposure increase.

When the mercury vapor lamp in quartz is used and the light is passed through a filter designated as *F*, which is opaque to all rays shorter than 286 mμ, only one minute's exposure is needed for complete protection. The author concludes that the most effective rays in curing rickets are within the limits of the solar spectrum at sea level, that is, 290 mμ or longer, and that rays shorter than 286 mμ have little or no antirachitic action.

Ultraviolet rays from the solar spectrum induce very little antirachitic action in lettuce, alfalfa, spinach, New Zealand spinach, and soybean plants, but rays from mercury vapor lamps passing through proper filters supply these plants with appreciable calcifying properties. Neither source of ultraviolet develops antirachitic substance in cabbage. Plants severed from the roots develop vitamin D under ultraviolet exposure much faster than intact plants. The irradiated plants in absence of an effective ultra-

violet source show no fall in protective action for 24 hours after irradiation, but there is some fall after 48 and 72 hours. The exposure of plants to ultraviolet rays necessary to give appreciable amounts of vitamin D is very much greater than the exposure of the animals themselves, sufficient to give complete protection against rickets.

It is evident that green plant materials grown in sunlight are a poor source of vitamin D and that irradiation of green plants with artificial ultraviolet sources is not economical not only because of the slow rate at which this vitamin is formed, but also because of the later degeneration of the vitamin during shipping and marketing.

Ultraviolet irradiation of plants [36] grown in soil under low light intensity or during cloudy weather causes an increased absorption of ash, including calcium or phosphorus, or both. Plants grown in soil under high light intensity and irradiated do not show a change in ash content. If plants are grown in midsummer when light intensity is high, the light for growing has to be cut down 65 per cent in order to make irradiation effective in increasing ash absorption. Short irradiation (15 seconds) increases the ash in the leaf and decreases that in the stem. Longer or repeated irradiation increases the ash in both stems and leaves; this involves increases in calcium or phosphorus, or both. Plants grown in sand show the response to irradiation even when under high light intensity. The manganese and magnesium content is not affected by irradiation. Plants grown in shade are more easily injured by short ultraviolet than those grown in high light intensity, but the effect on ash absorption occurred independently of injury. The effective rays lie between 290 and 313 mμ, coinciding with the effective antirachitic rays for the animal. Cabbage which is unable to form vitamin D showed no increase in ash absorption. Application of irradiated ergosterol to plants causes increased ash absorption. It is believed that ultraviolet irradiation increases the mobilization and absorption of calcium and phosphorus indirectly by activation of the ergosterol in the plant tissue, that is, by formation of vitamin D.

Later work [37] extends and clarifies these conclusions. Plants grown in absence of calcium and phosphorus show no increase in ash absorption due to irradiation. The level of supply of calcium or phosphorus, and not the ratio of calcium to phosphorus, determines the presence or absence of response to irradiation. With a high ratio of calcium to phosphorus in the nutrient medium, irradiation increases ash and calcium absorption; and in a high ratio of phosphorus to calcium, it increases ash and phosphorus absorption.

Shorter ultraviolet rays of a mercury vapor arc in a quartz tube are very injurious [11] to plants, but rays 290 mμ or longer are not injurious. The injury increases rapidly as the ray length shortens from 290 to 200 mμ. Radiations that give little or no injury with one application do not show cumulative injury when the dosage is repeated daily. Filters that transmitted rays as short as 286 mμ injured tomato plants with 16.5 hours'

exposure. It is evident that rays much shorter than those transmitted through the atmosphere from sunlight injure plants. Purified tobacco virus is inactivated by exposure to the open arc, but the virus within the plant is not inactivated. Irradiation did not stimulate growth or fruit set; the dominant effect is injury by the shorter rays.

It was claimed that *Digitalis* seedlings grown under ultraviolet-transmitting glass bear much more of the cardiac glycosides than similar seedlings grown under window glass, and that this difference persists even after the plants are grown for long periods in the open field. Contrary to this conclusion, Leonard and Arthur [21] find no difference between the glycoside content of plants grown under Sunlit and window glass when all other conditions are the same. They mention two other conclusions from the study that are of interest: the rosette stage of *Digitalis* requires three or four months of exposure to a low temperature (50° F [10° C] or lower) before it will produce a flower stalk, and the concentration of the glycosides in plants 4 to 6 months old is about twice that in plants 9 to 17 months old, whether flower stalks are formed on old plants or not.

Ultraviolet rays change inactive *trans*-cinnamic acid [43] to the isomer *cis*-cinnamic, which is an active plant hormone. This change occurs whether the acid is irradiated before or after application to plants. Other phenyl compounds, *p*-, *m*-, and *o*-nitrocinnamic acids, are similarly activated, as are β-naphthoylacetonitrile and tryptophane.

Minimum Light Intensity for the Survival of Green Plants

Green plants make two uses of the organic carbon materials they synthesize in sunlight; they oxidize them as a source of energy for growth or repair and they build them into new tissue leading to an increase in dry weight. Assuming that all other growth factors are held at a high level or near the optimum, but that the light intensity is gradually reduced, it will finally reach a point where the synthesis during the day is just sufficient to maintain respiration during the day and night, with no increase in dry weight, and the plant is able only to maintain its weight, if indeed it does not perish. All the plant materials we use as food or otherwise are possible because the synthesis during the day is in excess of the respiration during day and night. Plants on a forest floor have to grow in a low light intensity as well as modified light quality because some of the light reaching these plants has already passed through green foliage with differential absorption of the rays. Of course what is true of plants on a forest floor is true of weeds growing in the shade of crops or crops growing in the shade of weeds, and in part of plants growing in any sort of shade.

Shirley [32, 33] attacked anew the much investigated problems, What is the minimum daily light intensity in which various plants can persist? and What is the effect of varying light intensity and quality upon rate and nature of plant development? Experiments were run under different inten-

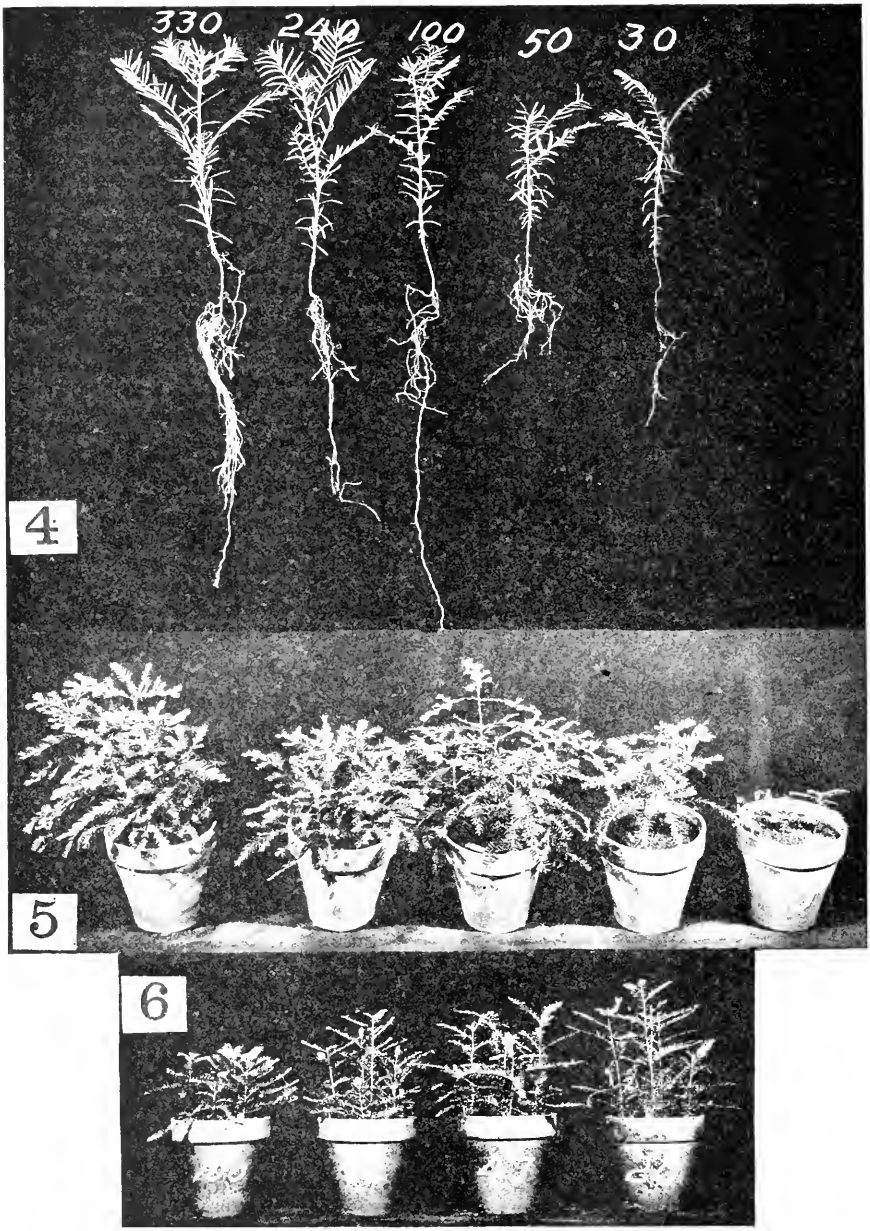

FIGURE 133. 4, Redwood plants grown for 55 days on 12-hour days in constant condition room. The figures above the plants show the light intensity in foot-candles. 5, Redwood plant from the greenhouse shades, grown from March 27 to October 22, 1928. The figures on the pots show the light intensity in percentage of full sunlight. 6, Redwood plants grown in outside shades June 1 to Sept. 26, 1928. Figures on the pots show the light intensities in percentages of full summer sunlight.

sities of light by screening the plants with one or more layers of cheesecloth or muslin in (*a*) the constant-condition artificial light room, (*b*) in the spectral glass houses, (*c*) in ordinary greenhouses, and (*d*) outdoors. This gives a great variation in both the intensity and quality of the light. The following plants were used in the study: buckwheat, a dwarfed sunflower, *Galinsoga*, *Geum*, redwood, wandering Jew, loblolly pine, hog peanut, tomato, and tobacco. This includes plants commonly growing in shade at least in the early stages, such as redwood, loblolly pine, *Galinsoga*, and hog peanut, and plants demanding or commonly growing in high light intensities, such as the sunflower and buckwheat.

Fig. 133 shows the growth of redwood under light of various intensities and sources. In the constant-condition artificial light room the growth was about the same in 330, 240, and 100 foot-candles, with a great reduction rate in 50 and 30 foot-candles. In the greenhouse good growth was shown in 71, 40, and 19 per cent of full sunlight, greatly reduced growth in 8 per cent, and little growth in 1 per cent of full sunlight; outdoors good growth occurred in 20, 47, and 74 per cent of full sun'ight, with slight dwarfing in stature in 100 per cent full sunlight. For the sunflower, as shown in Fig. 134, reducing the light in the artificial light room from 600 to 260 foot-candles caused a great reduction in growth. In the greenhouse, reduction of growth was evident in 19 per cent of full sunlight, marked in 8 per cent, and complete in 1 per cent; and in the open, good growth occurred in all intensities tried down to 21 per cent of full sunlight, the lowest intensity used.

Geum grown in the artificial light room (Fig. 135) showed a gradual reduction in growth as the light intensity fell from 470 to 41 foot-candles. In the latter there was very little growth even after 54 days. Sunflower grown in the spectral greenhouses with low but nearly equal intensities, except for house 5, gave much better growth in house 1 (full visible spectrum with ultraviolet excluded) and in house 2 (ultraviolet-transmitting glass) than in the other houses with only a part of the visible spectrum. House 3 transmitted the blue and violet and cut out all rays longer than 600 mμ; it also greatly dimmed the green and yellow. The glass on house 4 cut out most of the violet and dimmed the shorter green, but it transmitted a large percentage of the longer green and yellow, orange and red. House 5 cut out all the violet, blue, and shorter green but transmitted a considerable percentage of the long green and a greater percentage of yellow, orange, and red. The plants grown in houses 4 and 5 were small and spindling and those in house 3 were short but sturdy. *Geum* plants grown in the spectral houses were less unfavorably affected by the unbalanced spectrum than sunflower, although there is some evidence that house 5 was unfavorable. *Galinsoga* grown in the spectral greenhouses behaved more like the sunflower, except that the growth in height is considerable in houses 4 and 5.

The author concludes that light needed for the survival of all the plants studied is very low, less than 40 foot-candles, except for the sunflower, which needs much higher intensities. Redwood and loblolly pine survive

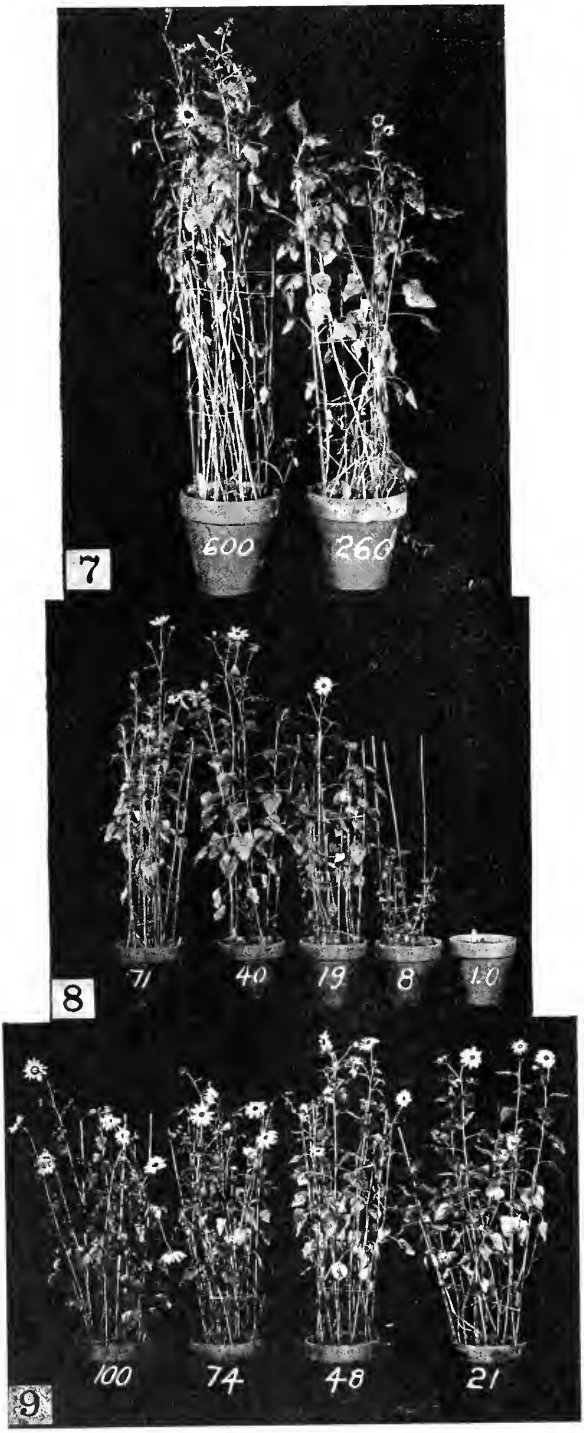

FIGURE 134. 7, Sunflowers from constant condition room grown for 48 days with 12 hours' daily illumination. The figures on the pots are light intensities in foot-candles. 8, Sunflowers from greenhouse shades June 5 to August 13, 1928. Figures on the pots represent light intensities in percentages of full summer sunlight. 9, Sunflowers from outside shades June 5 to August 20, 1928. Figures on the pots represent light intensities in percentages of full summer sunlight.

FIGURE 135. 13, *Geum* plants grown in constant condition room for 54 days with 12 hours' daily illumination. Figures on the picture show the light intensities in foot-candles. 14, Sunflower plants grown in the spectral house shades from June 5 to August 15, 1928. The Arabic figures on the pots show light intensities in percentages of outside sunlight. Roman numerals show the house number. 15, *Geum* plants grown in the spectral house shades from July 10 to October 24, 1928. Arabic figures show light intensities. Roman numerals show the house numbers. 16, *Galinsoga* plants grown in spectral house shades from May 30 to July 5, 1928. Arabic figures show light intensities. Roman numerals show house numbers.

for six months in light intensities scarcely sufficient to increase the dry weight. On the other hand, sunflower under this condition perishes within two or three weeks. At lower light intensities up to 20 per cent of full summer sunlight the increase in dry weight is proportional to the light intensity. At higher intensities the increased dry weight does not keep pace with the increased intensities. In shade plants the increase in dry weight begins to fall behind the increased intensities at lower light levels than in sun plants. With increase in light intensity go an increase in dry matter of tops, ratio of dry matter of roots to shoots, density of growth, strength of stem, and thickness of leaf. Leaf area and height of plant attain the maximum at about 20 per cent of full summer sunlight.

Chlorophyll concentration increases with decreasing light intensities until the low light intensities threaten survival. Still further decreases in intensity gave a decrease in chlorophyll concentration. Both of these findings are confirmed by Guthrie [18] in his study of many factors affecting the synthesis and decomposition of chloroplast pigments, which is to be discussed later in this chapter. The time of maximum flowering and fruiting was delayed by low intensities and flowering did not occur in intensities of 8 per cent of full summer sunlight. The entire solar spectrum, including the ultraviolet, was more effective than any portion of it and the blue region is more effective than the red.

In cases where the water supply is such as not to give waterlogging of the soil or to reach the wilting coefficient, light intensity is usually the limiting factor to vegetative growth under a forest canopy; quality of light is not a serious limiting factor.

Light Measurement

Shirley [34] developed a thermoelectric radiometer for ecological use on land and in water. It is rugged, light and compact, simple in construction and operation, gives readings within 7 seconds, and is sensitive to 0.1 per cent of full summer sunlight.

Effect of Radiation on Transpiration

A study [13] was made of the effect of artificial illumination of varying intensity and quality, including in some cases only infrared, on the rate of transpiration of tobacco plants. The experiments were run at two ranges of temperature (73° to 78° F [23° to 26° C] and 98° to 100° F [37° to 38°C]) and with 1000-watt lamps of both high efficiency (105-volt lamps on 120-volt lines) and low efficiency (120-volt lamps on 120-volt lines). A considerable range of humidity was also used. At the lower temperatures the radiant energy (visible and infrared) had to rise 2.3 times to double transpiration. This held within the range of humidity 50 to 88 per cent. At the higher temperatures rise in humidity lowered transpiration somewhat. At the lower temperatures the complete spectrum (visible and

infrared) gives 2.5 times the transpiration that the infrared alone gives, although the infrared represents over 90 per cent of the energy. At the higher temperatures the complete spectrum gives only 1.3 times the transpiration induced by the infrared alone. The authors conclude that all transpiration in the infrared is cuticular, for the stomates are completely closed in darkness. They emphasize strongly the significance of transpiration as a cooling factor which protects the leaf against overheating under strong irradiation. The fact that leaves carefully covered with Vaseline on both sides endure considerable irradiation without injury has been offered as an argument against the importance of transpiration as a cooling factor. The authors dispose of this argument by showing that Vaseline-treated leaves transpire rather freely when irradiated. They did find that transpiration was effectively stopped by enclosing the leaf in a snug-fitting cellophane envelope. Leaf temperatures of enclosed leaves at high radiation values (1.6 gram calories) rose from 87° to 127° F (31° to 53° C) in an exposure of four minutes, and the leaves were badly injured.

Miller,[25, p.495] in his critical review of the literature on transpiration in his text, says, "As previously mentioned, however, the average leaves are cooled by transpiration rarely more than 2° to 5° C — a difference which, so far as our knowledge of protoplasm goes, could be of no marked benefit in preventing injurious effects." In this statement does not Miller confuse the cooling effect of transpiration with the number of degrees of temperature below that of the atmosphere that the leaf sometimes attains under irradiation, at which time the cooling effect of transpiration is in full operation? Must we not stop transpiration entirely under irradiation to learn how much the cooling effect of transpiration really is, just as Arthur and Stewart have done? There is one adverse criticism of the Arthur-Stewart determination: they fail to show that the tight-fitting cellophane bag did not interfere with the loss of heat by thermal emissivity as well as by vaporization of water.

It seems to the writer that the plant physiologist who cannot see any advantage in transpiration as a cooling factor for the green leaf under irradiation ought to do as Brown and Escombe [15] did much earlier, namely, consider the leaf as a physical apparatus. The green leaf receives energy by absorption of radiant energy; indeed under high illumination in absence of the cooling processes of transpiration and thermal emissivity, it would absorb enough energy to raise it to the thermal death point within a few minutes. If the leaf has a lower temperature than the surrounding air it also gains energy by thermal emissivity, and a slight amount of heat is also added to the leaf by its own respiration. It loses heat not only by vaporization of water but by thermal emissivity as well, if the leaf has a higher temperature than the surrounding air; also there is a slight use of energy in C-synthesis. Brown and Escombe show that the great loss of energy by the leaf under irradiation is by transpiration if the leaf has about the temperature of the surrounding air; but the loss by thermal emissivity becomes

of rapidly increasing importance as the temperature of the leaf rises above that of the surrounding air. It seems that the dispute over the importance of transpiration as a cooling factor for the green leaf will be readily dissolved by answering two questions about the physics of the green leaf under illumination: How fast and to what temperature would the green leaf be heated if the cooling by both transpiration and thermal emissivity could be eliminated during illumination, and what part does each cooling factor play under various conditions?

Environmental Conditions and the Development of Chlorophyll Pigments

A study [18] was made of the effect of environmental conditions previously considered in this chapter on the amount and ratios of the five chloroplast pigments: chlorophyll *a* and *b*, carotene (*c*), xanthophyll (*x*), and a brown chloroplast pigment. Improved methods were developed for separating chlorophyll *a* and *b* that gave much higher yields of each; for determination of carotinoids; for preparing carotene from carrots; and for determining the brown pigment of the chloroplast.

Leaf tissue can be preserved in the frozen condition without loss of chlorophyll, but the *a*/*b* ratio decreases. Increasing the CO_2 concentration of the air or the duration of illumination during growth decreases the chlorophyll and carotinoid content of the leaf; the combination of both gives a still greater decrease, all without modifying the *a*/*b* or *c*/*x* ratios. Very young plants, such as seedling soybean, are a partial exception to this; in these increased CO_2 concentration increased the chlorophyll content. Keeping plants in darkness leads to a great decrease in chlorophyll content without a corresponding decrease in carotinoids, but with an increase in *c*/*x* ratio and in the brown pigment. Continuous artificial illumination of the tomato plant causes a marked reduction in chlorophyll and carotinoid in a few days and a significant lowering of the *a*/*b* and *c*/*x* ratios, but a rise in the brown pigment. Tomato mosaic causes a decrease in chlorophyll, which may be accompanied by a decrease in carotinoids but an increase in brown pigment. The *a*/*b* and *c*/*x* ratios show little change.

Filtering the ultraviolet rays from sunlight has no significant effect on the amount of chlorophyll and carotinoids in the living leaf growing under such light. Elimination of blue light gives a slight but significant decrease in chlorophyll and carotinoids provided the total light intensity is kept the same. Absence of the blue rays also lowers the *a*/*b* ratio. Filtering out the red rays leads to an increase of both chlorophyll and carotinoids, but this is partially due to reduction in total intensity. On the basis of a few experiments, the *a*/*b* ratio seems to be higher under blue rays. Blue rays also favor the development of the brown pigment.

Reduction of the light intensity to 12 per cent of full sunlight results in an increase of both chlorophyll and carotinoids without change in *a*/*b* and *c*/*x* ratios, but with a reduction in the brown pigment. This agrees with

Shirley's findings stated earlier in this chapter that maximum chlorophyll development occurs in plants in light intensities that are near the minimum of light intensity that insures survival of the green plant.

Limiting of nitrate supply leads to an increase in chlorophyll and carotinoids when plants are grown in the greenhouse during the winter; but the reverse is true with higher light intensities. Also under higher intensities the brown pigment increases. Limiting potash and phosphorus increases the chlorophyll on the wet weight basis but has little effect on the dry weight basis. Growth of plants in soil gives higher chlorophyll and carotinoids than growth in nutrient solutions. Limiting nitrates, potash, and phosphorus has little effect on a/b or c/x ratios.

Environmental Conditions Modify the Microchemistry and Anatomy of Plants

A study [27] was made of the effect on the microscopic, chemical, and anatomical changes brought about in plants by the several growth conditions mentioned early in this chapter. In one set of experiments the plants were grown in the artificial-light room under 5, 7, 12, 17, 19, and 24-hour day lengths. The conditions in the room were: temperature, 25° C (77° F); light intensity, 780 foot-candles at the beginning falling to 352 foot-candles at close; relative humidity, 80 per cent; CO_2 concentration, about 0.3 per cent. Plants were also grown in an ordinary greenhouse without extra light and CO_2, in a gantry crane house with 6 hours of artificial illumination at night, and in a gantry crane house with 6 hours of artificial illumination at night and about 0.3 per cent CO_2. The artificial illumination of the gantry crane started with 383 foot-candles and decreased during the experiment to 141 foot-candles. The experiments were run during March, April, and early May.

In short daily illumination the plants were low in carbohydrates and protein reserves and showed less differentiation of tissue. In the longer daily illumination the plants showed greater carbohydrate reserves without a corresponding increase in differentiation of tissues and protein reserves. Nitrates were abundant in the tomato plants in all the houses except the control and two gantry crane houses. Nitrates were low in buckwheat in the 17, 19, and 24-hour day lengths as well as in all the greenhouses, being lowest in the gantry crane CO_2 house.

In artificial light the maximum height and differentiation of tissue occurred in 12-hour day for tomato and the 17-hour day for buckwheat. The tomato plant was injured by artificial light in the 17-hour or longer day. Continuous illumination produced thinner leaves with palisade cells shorter or lacking. In the gantry crane houses the thickness of the leaves was variable, and the thinner leaves showed an increase in the number of stomates except in continuous light.

The size of the fibrous root system was proportional to the top growth of the plant. The storage root of the four-o'clock showed maximum develop-

ment in the gantry crane houses and in continuous light, while the maximum height of the top occurred in the 17-hour day. Increased development of the underground storage organs on long days is in contrast to the behavior in the Jerusalem artichoke and dahlia, which develop such storage organs on short days, but it agrees with Arthur's findings, mentioned above, that potato tuberizes well under long days if other conditions are favorable.

Table 35. Spectral Transmission of the Houses

Visible-spectrum house	Noviol O	720–390mμ
Full-spectrum house	Corex	720–290
Blue house	G403ED	585–335
Minus-violet house	Noviol C	720–471
Red house	G34	720–526

An anatomical study [28] was made of plants grown in several spectral greenhouses and outdoors as a check. The spectral transmission of several of these houses has already been described under the topic "Spectral Greenhouses," but since the glass on one of these houses was changed, the rays transmitted by each house are given in Table 35 and the light and radiant energy transmitted by each house in Table 36. Table 37 shows the relative

Table 36. Intensities in the Various Situations

Situation	MacBeth illuminometer (%)	Pyrheliometer (%)
Outdoors	100	100
Visible-spectrum house	59.8	52.7
Full-spectrum house	55.0	50.8
Blue house	8.6	8.0
Minus-violet house	50.7	33.2
Red house	31.3	30.2
Shade house	16.2	23.2

effectiveness of the several lights on the development of stems, leaves, and roots of *Mirabilis jalapa*, *Brassica rapa*, *Helianthus cucumerifolius*, and *Glycine soja*. Pfeiffer points out that both the intensity of illumination and the quality of the light varied. The light transmission (foot-candles) in the total spectrum, visible spectrum, and minus-blue houses was nearly identical, and exceeded 50 per cent of that outdoors. The illumination was very low in the blue house, about 8 per cent of that outside, and about twice that of the shaded house. The red house was about 31 per cent of that outside. This variation in intensity makes the results hard to interpret. This was partly overcome in the later work by Shirley, already discussed, where cloth screens were used to equalize more nearly the intensity in the several houses. Pfeiffer points out that plants grown in the full spectrum

Table 37. Summary: Degree of Development Listed in Order with the Highest First

Stem			Leaf		Root
Vascular development	Diameter	Height	Thickness	Differentiation	Development
Outdoors	Full-spectrum	Visible-spectrum	Outdoors	Outdoors	Outdoors
Full-spectrum	Outdoors	Full-spectrum	Full-spectrum	Full-spectrum	Full-spectrum
Visible-spectrum	Visible-spectrum	Minus-violet	Visible-spectrum	Visible-spectrum	Visible-spectrum
Minus-violet	Minus-violet	Outdoors	Blue	Shade	Minus-violet
Shade	Shade	Blue	Minus-violet *	Blue	Shade
Blue or red	Blue or red	Red and shade vary in position	Shade *	Minus-violet	Blue
Red or blue	Red or blue		Red	Red	Red

* Exception in sunflower.

house show better development in most cases than those in the visible spectrum, although the illumination and the total radiant energy are somewhat lower in the former than in the latter. This indicates that ultraviolet has minor importance in plant differentiation. Shirley's work reported earlier also indicated this. This work also emphasizes the fact that plants grown under long rays alone lack differentiation of tissues, have thin leaves and stems, and are spindling.

Some Low-Temperature Effects

In Chapter 3 there is a full discussion of the importance of keeping certain seeds at a low temperature with proper moisture and oxygen supply in order to after-ripen them. In some seeds that respond to this treatment the coats rather than the embryos seem to cause the delayed germination, but even in these seeds it is probable that the low temperature is effective, at least in part, through modifying the embryo. In other seeds of this group there is a sluggishness in the growth of the radical, as well as a dormancy in the epicotyl that produces a dwarfish plant. Both the sluggishness in the radical and the dormancy of the epicotyl are overcome by the low-temperature treatment. In Chapter 7 there is a discussion of the effect of low temperature exposures on the after-ripening of buds of trees, bulbs, and tubers. In most, but not all, cases mentioned in this paragraph the low-temperature exposures overcame dormancy in vegetative organs and increased the vigor of the resulting vegetative growth.

Periods at low temperatures in many cases also shorten the vegetative period and hasten the appearance of the reproductive phase of plants (yarovization or vernalization). The treatment may be applied to imbibed and slowly germinating seeds, to seedlings [38, 39] such as celery, beet, and cabbage, or to the well-developed plants,[38] such as cabbage and onion.

Lojkin [23] reviewed the seventy-odd years of observation and research that led up to Lyssenko's conception of yarovization of seeds and tested his conclusions by investigations on six varieties of western winter wheats, on Blue Stem, a spring wheat, and on Clydesdale, a spring oat. Yarovization did not proceed at freezing temperatures, but 1° to 3° C (33° to 37° F) was satisfactory for yarovizing Turkey Red and Leap's Prolific wheats. But 5° C (41° F) was too high, for it led to molding and excessive germination. There must be some growth in the refrigerator to insure complete yarovization. Moisture content ranging from 50 to 70 per cent was adequate, and at least 60 per cent initial moisture was found necessary if the moisture was kept constant during the entire yarovization period. Turkey Red, Leap's Prolific, and Blackhull wheats required nine to ten weeks for complete yarovization, and Ilred, Wisconsin Pedigree #2, and Tenmarq eight weeks. Early spring sowing, which gives a cold period in the soil, shortens the required period for complete yarovization. Fig. 136 shows the effect of yarovizing grains of Turkey Red and Leap's Prolific on the future development of the plants.

Prolonged treatment of the grains beyond the optimum time did not nullify the yarovization, but drying the yarovized grains and exposing them to warm temperatures did so. When sown during May with the longer days and higher temperatures, the plants from yarovized grains headed in 56 days, whereas those sown in early spring required 80 days.

Non-yarovized Turkey Red and Leap's Prolific wheat grown at 16° to 22° C (60° to 72° F) with a day length of 15 to 16 hours headed in about 150 days after sowing, but the completely yarovized grains required 110 to 120 days from the time of beginning of yarovization to heading under optimum conditions. Yarovization always decreased the percentage germination in the field. Low-temperature treatments did not shorten the vegetative period of the two spring cereals. In practice the winter cereals, of course, get the cold period necessary for hastening the shooting and heading in the rosette stage during the winter. Only temperatures a little above freezing are effective and temperatures below freezing are without effect, as is the case in the after-ripening of dormant embryos.

Arthur and Harvill [10] grew many plants of *Digitalis purpurea* in the rosette stage in the greenhouse for years with night temperatures as low as 55° to 60° F (13° to 16° C) without having a single plant produce stems and flowers. Well-grown rosettes left out-of-doors during the winter flower in June. Rosettes kept in cold frames until December 18 flowered within two and one-half months when put into the greenhouse and given a long day. Low-temperature treatment at 41° F (5° C) caused the plants to flower within a month in the greenhouse, the day length being increased by artificial illumination. They used several methods of exposing the rosettes to the low-temperature treatments necessary to induce flowering. Two of these are shown in Fig. 137. The authors propose the term *thermoperiodism*

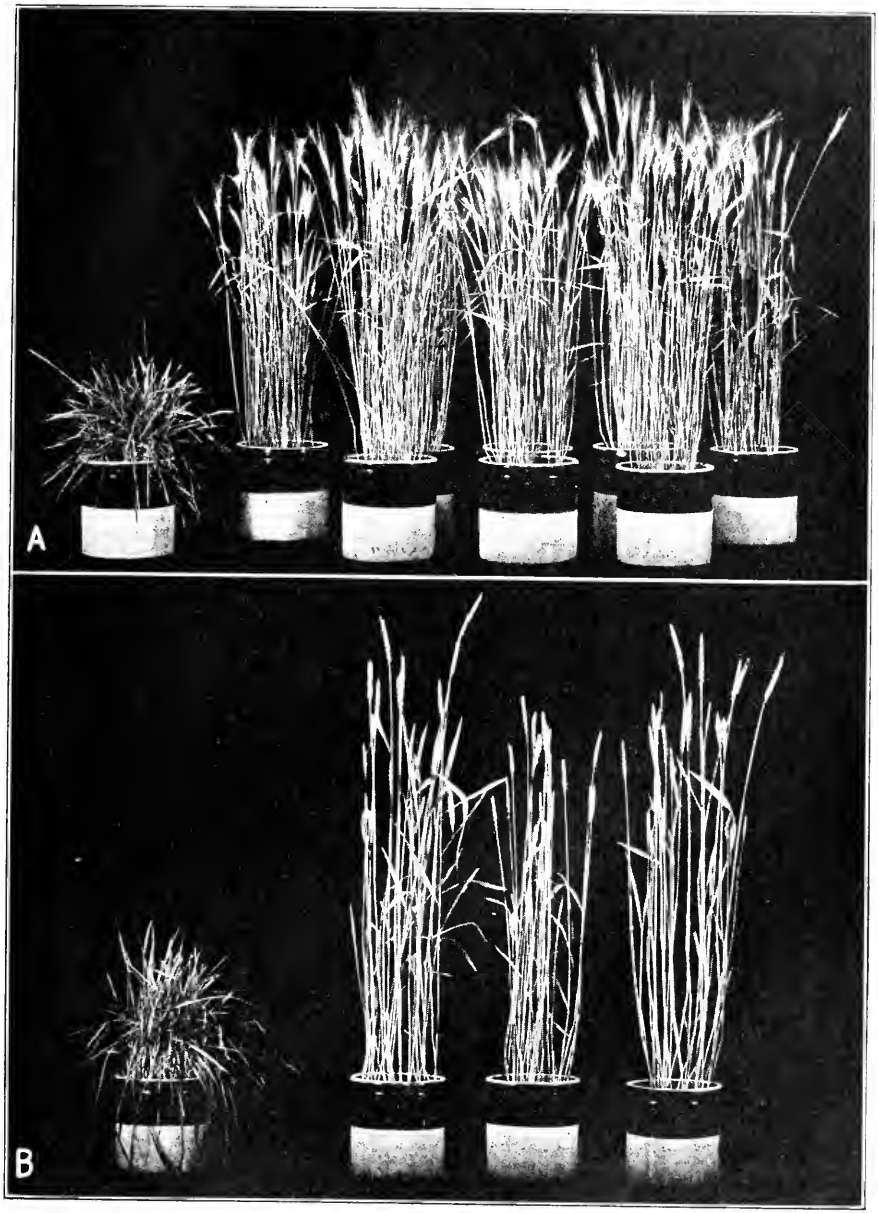

FIGURE 136. Wheat yarovized for 137 days. A, Turkey Red. B, Leap's Prolific. *Extreme left:* control.

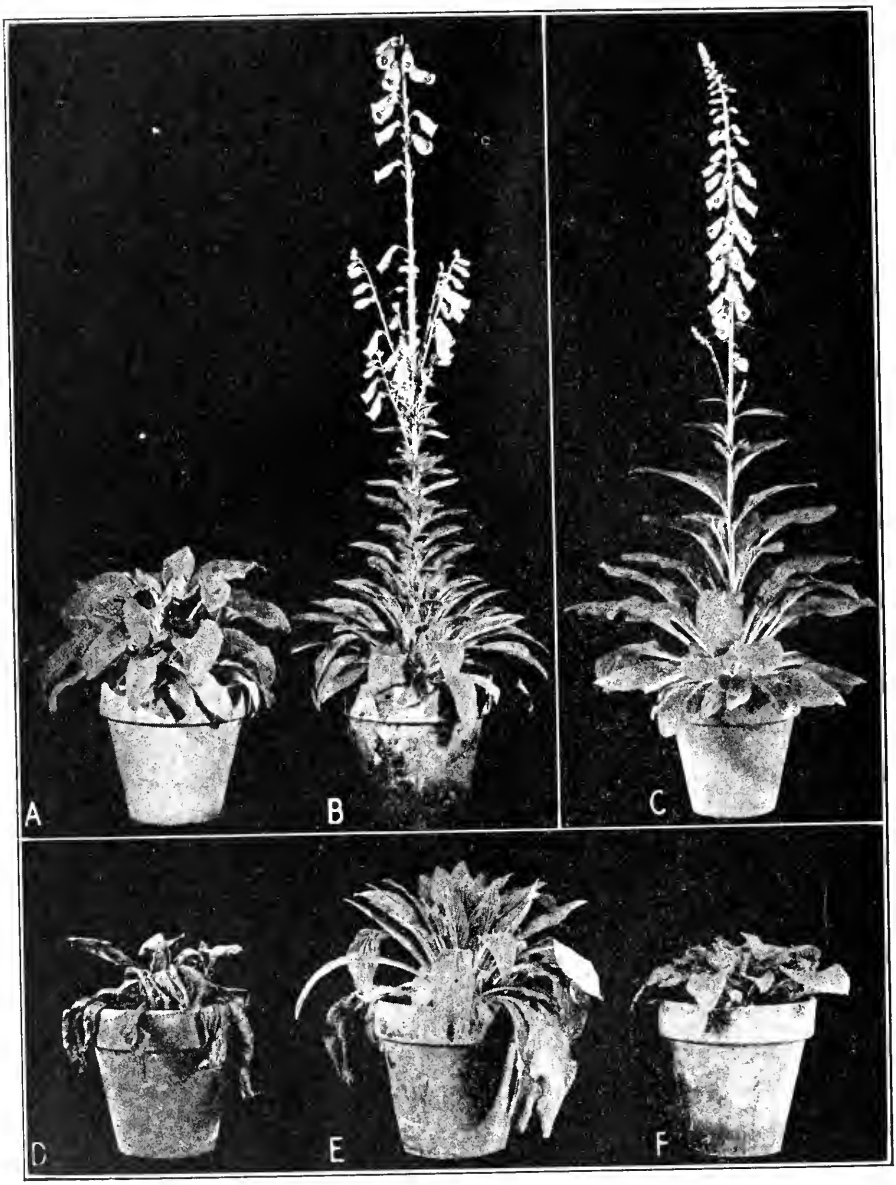

FIGURE 137. *Digitalis* after low temperature treatments. A and B, November 18, 1938. A, Control held continuously in greenhouse. B, Dark room at 50° F (10° C) each night, May 14 to September 30, then long days in warm greenhouse. C, January 4, 1940. Same dark room as B except week-ends only outside from May 10 until September 12. D, Held continuously in 41° F (5° C) dark room from May 10 to September 12. E, Same as D, except returned to outside each week-end. F, Cold dark room each night and outside each day. (D, E, F, Condition of plants at end of cold treatment.)

for the flowering response brought about by periods of low-temperature exposure.

Arthur [5, and unpublished work] confirms the findings of Slogteren and others that exposing the planted bulbs of hyacinth and daffodil to low temperatures for a few weeks induces flowering. Tulip bulbs and many other mature plants show similar responses. A large plant of *Crassula rubicunda*, which had been growing in the greenhouse for many years without flowering, was induced to flower profusely by exposure to low temperature followed by a period in the greenhouse. In many cases low temperatures during the night are sufficient to induce flowering. Thermoperiodism seems to approach photoperiodism in importance as a flower-inducing factor.

Summary

(1) When the Institute was built, three major pieces of equipment were installed for growing plants on a large experimental scale under a rather wide range of controlled conditions, with special emphasis on light of various intensities, quality, and daily duration. The two constant-condition rooms, one continuously illuminated and the other dark, provided for growing plants entirely in artificial light with various day lengths, with controlled temperatures and humidities, and with increased concentrations of CO_2. The two gantry crane houses provided means of growing plants under sunlight during the day, supplemented by artificial light at night. The five spectral greenhouses provided for growing plants under a full solar spectrum and under various portions of the solar spectrum. Finally, the insulated greenhouse provided means of growing plants under sunlight during the day with intermittent artificial light at night as the sole source of heat. Several other types of small-scale equipment were used to grow plants under different kinds of light with other environmental conditions controlled. The plants produced under the several conditions were studied as to rate and nature of growth, dry and wet weight increase, chemical composition, pigment development, and content, size, form, and anatomy of the several organs, and as to vegetative or reproductive development.

(2) Several runs of a number of different kinds of plants were made in the constant-condition rooms with 5, 7, 12, 17, 19, and 24 hours of daily illumination. Some runs were made at 68° F (20° C) and some at 78° F (26° C). The humidity was held mainly at 80 per cent and the CO_2 at 0.3 per cent. Salvia, a short-day plant, flowered on 5, 7, 12, and 15-hour days and did not flower on the 19 or 24-hour days. Lettuce and radish, long-day plants, flowered on the 17, 19, and 24-hour days but not on the shorter days. The spring cereals were also hastened greatly in heading by longer days. Buckwheat, which is indifferent to day length in flowering, flowered on all day lengths. Tomato, another day length-indifferent plant, flowered on all day lengths except 5 and 24 hours. Continuous artificial light soon killed all foliage of the tomato. Artificial light acted like sunlight or a

combination of sunlight and artificial light so far as day lengths and flowering are concerned.

(3) Under artificial light the dry weight, percentage of assimilable carbohydrates, and size of plants increased and the nitrogen content decreased with lengthening of day up to 17 or 19 hours. Increasing the day length from 17 to 19 hours up to continuous illumination reversed these changes, which probably means that all the species of plants grown under continuous illumination with this particular artificial light showed incipient injury. The tomato was especially sensitive to continuous illumination by this light, the foliage being completely killed within a few days. Geranium, coleus, and tobacco also showed foliar injury.

(4) Buckwheat plants grew well for 8 to 11 days under continuous illumination from Mazda, neon, sodium vapor, and mercury vapor lamps (700 to 800 foot-candles) and sodium vapor showed much higher efficiency than the other lights. Sodium vapor in this intensity killed all the foliage of the tomato within two months under continuous illumination at a temperature somewhat above 70° F (21° C). If the sodium vapor lamp was supplemented for two hours a day by a mercury vapor or Mazda light, no foliar injury was produced by continuous illumination by the former light and the geranium thrived indefinitely under this combination of light at a temperature of 63° F (17° C). The light in the constant-light room cost 15 cents a square foot day (900 foot-candles) for current and the sodium vapor combination 3 cents a square foot. The former gave foliar injury under continuous illumination and the latter none.

(5) Combination of daylight in winter with 6 hours of artificial light at night from the gantry crane gave excellent conditions for the growth of plants. The artificial illumination gave a great increase in size, dry weight, and carbohydrate percentage. Under this condition all long-day plants flowered promptly and the spring cereals headed much earlier while the short-day plants failed to flower. Increasing the CO_2 content of the air in the gantry crane house ten-fold, to 0.3 per cent, gave a great additional increase in size, dry weight, and carbohydrate content of plants, but had no effect comparable to day length on flowering. It did seem to increase the profusion of flowering in the rose and nasturtium.

(6) The insulated greenhouse as well as other greenhouses were used for growing plants in daylight supplemented by intermittent artificial light at night. Because of lack of air exchange, the insulated greenhouse had to be enriched with CO_2 to give maximum growth. Intermittent light at night had the same effect as lengthening the day by a continuous period of artificial light. *Calceolaria* and many other long-day plants were forced to bloom. The intermittent light also increased the dry weight, size, and carbohydrate content of the plants. At favorable temperatures intermittent artificial light 5 seconds on and 5 seconds off gave about one-half as great increase in dry weight as the same light on continuously, although the plants were nearly the same size.

(7) Underground storage organs of most plants develop on a short day. The potato is an exception; at 68° F (20° C) it tuberizes well on long days or even under continuous illumination. Storage of foods in the root of four-o'clock is also favored by the long day.

(8) The ratio of assimilable carbohydrates to total nitrogen in plants was varied greatly by the several conditions of growth in the constant-light rooms and gantry crane greenhouses. This was especially true in the radish, lettuce, and others, but was far less marked in salvia, small grains, corn, and others in which nitrate absorption is apparently regulated. The day length rather than chemical composition, or C/N ratio, determined the flowering of all photoperiodic plants. Plants indifferent to day length as regards flowering, flowered over a wide range of C/N ratio, as did the photoperiodic plants.

(9) Cultures in the spectral greenhouses indicate that the complete visible solar spectrum is necessary for perfect development of green plants and that ultraviolet has some, though a minor, importance for the form and composition of plants. Plants grown in light from the long end of the visible spectrum alone are spindly with weak stems and small thin leaves, or are otherwise inferior. Plants grown in light from the short end of the spectrum are sturdy, with well-formed deep green leaves, but are low in stature. It is possible that if sufficient intensity of blue-violet were available it would yield plants similar to those produced by the complete spectrum. A balanced spectrum seems to be necessary for plants such as we see in nature. There are indications that sodium vapor lamps supplemented by mercury vapor or tungsten lights also give a balanced spectrum.

(10) Although ultraviolet rays are of minor importance in determining the stature and form of green plants, at least at sea level, they affect plants in several other ways. (*a*) Rays of the solar spectrum shorter than those transmitted by window glass, 312 to 290 mμ, give very rapid development of the anthocyanin pigment in McIntosh apples. This pigment does not develop in darkness and only slowly in long ultraviolet and shorter visible rays. These rays also hasten the development of the pigment in cranberries and other fruits that develop the pigment in darkness. Low temperature, 15° C (59° F) is favorable for the anthocyanin production in McIntosh apples. (*b*) Short ultraviolet rays, 290 mμ or longer, induce the formation of vitamin D in most green plants, but it is much more economical to irradiate the animal directly than to feed irradiated green plants. (*c*) Ultraviolet irradiation of plants increases phosphorus and calcium absorption by increasing vitamin D in the plants. (*d*) Shorter ultraviolet rays from mercury vapor quartz tube are very injurious to plants, but rays longer than 290 mμ do no injury. (*e*) Ultraviolet irradiation of *Digitalis* seedlings does not increase the cardiac glycosides in the seedlings or in older plants grown from such seedlings. (*f*) Ultraviolet rays transform inactive *trans*-cinnamic acid into *cis*-cinnamic acid, an active plant hormone. They transform a number of other inactive compounds into active hormones.

(11) Some plants (redwood, loblolly pine, *Geum*, hog peanut, etc.) will survive in light intensities of 40 foot-candles. The sunflower needs much higher intensities for survival. Redwood and loblolly pine survived for six months in intensities that gave scarcely any increase in dry weight. In light intensities up to 20 per cent of full sunlight the increase in dry weight is proportional to the light intensity. Above that the dry weight does not increase as fast as the light intensity. Chlorophyll content increases with fall in light intensity down to about the minimal survival intensity.

(12) Transpiration plays an important role in preventing overheating of the leaf under high insolation.

(13) The great range of environmental conditions discussed above modified the amount and proportions of the five chloroplast pigments: chlorophyll *a* and *b*, xanthophyll, carotene, and a brown pigment.

(14) Lyssenko's conclusions on the vernalization of winter grains were confirmed in the main, but it was found that spring cereals do not respond to vernalization.

(15) Rosettes of *Digitalis purpurea* flower only after several months of exposure to low temperatures, such as 40° to 50° F (4° to 10° C). Large plants of *Crassula rubicunda* showed similar response. In many cases low temperatures during the night were sufficient to induce flowering. The term *thermoperiodism* was adopted to cover the flower inducing response to low temperature exposures. It seems to approach the importance of photoperiodism in flower induction.

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CHAPTER 10

Research on Insecticides

ALBERT HARTZELL

The work on insecticides at Boyce Thompson Institute covering a period of twenty years is briefly summarized in this chapter. This research has centered mainly on contact poisons, such as pyrethrum, organic thiocyanates, and piperine, and also on greenhouse fumigants including naphthalene and β,β'-dichloroethyl ether. The method of penetration of contact sprays into insect integument and tracheae, and the mode of action and neurotoxic effects on insects are described and illustrated. Such neurotoxic action was found to be indicative of active compounds used in housefly sprays. This led to a search for other neurotoxic compounds which has followed two main lines: the preparation and testing of synthetic compounds, and the extraction of plant products, which resulted in the discovery that the alkaloid, piperine, a product of black pepper, is more toxic to houseflies than pyrethrum.

In addition to the main insecticide projects mentioned above, some local insect control problems are briefly described.

There is a great dearth of fundamental knowledge on the action of insecticides. If the present account may be the means of inducing some young scientists trained in the latest techniques in chemistry, physics, and biology to become active in this field of research, it will have served its purpose; for it is only by a fuller knowledge that the ever-present demand for insect control will be alleviated.

Efficiency and Mode of Action of Contact Insecticides

A grant from the Herman Frasch Foundation for Research in Agricultural Chemistry made it possible to direct attention to the factors affecting the efficiency of contact insecticides [34] and the mode of action of some of our common insecticides on insects. Contact insecticides were selected for this study because less was known of their action on insects. Contact insecticides had been accepted and generally applied with little or no definite knowledge as to the basis of their efficiency. It was assumed by many that contact sprays entered the spiracles and tracheae of insects, thereby suffocating them.

Microscopic examinations of dissected insects showed that spray solutions fulfilling certain physical requirements entered the tracheae. The

penetration in most cases was not complete, especially with aqueous solutions; therefore, the view that death is due entirely to suffocation is untenable. The tracheae serve to conduct the active ingredient of the spray into close proximity to certain tissues, especially the nerves.

The importance of surface forces in the performance of contact insecticides had long been recognized, but few careful measurements had been made. Preliminary observations had shown that spray solutions wet insects poorly because of the nature of the integument and did not spread and form a film unless a suitable spreading agent was present. Drops of water that had been sprayed on an aphid, for example, retained their spherical shape and evaporated before spreading to a film. By adding various spreading agents, such as calcium caseinate, saponin, and gelatin to each of a series of 0.1 per cent nicotine solutions, it was noted that only in the case of soap solutions containing 0.5 per cent or more of soap were many of the drops observed to flatten out and spread to a thin film.[42]

Spray solutions were rated both with respect to their tendency to spread on insect integument, as evidenced by their spreading coefficients, and as to their toxicity to the bean aphid (*Aphis rumicis* L.). As a result of this work it was demonstrated that spreading is correlated directly with toxicity. An aqueous solution of nicotine (0.1 per cent), for example, gave a kill of approximately 60 per cent of *Aphis rumicis* without a spreading agent. When 0.5 per cent of sodium oleate was added, the kill was increased to 97 per cent.

The effectiveness of contact insecticides is greatly increased if the spray solution penetrates the tracheae of the insect. When excised portions of the tracheae from the common tomato worm (*Phlegethontius quinquemaculata* Haw.) were submerged at one end in drops of spray under a microscope, it was observed that water and several other spray solutions did not enter the tracheae, the surface forces between the liquid and the tracheal wall evidently being opposed by hydrostatic pressure tending to cause entrance. This is quite different from the behavior of such solutions in capillary glass tubes.

Soap solutions containing 0.5 per cent or more of soap entered the tracheae and exhibited a contact angle within the tracheal walls of slightly less than 90°. This large contact angle indicates that even in the case of soap solutions the capillary forces tending to cause entrance are relatively feeble. It will be remembered that when the contact angle is 90° the capillary force is zero.

A study was made of the actual penetration of the spray solutions into the tracheae of the common tomato worm by immersing the insect in the spray solution, removing and immediately dissecting the larva without the use of water or other liquid. Photomicrographs were taken of these dissections (Fig. 138). Nicotine-soap solutions were plainly visible in the tracheae. Even with the use of soap as a spreader the solutions did not penetrate the tracheal system of a tomato worm that had been previously

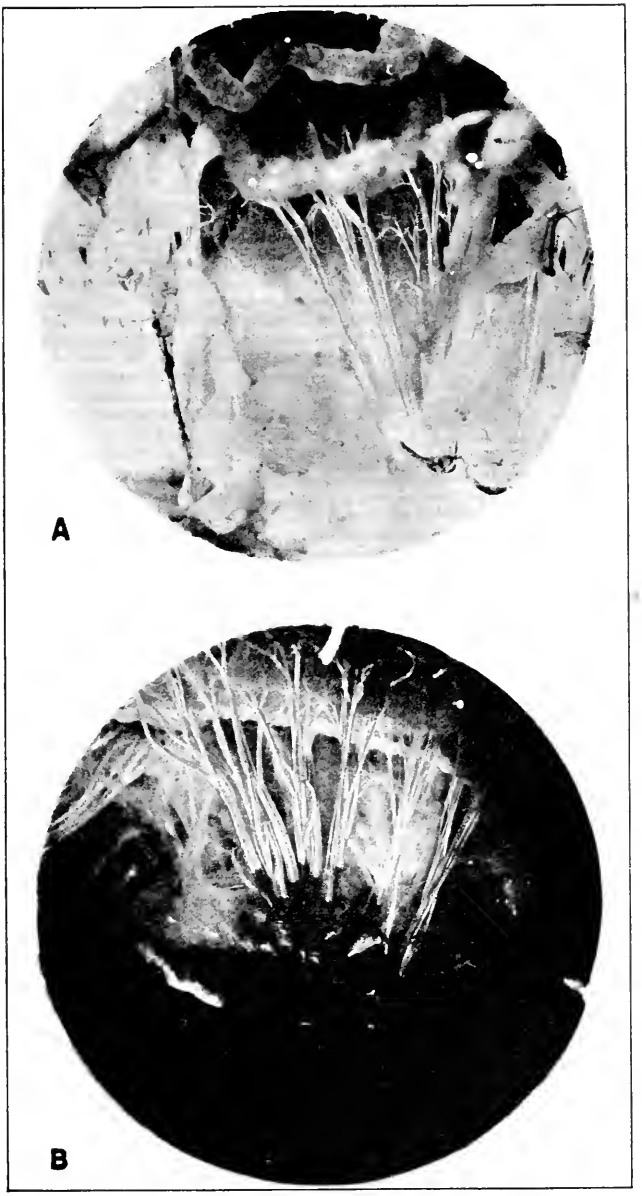

FIGURE 138. A, Tomato worm larva that had been previously killed with KCN and then immersed in a soap and nicotine solution. It will be noted from the dissection that the tracheal tubes do not contain any of the soap solution. B, Tomato worm larva that was immersed alive in a soap and nicotine solution prior to dissection. The tracheal tubes, it will be noted, are partly filled with spray solution.

killed with potassium cyanide, indicating that respiratory movements or at least vital activity are necessary for penetration to take place.

Following the study of the use of soap as a contact insecticide, the toxicity of some of the common fatty acids was determined by Dills and Menusan,[2] using *Aphis rumicis* and the rose aphid (*Macrosiphum rosae* L.) as experimental insects. Capric and lauric acids were found to be more toxic than oleic, caprylic, myristic, caproic, and palmitic acids, while stearic was the least toxic of the fatty acids tested. The insecticidal value of the potassium soaps in decreasing order of toxicity was found to be: oleate, laurate, caprate, followed by the equally toxic caprylate, myristate, and palmitate, which are more toxic than the stearate and caproate. The addition of nicotine to the soap solutions did not alter the order of toxicity. When the soap and nicotine sulphate were combined, the toxicity due to nicotine was not strictly additive; the better-spreading soaps increased the effectiveness of the nicotine.

Potassium soaps made from olive, coconut, castor, corn, palm, cottonseed, and menhaden fish oils were tested on aphids and several other species of insects. Olive-oil soap, containing the highest percentage of oleate, was found to be the most toxic. The phytotoxicity of the fatty acids was found to be in the same order as that for their toxicity to insects.

Pyrethrum. Although the results obtained by microscopic examination and the application of physical chemistry to nicotine-soap solutions explained at least in part the mechanism by which nicotine acts on insects, these results did not explain the mode of action of pyrethrum (*Chrysanthemum cinerariaefolium* Vis.), an insecticidal plant.[3, 9] In the case of pyrethrum sprays, a wetting agent was also found to be desirable. Nevertheless, with pyrethrum, killing can take place without penetration of the tracheae. This is shown by the fact that aqueous emulsions of pyrethrum are quite toxic even without a wetting agent, and also by the fact that concentrated pyrethrum preparations, when placed on portions of insects far removed from the spiracles and other body openings, are able to cause characteristic pyrethrum intoxication and death.[19] In an attempt to explain this phenomenon, the possible penetration of the material through the integument was studied by means of dyes dissolved in pyrethrum concentrate. By using Sudan III and other vital stains, evidence was obtained that pyrethrum could penetrate the integument of insects at least in certain regions.[20, 43]

Meal worms (*Tenebrio molitor* L.) were painted on the dorsal surface with pyrethrum extract colored with Sudan III, care being taken that none of the material came in contact with the spiracles. After the insects were dead, they were sectioned with a freezing microtome and examined immediately under a microscope for the presence of the dye in the tissues. It was noted that the trichogen and hypodermal cells were stained red (Fig. 139B). It would appear that the pyrethrum extract with the dye had entered through the articular membranes and the trichopores and penetrated the cells of the hypodermis. Similar membranes are found between the segments and at the

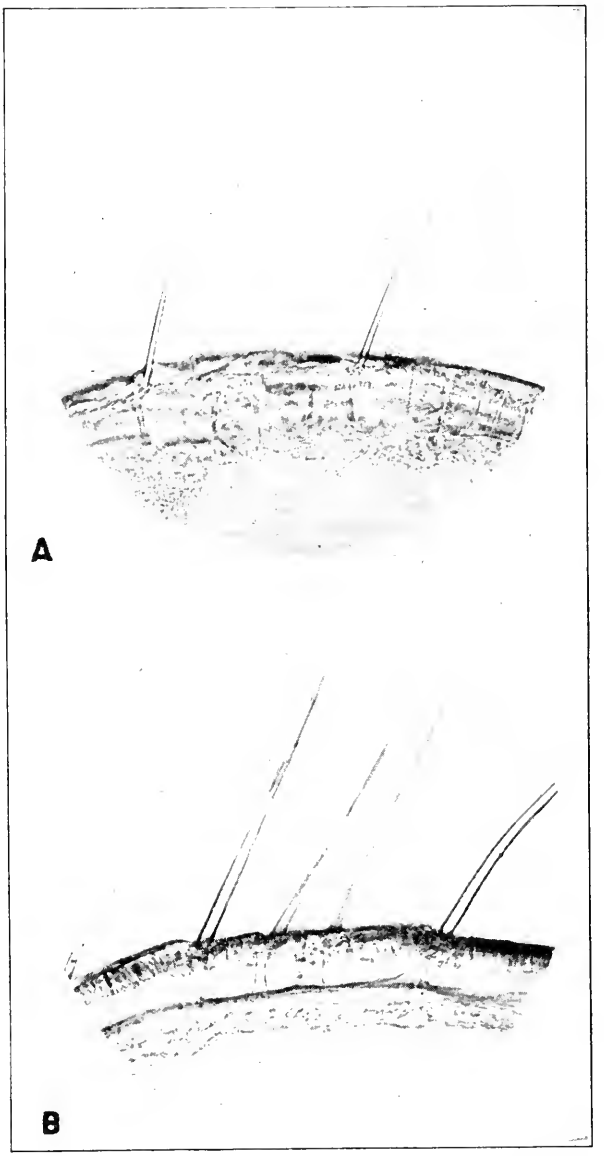

FIGURE 139. Penetration of insect tissue by pyrethrins. A, Section of the integument of an untreated *Tenebrio molitor* larva (200 ×). B, Section of the integument of a *Tenebrio molitor* larva that had been painted on the dorsal surface with Sudan III, dissolved in pyrethrum extract. The trichogen cells at the base of the setae were stained red and appear black in the photograph. The hypodermis also stained red and appears as a black line below the trichogen cells (200 ×).

attachments of the appendages. While it is conceded that the pyrethrum could penetrate to a greater depth than the stain, it seems improbable that the reverse could be true.

As pyrethrum extract is slightly soluble in water, it is probable that it would be soluble in the body fluids and be carried to the nerve ganglia. Portions of the ventral nerve cord were dissected from insects that had been killed with pyrethrum extract. The tissue was fixed in 95 per cent alcohol and stained for 5½ hours with 0.1 per cent aqueous toluidine blue, and imbedded and sectioned by the usual paraffin-xylol method. Cross-sections of the ventral ganglia and nerve cord stained blue throughout in all cases, except in larvae that had been killed with pyrethrum. Scattered among the blue-stained cells, in this case, were areas in which the cells stained violet. In addition there were other areas that appeared vacuolated, the margins of which stained dark blue (Fig. 140B). The contrast was so striking that one could readily distinguish between the controls (Fig. 140A) and larvae treated with pyrethrum extract. Insects killed with nicotine sulphate, rotenone, and lead arsenate did not show visible differences from the controls.[20, 43, 44]

Krüger [32] noted morphological changes in the nerves, muscles, and hypodermis of *Corethra* larvae that had been treated with suspensions of pyrethrum flowers in water. The ventral nerve ganglia of the treated larvae showed vacuoles that were not present in untreated larvae.

It seemed desirable to pursue this investigation further and determine more precisely the region of the nervous system affected. Lesions were found throughout the main part of the central nervous system, in the brain, suboesophageal ganglion, thoracic ganglia, abdominal ganglia, and the connectives of meal worm larvae and adult grasshoppers (*Melanoplus femur-rubrum* De Geer) killed by pyrethrum.[8] From this histopathological study it was concluded that death is caused by the destruction of the central nervous system, followed by paralysis. That pyrethrum causes nerve lesions in insects was later confirmed by Klinger,[31] who made a histological study of the nerve lesions of the larvae of the gypsy moth (*Porthetria dispar* L.) after application for 24 hours of a 15 per cent pyrethrum extract. He reports that the nerves appeared to be isolated and surrounded by spaces, as contrasted with the check where the tissue was not distorted. A similar histological change was reported by him for moribund insects.

The use of activators with pyrethrum in housefly sprays presented new problems in the mode of action of the combined spray. The percentage of flies that are knocked down is fully as important in fly sprays as the per cent killed. Interest was therefore centered on the action of pyrethrum and activators on the nervous system of moribund flies and in individuals that fully recovered after spraying.

The foremost effect of pyrethrum [14] by the Bodian technique consists of drastic destruction of the fiber tracts of the brain. The general disorganization is apparently due to actual dissolution of the fibers. In addition to

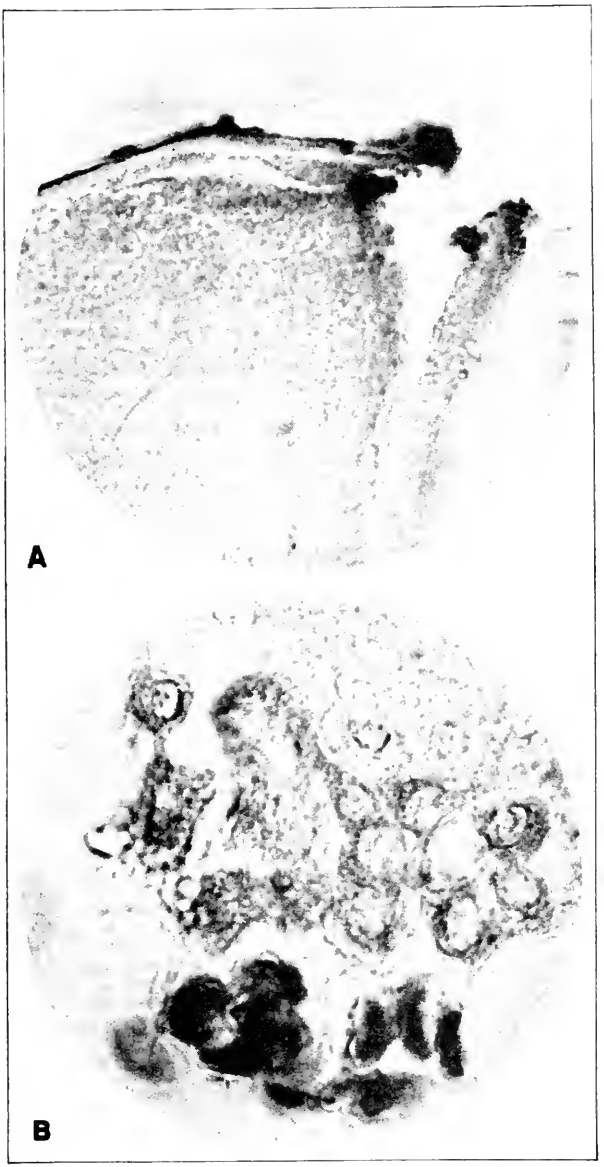

FIGURE 140. Effect of pyrethrum concentrate on nerve ganglia. A, Cross section of the ventral nerve ganglion of *Tenebrio molitor* larva killed by decapitation and stained with toluidine blue (1350 ×). B, Cross section of the ventral nerve ganglion of *Tenebrio molitor* larva that had been killed with pyrethrum extract and stained with toluidine blue (1350 ×). Note the vacuolated tissue with dark stained margins; the violet stained areas in the lower part of the photograph appear black.

the destruction of the nerves, there is a widespread clumping effect on the chromatin of the nuclei in all the body tissues. In the muscles, stained with iron hematoxylin and erythrosin, the nuclei are clumped into rod-like, oblong masses, and the cytoplasm is fenestrated. There is a loss of sharp stainability of the striations. This makes one realize why there is a loss of locomotor faculties.

Isobutylundecyleneamide, the activator of Pyrin,* causes chromatolysis, as opposed to clumping action of pyrethrum of the nuclei. This activator shows the same effect on the muscles as pyrethrum, but does not alter the stainability of the striations.

Pyrin combines the effects of both pyrethrum and activator. The combination of these two agents (as in Pyrin) shows a histological picture that is a summation of the effect of both. The interaction of these two types of nuclear destruction is believed to be the basis of activation.

The toxic agents in pyrethrum flowers are two esters called pyrethrin I and pyrethrin II. The relative toxicity of pyrethrins I and II was a controversial subject.[3, 4, 39, 40] The discrepancies of the findings of various investigators might be due either to different susceptibilities in insects used or differences in the physical state of the pyrethrins at the moment of application. Experiments were designed to test this hypothesis. The comparative toxicity of pyrethrum extracts varying in ratios of pyrethrins I and II was determined on *Aphis rumicis*, using acetone and a miscible oil as solvents for pyrethrins with water. When extracts high in pyrethrin I were compared with those high in pyrethrin II using acetone as a solvent, the former extracts were considerably more toxic than the latter. When a miscible oil such as Penetrol was used as a solvent, the difference in toxicity tended to disappear.[23]

When similar extracts were tested on houseflies (*Musca domestica* L.) by both the Peet-Grady and the modified Nelson methods the differences in toxicity of the extracts high in pyrethrin I and those high in pyrethrin II were not statistically significant.

The results indicated that the physical condition of the pyrethrins at the time of application is a determining factor in the relative toxicity, at least so far as *Aphis rumicis* and *Musca domestica* are concerned. The relative toxicity of pyrethrins I and II depends almost entirely on the method of application.

An improvement in the method of the determination of pyrethrin I was made by Wilcoxon.[41] The reaction of chrysanthemum monocarboxylic acid with Denigés' reagent was adapted to the quantitative determination of pyrethrin I in pyrethrum flowers and extract. With slight modification this has been adopted as the official method for the determination of pyrethrin I by the Association of Official Agricultural Chemists.[1, 5, 37]

Various esters of chrysanthemum monocarboxylic acid were prepared by Harvill.[28] The lauryl, myristyl, cetyl, and diethanolamine esters at a con-

* A product of John Powell & Company, Inc., New York, N. Y.

centration of 0.03 per cent gave a kill of 60.4, 62.0, 65.3, and 63.6 per cent to *Aphis rumicis*, as compared to 70 per cent for the pyrethrins at the same concentration. Most of these compounds showed no signs of decomposition or loss of toxicity for six months. The instability of the pyrethrins is believed to be due to the ketonic alcohol, pyrethrolone. None of the esters produced the typical pyrethrin symptoms when applied to various parts of the cockroach (*Periplaneta americana* L.).

Organic thiocyanates. In view of the fact that a number of compounds containing the SCN group had been reported [35, 36] to be toxic to insects, it was decided to prepare and test a number of thiocyanates representing various types. A score or more of organic thiocyanogen compounds including both aliphatics and aromatics were prepared and tested on *Aphis rumicis*.[21, 22, 45] Several of these compounds exhibited marked toxicity at a concentration of 0.1 per cent, but also caused injury to nasturtium foliage. When injury and toxicity are considered, γ-thiocyanopropyl phenyl ether proved to be one of the most promising compounds by giving excellent control of *Aphis rumicis*, mealy bugs, and red spider. The action of this compound on insects was found to be that of a paralytic agent, producing nerve lesions in the meal worm similar to those described for pyrethrum.

Trimethylene dithiocyanate was found to be equal to or better than γ-thiocyanopropyl phenyl ether. Trimethylene dithiocyanate controlled *Aphis rumicis*, the melon aphid (*Aphis gossypii* Glover), the citrus mealy bug (*Pseudococcus citri* Risso), the long-tailed mealy bug (*Pseudococcus adonidum* L.), the lesser European bark beetle (*Scolytus multistriatus* Marsh), the potato flea beetle (*Epitrix cucumeris* Harris), and red spider. Of 75 species and varieties of plants tested in regard to their tolerance to trimethylene dithiocyanate (0.1 per cent), 64 were tolerant as compared with 59 for γ-thiocyanopropyl phenyl ether used at the same concentration.

When compared at equal concentrations of SCN, lauryl thiocyanate was found to be more toxic than trimethylene thiocyanate to *Aphis rumicis*, but less toxic than rotenone.

The toxicity of mixtures of equal parts of rotenone and lauryl thiocyanate was the same as that of a spray containing rotenone alone at a concentration equal to the total concentration of the mixture, and greater than that of a spray containing thiocyanate alone at this concentration.[22]

Both trimethylene thiocyanate and phenacyl thiocyanate were ineffective as insect stomach poisons. Guinea pigs fed cabbage leaves sprayed with the first-named compound showed no acute symptoms.[22]

Piperine. It was found by Harvill and others [30] that the addition of piperine, the alkaloid found in the dry fruit of black pepper, to a pyrethrum solution gives a product that is extremely efficient as a housefly spray. Piperine was found to be more toxic than pyrethrum to houseflies, but its paralyzing action was too slow to produce the knockdown required of fly sprays. At concentrations of 0.10 per cent, piperine killed 75 per cent, and

the pyrethrins killed 51.1 per cent of the flies by the Peet-Grady method. Fly sprays containing 0.05 per cent piperine and 0.01 per cent pyrethrins were more toxic than sprays containing pyrethrins alone at a concentration of 0.10 per cent.

Acetone extracts of black pepper are unsuited for use in fly sprays, since they contain an oil with a very sharp, pungent odor. Piperine is almost without odor and causes little or no nasal irritation.

Tests were made of solutions of various substituted amides and pyrethrum. The presence of a methylenedioxyphenyl group increased the effectiveness of the amides. Increasing the side-chain attached to the methylenedioxyphenyl group increased the effectiveness of the amide.

Piperine was found to produce characteristic effects on the central nervous system and muscles of the housefly,[15] using the Bodian technique. The foremost effect was the destruction of the fiber tracts and vacuolation of the nerve tissue of the brain, but the widespread clumping effect of the chromatin of the nuclei characteristic for pyrethrum was not observed with piperine.

The action of piperine on the muscles of the housefly also appears to be different from that of pyrethrum. The head muscles of houseflies that were sprayed with piperine (0.5 per cent) showed evidence of tetanus and Krause's membrane appeared to be enlarged so that it stood out prominently. With pyrethrum there is fenestration of the cytoplasm, clumping of the chromatin of the nuclei, and loss of striation of the muscles.

Fumigants

Naphthalene. A severe infestation of red spider (*Tetranychus telarius* L.) in the Institute greenhouses focused attention on a chemical means of controlling this pest. At that time the only means known to eradicate red spider were cultural practices and hosing the plants with water from a high-pressure nozzle. Naphthalene was tried as a fumigant. This was probably the first time that it was successfully employed for that purpose in the United States, although it had been used in England a few months previously.[6, 38]

At first, flake naphthalene was volatilized by means of heat, either over lamps or on electric hot plates.[7] It was soon found, however, that if the volatilization was too rapid, the foliage of the plants was injured and the mites were not killed. A slow, even distribution of naphthalene vapor results in a satisfactory kill of the mites with little or no injury to the plants, but this is difficult to accomplish when naphthalene is volatilized by means of heat. The aid of two physical chemists was enlisted to devise a method that would overcome this difficulty.

The first suggestion was to pass a current of air from an outside source over naphthalene balls arranged on shelves in a metal box.[18] The naphthalene-laden air was blown into the greenhouse to be fumigated. By substi-

tuting solid solutions of naphthalene-sulphur mixture for naphthalene balls on the shelves, the air could be recirculated through the apparatus rather than drawn in from an outside source. This was a decided advantage when fumigating in cold weather. Both methods of fumigation would maintain practically a constant concentration under ordinary greenhouse temperatures. This apparatus unfortunately proved to be too clumsy to pass along the narrow aisles of a greenhouse.

The next development was to dissolve naphthalene in a motor oil of definite physical properties and spray the oil by means of a motor-driven pump in a cylinder open at the top protected with suitable baffles.[48] In this way the naphthalene was volatilized from the oil and dispersed in the greenhouse air and could be maintained at a constant concentration. This machine was small enough to be rolled along the greenhouse aisles. It is still in use.

Naphthalene fumigation suffers from the disadvantage that fumigations repeated at too short intervals cause the tissues of plants to harden. Many varieties of roses also are intolerant to naphthalene vapor. In spite of these limitations naphthalene has come into general use as a greenhouse fumigant both in England and in the United States.

β, β′-Dichloroethyl ether. This compound was tested as a greenhouse fumigant.[46] It was found to control *Aphis rumicis*, red spider, gladiolus thrips (*Taeniothrips simplex* Morison), and adult white fly (*Trialeurodes vaporarium* Westw.). The use of the pure compound in shallow pans with vertical porous plates dipped in the liquid to give increased evaporation was found to be the most practical method. An electric fan was used to maintain circulation of the air. Overnight fumigations of from 14 to 17 hours were used. Among 44 species and varieties of plants tested, rose (*Rosa* [hybrid tea] sp. vars. Briarcliff, Hollywood), peach (*Prunus persica* [L.] Stokes), carnation (*Dianthus* sp.), and castor bean (*Ricinus communis* L.) were among the most susceptible.

Gases. The toxicity of ammonia, chlorine, hydrogen cyanide, hydrogen sulphide, and sulphur dioxide to insects has been discussed in Chapter 5.

Search for New Insecticides

Colloidal sulphur. The earliest work on insecticides at Boyce Thompson Institute was on sulphur [12] under a joint project financed by the Crop Protection Institute and Boyce Thompson Institute. A new method of making colloidal sulphur by distilling flowers of sulphur into a soap and water solution was discovered.

The search for new insecticides has followed two main lines of attack. Taking advantage of man's long experience with plants possessing potent chemicals, particularly with drug plants, many of these have been tested for insecticidal properties. Another line of attack has been the preparation and testing of synthetic products.

Plant products. A survey of plant products for insecticidal properties has resulted in testing some 300 species and varieties of plants on mosquito larvae (*Culex quinquefasciatus* Say). As mosquito larvae are relatively easy to kill with insecticides, any substance that shows promise is likely to be detected. Another advantage of mosquito larvae as test insects is that they may be tested in an aqueous solution, thus insuring proper wetting rather than having the spray atomized on the body of the insect, which results in a variable coating even under the most precise conditions of spraying. Both acetone and water extracts have been made of all the plant products tested. Usually the products are tested at several concentrations. Mosquito larvae tests are made at a temperature of 29° ± 1° C (84° ± 2° F) for periods of 16 hours, following a standard method.[10, 26]

The median lethal dose (LD50) for mosquito larvae for balm of Gilead (*Populus* sp.) buds was found to be 5.8 ppm,[26] for filicin, a product of male fern (*Aspidium filix-mas* [L.] Sw.) 11 ppm,[47] for echinacea (*Brauneria* sp.) root 16.5 ppm, for sage (*Salvia officinalis* L.) root 18.3 ppm,[26] for cubeb (*Piper cubeba*) berries 25 ppm, for oil of sweet basil 28 ppm, for black pepper (*Piper nigrum*) 29 ppm,[10] for oil of cypress 31 ppm, and for oil of rosemary 78 ppm. In comparison the LD50 for mosquito larvae for the isolated toxin rotenone is 0.06 ppm.[26]

The fact that a plant extract is toxic to mosquito larvae does not necessarily indicate that it possesses toxicity to other species of insects. An acetone extract of balm of Gilead buds is very toxic to mosquito larvae but possesses little or no toxicity to *Aphis rumicis;* on the other hand, filicin possesses a toxicity comparable with pyrethrum to mosquito larvae, *Aphis rumicis,* and houseflies. Satisfactory kills were obtained with Mexican bean beetle (*Epilachna varivestis* Muls.) when bean plants were dusted with black pepper and the adults allowed to feed on the foliage.[10] The toxicity of piperine, a product of black pepper, was discussed in the previous section.

Synthetic products. The need for synthetic compounds as insecticides has become very acute due to the shortage of natural products such as pyrethrum and rotenone-containing plants as the result of the war. An example of this type of research is the recently published paper by Harvill and Arthur [29] on the allyl phenols as fly sprays. Allyl phenols were found to possess insecticidal properties. The median lethal dose of *o*-allylphenol, *o,o'*-diallylphenol, and *o,o',p*-triallylphenol was 18.5, 10.5, and 2.63 per cent respectively.

The γ-thiocyanopropyl and β-thiocyanoethyl esters of phenols were found to be very toxic to houseflies and had a very rapid paralyzing effect. Likewise, the γ-thiocyanopropyl ether of 1,3,5-xylenol was found to be an excellent fly spray because of its high toxicity, rapid paralyzing effect, and lack of objectionable odor.

Some Local Problems in Insect Control

Spray residues. Anxiety having arisen regarding the safety of sprayed fruit for human consumption, analyses were made of apples which had received five applications of lead arsenate (4 lb to 150 gal) during the seasons of 1926 and 1927 at Yonkers, New York.[16, 17] The average amount of arsenic as As_2O_3 was 0.173 mg per kg of fruit in 1926, with a rainfall exposure of 17.85 to 19.51 inches, while in 1927 the average value was 0.099 mg per kg with a rainfall exposure of 33.24 inches. No sample analyzed in either year exceeded the limit adopted by the Royal Commission of Arsenical Poisoning in 1903 (1.429 mg per kg), the then generally accepted standard of tolerance for arsenic. An average of 0.912 mg of metallic lead per kg of fruit and a maximum of 1.80 mg per kg were found in 1927, the only year in which analyses for lead were made.

Failure to control cankerworms by banding leads to the discovery that the larvae are wind-borne. An outbreak of cankerworms in 1933 practically defoliated a tract of woodland belonging to the Institute. Control by spraying was not considered practical because of the rough terrain. It was decided, therefore, to band the trees of a section of this woodland to test the efficacy of this method of control with the view of banding the whole tract to prevent defoliation in future outbreaks. Accordingly, trees in a somewhat isolated section were banded with tree tanglefoot in the fall of 1933, and the bands were renewed the following spring. The failure of this method to give any appreciable control led to the study of the efficiency of banding and a search for the cause that made it ineffective.[27]

In the United States the term "cankerworm" is restricted to two species of Geometridae, the fall cankerworm (*Alsophila pometaria* Harris), and the spring cankerworm (*Paleacrita vernata* Peck). The larvae of these two species are commonly called inchworms, measuring worms, span-worms, or loopers, because of their size and peculiar looping habit of locomotion. When disturbed the larvae drop, supported by silken threads. The two species have much in common and outbreaks often occur simultaneously. They attack elm and apple and feed on a wide range of deciduous host plants.

The adult female moths of both species are wingless and ascend the trunks of the trees by crawling to lay their eggs on the bark of the branches and twigs. The fall cankerworm female moth ascends the trunks of the trees in the fall to lay its eggs, while the spring cankerworm female moth ascends the tree trunks in the spring for egg-laying purposes. The eggs of both species hatch in the spring about the time apple blossoms show pink.

If trees are banded both in the autumn and spring well in advance of the emergence of the females of the fall and spring cankerworms, the female moths are impaled and thus oviposition is prevented. Control of cankerworms by various methods of banding has been a common practice for nearly a century in eastern United States.

Weighings of samples of 100 leaves were made from American elm trees in 1934 and again in 1935 as compared with an equal number of unbanded trees. The difference in leaf weight resulting from banding was less than 10 per cent, indicating no appreciable control.

Since cankerworm larvae had occurred on banded trees in numbers sufficient to defoliate them in spite of the fact that neither in the fall nor in the spring were any female moths observed that had succeeded in crawling over the bands, the larvae apparently had infested the banded trees by some other means than by hatching from eggs laid above the bands.

It was decided to investigate the possibility that the infestation on the banded trees had been brought about by larvae being blown by the wind from unbanded trees. Eight stationary shields covered with tanglefoot and directed toward the cardinal and intercardinal points of the compass were erected on a pole 20 feet high just north of the banded area. From time to time the shields were examined for the presence of cankerworm larvae and the tanglefoot renewed when needed. The site selected was a clear space where no larvae could reach it except by passing through an air space of at least 25 feet. A tanglefoot barrier was maintained on the pole to prevent any larva from climbing up from the ground. To the west, the land sloped away rapidly and there were no infested trees in direct line of the shields for about 500 feet. It was demonstrated by means of captures on the tanglefoot shields that cankerworm larvae are disseminated by wind. A total of 99 larvae were collected on the shields. Larvae were collected on all shields. Toward the west and the northwest there were no infested trees nearer than 500 feet, yet more than 20 per cent of the larvae were collected from shields facing these directions. It was estimated that if the density of the larvae per cubic foot of air space was the same at all heights above ground as surrounding the shields, in an air space occupied by a tree 60 feet high and 20 feet wide approximately 5000 larvae would have drifted from May 18 to June 12 during the period of observation.

As is often the case in research, the failure of an accepted method led to a discovery, namely that cankerworm larvae are wind-borne, the silk strands buoying the insects through the air like parachutes.

Japanese beetle. β,β'-Dichloroethyl ether was tested as a soil fumigant against the larvae of the Japanese beetle (*Popillia japonica* Newm.). The saturated aqueous solution, with the addition of 0.1 per cent Tergitol 7 penetrant* (a sodium alkyl sulphate) as wetting agent, was found to give satisfactory control of the grubs, when two applications were made about one week apart when the grubs are near the surface of the soil.[24] No injury to the turf resulted with this treatment. When it is desired to avoid the use of materials which leave poisonous residues, such as lead arsenate, and where a rapid kill is required, this mixture may offer a promising substitute.

It was found, however, that β,β'-dichloroethyl ether with Tergitol 7 penetrant as a wetting agent was not very effective in the control of adult

* A product of Carbide and Carbon Chemicals Corporation, New York, N. Y.

Japanese beetles. It was found, nevertheless, that Tergitol 7 penetrant can function both as a solvent and as a spreading agent of pyrethrum resins and possesses definite insecticidal properties of its own.[25] An aqueous solution containing 0.02 per cent total pyrethrins and 0.5 per cent Tergitol 7 penetrant gave a satisfactory control of adults (85 to 100 per cent). Of 26 species and varieties of plants tested, the foliage of only one (apple) was severely injured, and four species were slightly injured.

A survey was begun in 1939 to find new wetting agents to improve agricultural sprays especially for the control of Japanese beetle. A series of 34 wetting agents [33] were tested in combination with lead arsenate and rotenone sprays for the control of adult Japanese beetles. Ultroil * (a sulphonated vegetable oil), when used in combination with lead arsenate, left no visible residue and caused no injury to the foliage of 48 species and varieties of plants tested. This combination appears promising for use on ornamentals where a minimum visible residue is desirable.

Holly leaf miner. Preliminary results [13] had indicated that a spray consisting of two quarts of fish oil, and one quart of nicotine sulphate made up to 100 gallons with water and sprayed on the foliage gave a reduction in mines of 91 per cent of the holly leaf miner (*Phytomyza ilicicola* Loew). Spray applications were made the second and third weeks in May, followed by an application about the middle of June, and a final application around the middle of July. These results were later confirmed by extensive field experiments [11] made during the season of 1942. It is probable that two applications would be sufficient in a normal year if carefully correlated with the life cycle of the insect, if the first application is made two or three days before the peak, and the second two or three days after the peak of emergence of the adults.

Summary

The role of surface forces in determining the efficiency of contact insecticides has been one of the principal subjects of investigation.

Spray solutions wet poorly and do not spread over the insect and form a film unless a suitable spreading agent is present. It was also found that spray solutions do not penetrate the tracheal system of insects without a wetting agent. Even with the use of soap as a spreader, the solution did not penetrate the tracheal system of an insect killed with KCN, indicating that respiratory movements or at least vital activity are necessary for penetration to take place. The angle of contact exhibited by a soap solution with the trachea also indicates that the capillary forces involved cannot account for penetration by this means alone.

The toxicity of spray solutions containing nicotine and various spreaders follows the same order as the spreading coefficient.

In the case of pyrethrum it was found that intoxication and death may

* A product of Hercules Powder Co., Providence, R. I.

occur from external applications of pyrethrum concentrates under conditions where no tracheal penetration takes place. Evidence is presented that pyrethrum can penetrate the integument of insects through the articular membranes into the trichopores and permeate the cells of the hypodermis.

Histological changes were detected in the central nervous system of insects killed by pyrethrum, piperine, and organic thiocyanates by means of stains.

The uses of activators with pyrethrum presented new problems in the mode of action of the combined sprays. The histological effects of pyrethrum and isobutylundecyleneamide, an activator of pyrethrum, were studied. A widespread clumping effect of the chromatin of the nuclei was observed for pyrethrum, while the activator isobutylundecyleneamide caused chromatolysis or dissolution of the chromatin in preparations stained with Bodian's method. The combination of these two agents showed a histological picture that is a summation of the effect of both.

Piperine causes destruction of the fiber tracts of the brain of the housefly.

The physical condition of the pyrethrins at the time of application was found to be the determining factor in their relative toxicity. When extracts high in pyrethrin I were compared with extracts high in pyrethrin II, using acetone as a solvent, the pyrethrin I extracts were considerably more toxic than extracts high in pyrethrin II. When a miscible oil was used as a solvent the differences tended to disappear.

An improvement in the method of the determination of pyrethrin I was made by Wilcoxon, which with slight modification has been adopted by the Association of Official Agricultural Chemists.

Various esters of chrysanthemum monocarboxylic acid were prepared by Harvill. The lauryl, myristyl, cetyl, and diethanolamine esters were slightly less toxic than the pyrethrins at the same concentrations.

A number of organic thiocyanates were prepared. γ-Thiocyanopropyl ether gave excellent control of *Aphis rumicis*, mealy bugs, and red spider. The toxicity of mixtures of equal parts of rotenone and lauryl thiocyanates was the same as that of a spray containing rotenone alone at a concentration equal to the total concentration of the mixture and greater than a spray containing thiocyanates alone at this concentration.

A method of fumigation with naphthalene was devised which permits control of the concentration of naphthalene vapor in the air of a greenhouse, which insures that the desired concentration will be maintained throughout the fumigation period. The method involves the continued recirculation of the greenhouse air through a saturator containing a solution of naphthalene in a solvent. The concentration of the naphthalene in the solvent determines the maximum concentration which can be reached in the greenhouse air. It was found that a satisfactory control for red spider could be obtained by fumigation with this method without injury to most plants usually considered sensitive to naphthalene fumigation, if a sufficient time interval elapses between fumigations to avoid hardening of the plant tissues.

β,β′-Dichloroethyl ether was tested as a greenhouse fumigant and found to control *Aphis rumicis*, red spider, gladiolus thrips, and adult whitefly.

A survey was made of plant products for insecticidal properties representing some 300 species and varieties of plants. The median lethal dose (LD50) for mosquito larvae (*Culex quinquefasciatus*) for balm of Gilead (*Populus* sp.) buds was found to be 5.8 ppm, for filicin, a product of male fern (*Aspidium filix-mas* [L.] Sw.), 11 ppm, for echinacea (*Brauneria* sp.) root, 16.5 ppm, for sage (*Salvia officinalis* L.) root, 18.3 ppm, for cubeb (*Piper cubeba*) berries, 24 ppm, for oil of sweet basil, 28 ppm, for black pepper (*Piper nigrum*), 29 ppm, and for oil of cypress, 31 ppm.

Piperine, the alkaloid present in the dry fruit of black pepper (*Piper nigrum* L.), was found to be more toxic than pyrethrum to houseflies, but its paralyzing action was too slow to produce the knockdown required by fly sprays. At a concentration of 0.10 per cent, piperine killed 75 per cent, and the pyrethrins killed 51.1 per cent of the flies by the Peet-Grady method. Fly sprays containing 0.05 per cent piperine and 0.01 per cent pyrethrins were more toxic than sprays containing pyrethrins alone at a concentration of 0.10 per cent. Thus by making suitable combinations of piperine and pyrethrum in fly sprays large savings of pyrethrum may be accomplished without reduction in toxicity or knockdown effects.

The allyl phenols were found by Harvill and Arthur to possess insecticidal properties on houseflies.

Several local insect control problems have been investigated. These have been undertaken as the need has arisen in Yonkers, New York, and vicinity. Spray residues on apple trees that had received five applications of lead arsenate (4 lb to 150 gal) fell below 1.429 mg of As_2O_3 per kg of fruit, indicating that with normal rainfall arsenic spray residues may be kept within reasonably safe limits.

The failure to control cankerworms in a tract of woodland by banding led to the discovery that the larvae are wind-borne.

β,β′-Dichloroethyl ether with 0.1 per cent Tergitol 7 penetrant was found to be effective in the control of Japanese beetle grubs in turf without the possibility of leaving a poisonous residue in the soil. It was found in tests to control Japanese beetle adults that Tergitol 7 penetrant, a sodium alkyl sulphate, can function both as a solvent and spreading agent for pyrethrum resins and possesses definite toxicity of its own.

A spray consisting of 2 qt fish oil soap and 1 qt nicotine sulphate made up to 100 gal with water was found to be effective in the control of the holly leaf miner, the most serious pest of holly in eastern United States.

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Note: The common greenhouse red spider in the United States is now recognized as *Tetranychus bimaculatus* Harvey.

CHAPTER 11

Fungicide Investigations

S. E. A. McCallan

The work of the fungicide laboratory has been along three main lines. These are: (*a*) studies on the nature or mechanism of fungicidal action; (*b*) development and improvement of laboratory and greenhouse methods of evaluating the effectiveness of fungicides; and (*c*) research on new chemicals as possible fungicides. Since these three approaches are closely interdependent on one another, advances made in any one have been of considerable help in the furthering of the others and in our general understanding of fungicides.

Fungicidal Action of Sulfur

The initial research on fungicides was begun in 1929 by Dr. Frank Wilcoxon and the author under a grant from the Herman Frasch Foundation for Research in Agricultural Chemistry, and coincidentally but very appropriately the nature of the fungicidal action of sulfur was first chosen for study.

The earliest use of sulfur as a fungicide probably is shrouded in the mists of antiquity. About the beginning of the 19th century various intelligent gardeners were advocating it for the control of mildews. Today sulfur is one of our most important fungicides and annually about 150,000,000 pounds are consumed for this purpose in the United States. Because of such a long history and extensive use, there have been many theories to account for the manner in which insoluble sulfur acts as a fungicide.

Pentathionic acid. A recent theory which had received some publicity was first advanced in 1922 by Young,[40] who contended that traces of pentathionic acid, associated with sulfur and formed from it, constituted the active fungicidal agent. Because of the relative newness of this theory, a critical study of it was undertaken.[33] Potassium and barium pentathionates were prepared for the first time in sulfur toxicity studies and from these, solutions of the uncontaminated pentathionic acid were obtained.

Comparative toxicity tests were made of pentathionic acid, sulfuric acid, and hydrogen sulfide by means of the slide-germination method. In the case of hydrogen sulfide it was necessary to work in a closed system in order to prevent its escape — a precaution ignored by some earlier workers. Four representative fungi exhibiting different degrees of sulfur sensitivity

were used, namely *Sclerotinia fructicola* (cause of brown rot of stone fruits), *Botrytis* sp. (*cinerea* type) (cause of marigold blight), *Macrosporium sarcinaeforme* (cause of leaf spot of red clover), and *Uromyces caryophyllinus* (cause of carnation rust). In all four cases the toxicity of the pentathionic and sulfuric acids was found to be identical and apparently was due to the acidity. Hydrogen sulfide was found to be from 6 to 200 times as toxic as the pentathionic or sulfuric acid. Neutral salts of pentathionic acid were non-toxic except when decomposed with alkali to give fungicidally active colloidal sulfur. Most samples of sulfur gave water extracts containing pentathionic acid, but these were found to be non-toxic. Finally a commercial sulfur dust was treated with alkali to remove the pentathionic and sulfuric acids and a comparative toxicity test with the same material before treatment showed no difference. Thus it was concluded that pentathionic acid is not a factor of importance in the fungicidal action of sulfur.

Hydrogen sulfide. As early as 1875, Pollacci in Italy noted that grape leaves treated with sulfur evolved H_2S, which he believed was thus responsible for the fungicidal action of sulfur. Marsh [27] in England had shown that certain leaves, when sulfured, evolved H_2S and also that H_2S was toxic to fungus spores.

In view of these findings and the high toxicity of H_2S observed in the pentathionic acid work, a detailed examination of this theory was undertaken.[19] It was found that all species of plants tested evolved H_2S when in association with sulfur. The plants included the attached leaves of 26 species of higher plants, the spores of 16 species of fungi, the sporophores of 3 species of Agaricaceae, and the expressed and filtered juice of *Pleurotus ostreatus*. A quantitative determination on a potted strawberry plant showed at 35° C (95° F) an evolution of 0.002 mg of H_2S per hour per sq dm of leaf surface.

Quantitative determinations were also made on the spores of eight different species of fungi, namely: *Venturia inaequalis, Uromyces caryophyllinus, Puccinia antirrhini, Sclerotinia fructicola, Macrosporium sarcinaeforme, Pestalotia stellata, Glomerella cingulata,* and *Botrytis* sp. (*cinerea* type). The spores were mixed with an aqueous paste of sulfur in a small stoppered bottle with a strip of lead acetate paper suspended from the stopper, and the time was determined for the lead acetate paper to attain a known degree of blackening. Thus rate of production curves for H_2S were obtained and the total amount produced calculated. This amount was found to be directly proportional to the number of spores, but varied with the different species. In 12 hours at 30° C (86° F), the most active fungus, *Glomerella,* produced an amount of H_2S equivalent to 9.8 per cent of the weight of the spores, while the least active fungus, *Macrosporium,* produced only 0.14 per cent of its weight of H_2S. The effect of temperature was marked with a well-defined maximum at about 35° C (95° F), but at 60° C (140° F) the reaction was entirely inhibited. These temperature relations indicate an enzymatic reaction. Within a pH range of 4.0 to 8.0 the rate of evolution

was independent of acidity. Actual contact between the sulfur and spores was not found to be necessary for the production of H_2S, since it was found that the reaction can take place through a collodion membrane with the H_2S being produced on the spore side and not the sulfur side, as illustrated

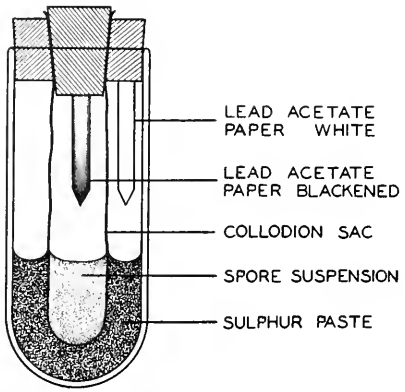

FIGURE 141. The production of hydrogen sulfide by *Sclerotinia* spores separated from sulfur by a collodion membrane. Note that the evolution of hydrogen sulfide takes place on the spore side of the membrane and not on the sulfur side.

in Fig. 141. It is thus evident that the production of H_2S takes place on or within the spore. The reaction can even take place across an air space of several millimeters, as shown in Fig. 142. The presence of the sulfur-reducing —SH group was also demonstrated in the spores of *Sclerotinia fructicola*.

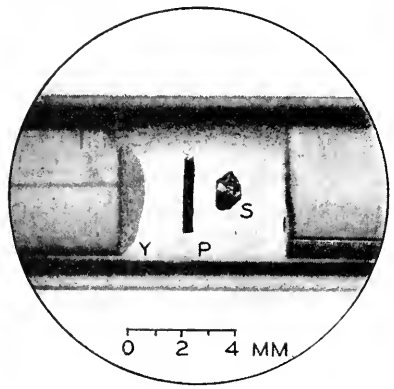

FIGURE 142. The action of sulfur (*S*) across an air space on yeast spores (*Y*), as indicated by the blackening of lead acetate paper (*P*).

Hydrogen sulfide gas was found to be highly toxic to the spores of these eight fungi, the toxicity varying with the different species. When these eight species were compared as to their sensitivity to H_2S and to sulfur the order was identical. The order of decreasing sensitivity was as follows: *Venturia*, *Uromyces*, *Puccinia*, *Sclerotinia*, *Macrosporium*, *Pestalotia*, *Glomerella*, and *Botrytis*. When the production of H_2S by these spores was expressed in units equal to the amount of H_2S required to reduce their germination 50 per cent, the following relation appeared, as may be seen in Table 38: the four sulfur-sensitive species (*Venturia*, *Uromyces*, *Puccinia*,

Table 38. Comparison between the Toxicity and the Production of Hydrogen Sulfide, Expressed in Units Equal to the Amount of Hydrogen Sulfide Required to Reduce Germination 50 Per Cent.

Species	Mg H_2S required to reduce germination of 1,000,000 spores 50%	Mg H_2S produced by 1,000,000 spores in 12 hours	Production of H_2S expressed in units equal to the amount of H_2S required to reduce germination 50%
Venturia inaequalis	0.001	0.002	2.0
Uromyces caryophyllinus	.002	.019	9.5
Puccinia antirrhini	.006	.013	2.2
Sclerotinia fructicola	.013	.039	3.0
Macrosporium sarcinaeforme	.043	.013	0.30
Pestalotia stellata	.049	.001	.02
Glomerella cingulata	.523	.027	.05
Botrytis sp. (*cinerea* type)	.665	.002	.003

and *Sclerotinia*), which can be controlled in the field with sulfur, produce more than one unit, while the four resistant species not controllable by sulfur produce considerably less than one toxic unit of H_2S. Very recently Horsfall [6] has demonstrated that when spores of *Macrosporium* and *Sclerotinia* are placed together in the same drop of water in the presence of sulfur, the sulfur "resistant" *Macrosporium* is killed, presumably because of the excess H_2S produced by *Sclerotinia*.

As a result of these studies the fungicidal action of sulfur is explained thus:[19, p.35] "Sulfur in the vicinity of fungous spores, by reason of its vapor pressure, gives off sulfur vapor which diffuses into the spores. Here reduction takes place within the spores with hydrogen sulfide as a final product. The reaction is enzymatic in nature and is probably concerned with —SH compounds. The toxic product, hydrogen sulfide, being produced in intimate contact with the living cell, is able to exert its maximum effect. It is not believed that the hydrogen sulfide produced from the leaves in the open affects the spores. . . . Each individual spore, therefore, by reason of its ability to reduce sulfur to hydrogen sulfide, is thus instrumental in bringing about its own death."

In order to throw further light on the mode of action of sulfur as a fungicide, a study was made of the comparative toxicity of sulfur and its analogous elements, selenium and tellurium.[35] These were found to be much less toxic than sulfur to spores of *Sclerotinia fructicola*, *Pestalotia stellata*, and *Uromyces caryophyllinus*. Even in colloidal form, selenium showed no appreciable toxicity. The toxicity of hydrogen selenide, however, while difficult to measure accurately because of the instability of this compound, appeared to be comparable to that of H_2S. But the formation of hydrogen selenide and hydrogen telluride by yeast cells or by glutathione took place less readily than the reduction of sulfur to H_2S under similar conditions. Thus the lesser toxicity of selenium and tellurium is readily understood, and is in accord with the explanation offered for the fungicidal action of sulfur.

It seems probable that the phytotoxicity of sulfur fungicides, especially lime sulfur, is also due to the production of H_2S, for in the work on the toxicity of H_2S to green plants [12] discussed in Chapter 5 it was found that the symptoms of lime sulfur injury were identical with those of H_2S. Studies on the toxicity of other industrial gases to fungi [13, 14] and to green plants are also described in detail in Chapter 5.

Particle size. Various commercial sulphur dusts were found to differ in their toxicity toward conidia of *Sclerotinia fructicola*.[34] The difference could not be ascribed to the rate of formation of H_2S nor to the acidity of the water extracts; it is due to the size of the sulfur particles, and the toxicity increases with a decrease in the size of particles. When straight unmodified sulfur dusts, particles of which differ significantly in their mean diameter, were compared on an equal weight basis, there were significant differences in toxicity. However, when these dusts were compared on the basis of an equal number of particles per unit area, there was no difference in toxicity, as is graphically demonstrated in Fig. 143.

A convenient method for determining the mean particle size of sulfur dusts was developed. This involves counting the number of particles furnished by a known weight of sulfur dusted on a surface of known area.

The tenacity of sulfur dusts to glass slides, following laboratory "rain" tests, was found to depend on the degree of fineness of the dust, the smaller particles being the most tenacious. With a given dust, an increase in the amount applied results in a decrease in the percentage adhering. It was found that in general dusts adhere better to leaves than to glass slides, and that the tenacity improves with the roughness and hairiness of the leaf surface.

A useful measure of the dusting qualities of sulfur dusts was found in the "angle of slope," that is, the angle between the side and base of a cone of dust carefully built up to the maximum height attainable; the smaller the angle the better the dusting quality.

Thus the most important single factor determining the toxicity of sulfur applied as a dust is the number of particles furnished by a unit weight of the material. The greatest number of particles is furnished by the dust of smallest mean particle diameter; this is also the most tenacious and will give a denser distribution for a given weight. This relation between particle size and toxicity seems to hold within wide limits from relatively coarse particles down to those of colloidal dimensions. However, the dust having the highest toxicity does not necessarily have the best dusting qualities.

Fungicidal Action of Copper

Since the classical discovery of Bordeaux mixture by Millardet in 1882, the copper fungicides have become our most effective group. About 100,000,000 pounds of copper sulfate are consumed annually in the control of plant diseases in the United States. Although in recent times numerous substitutes have been developed, Bordeaux mixture remains by far the

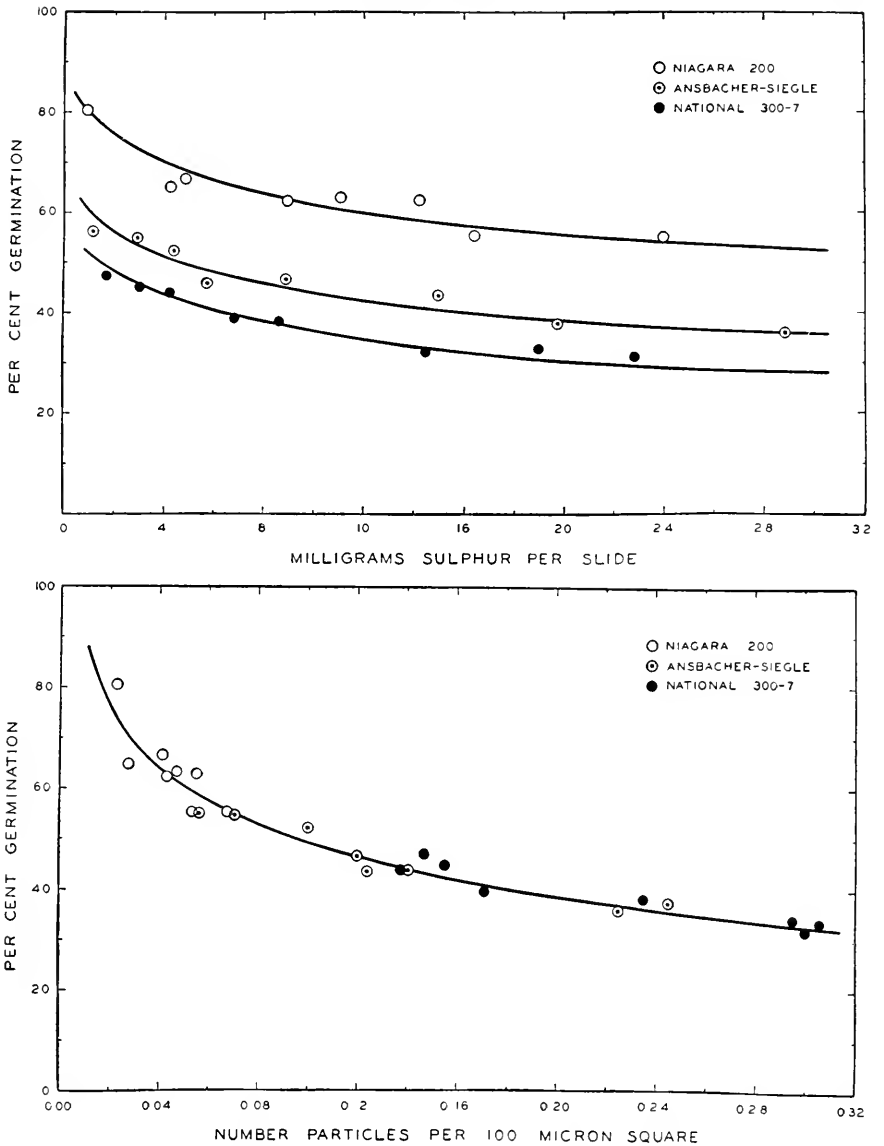

FIGURE 143. (*Above*) Toxicity curves, for three unmodified sulfur dusts of significantly differing mean particle size, plotted on a weight basis and showing a significant difference in toxicity. Germination of conidia of *Sclerotinia fructicola*. (*Below*) The three upper curves plotted on the basis of particle numbers become one curve showing that the distribution or number of sulfur particles determines the toxicity.

most important copper fungicide. Bordeaux is a complex mixture of copper sulfate and hydrated lime, and despite much research its chemistry is still not entirely understood, though careful work by Martin [28] indicates that it is cupric hydroxide stabilized by adsorbed sulfate ions. The copper is present

in an insoluble form, and various theories have been advanced to explain its fungicidal action.[8] These may be divided into three groups: (*a*) that atmospheric agents such as CO_2 and rain liberate soluble copper; (*b*) that the fungus itself secretes materials which free the copper; and (*c*) that excretions from the host plant act on the insoluble copper.

Studies were made of the action of fungus spores on Bordeaux [23] and of the weathering of Bordeaux mixture.[38] The amount of copper that goes into solution in distilled water above a dried deposit of Bordeaux was determined to be less than 0.3 ppm, an amount insufficient to affect materially the germination of most fungus spores.

Action of spores. Fungus spores were obtained by a vacuum technique which prevented their contamination by the nutrient medium.[23] These were suspended in water, allowed to stand for several hours, and filtered. The filtrates were then placed over dried Bordeaux mixture and agitated overnight. The amount of copper rendered soluble varied with the seven different species tested and was directly proportional to the number of spores. For example, the water extracts from 100,000,000 spores of the most active fungi, *Uromyces caryophyllinus* and *Sclerotinia fructicola*, brought into solution 1.01 and 0.76 mg of copper respectively, whereas the least active fungus, *Alternaria solani*, liberated 0.013 mg of copper. Determinations of total solids excreted by the spores showed that those species excreting the greatest amount of solids were also the most active in bringing copper into solution. It was also found that those spores most sensitive to the toxicity of Bordeaux mixture were in general the ones most active in solubilizing copper.

By means of ultrafiltration tests it was further found that the active solubilizing material in the spore excretions is all in true solution. Also, practically all of it may be removed in the first washing of the spores. A quantity of spore excretion was obtained from approximately 360,000,000,000 spores of *Neurospora sitophila*, and an analysis indicated 3.1 per cent of malic acid present in the solid matter. There was also found 0.75 per cent of amino nitrogen, thus indicating the presence of amino acids. Since the spore excretions are practically neutral, their action cannot be due to any acidic properties. It is well known that certain amino acids form soluble complexes with copper oxide, and it was found that glycine and aspartic acid as well as neutral sodium malate will dissolve large amounts of copper from Bordeaux mixture. Finally, comparative toxicity tests of sodium cuprimalate and of a copper glycine derivative showed that these forms of copper exert substantially the same action as copper sulfate. Thus, in view of these findings, it is believed that the salts of hydroxy acids such as malic and perhaps others, as well as of amino acids present in the spore excretions, act on Bordeaux mixture to form soluble toxic copper hydroxy and copper amino salts.[23]

Weathering action. The action of the spores in liberating soluble copper from Bordeaux mixture was of course demonstrated on glass in the labora-

tory; under actual field conditions other agencies such as rain may also be a factor. Although considerable research had been done on the action of weathering, possible changes which may occur in the copper-lime-sulfate ratio of Bordeaux under the influence of rain had not been studied.

It was found that when glass plates are sprayed with an 8–8–100 Bordeaux mixture and exposed outdoors, the sprayed film undergoes a change in composition under the leaching influence of rain and dew, leading to a mixture relatively richer in copper.[38] This change in composition is accompanied by an increase in soluble copper. The highest amount observed was 0.45 mg per plate (225 sq cm), when the plate was agitated with 50 cc of water. Carbonation of the excess lime was complete in a few hours, as judged by pH measurements, but the increases in soluble copper did not occur until much later. The results could be duplicated in the laboratory using artificial rain, but only if a sprayed, dried film of Bordeaux was used. Washing the Bordeaux precipitate in bulk by centrifuging, or on a Büchner funnel, did not lead to substantial increases in soluble copper. When Bordeaux mixtures low in lime were subjected to the leaching action of rain, soluble copper appeared sooner than with an 8–8–100 mixture. Treatment of the sprayed films with CO_2, either wet or dry, did not lead to much increase in soluble copper. It is considered that the increases in soluble copper observed can be best explained by assuming that the weathered Bordeaux precipitate is an adsorption complex, or a solid solution containing copper, lime, and sulfate, the copper of which is soluble in water to an extent which varies with its composition. The appearance of small amounts of soluble copper must be considered as a factor in connection with foliage injury, as well as in fungicidal action where it may supplement the solvent action of spore excretions.

The Laboratory Slide-Germination Method of Evaluating Fungicides

Although the ultimate evaluation of fungicides must depend on their efficiency under actual field conditions, such tests are costly and time-consuming. It is most desirable to be able to test fungicides under the rapid and simple conditions of the laboratory and greenhouse and considerable effort has been devoted to developing such methods and to a clarification of the important factors involved.[9, 10, 11, 15, 16, 17, 18, 20, 21, 24, 25, 26, 29, 30, 31, 32, 39]

Theoretical principles. In the beginning of laboratory testing[7] little or no attention was paid to such factors as concentration or deposition and variability of results, while statistical interpretation was unheard of. Pioneering studies were undertaken in these matters. The relative precision of spore germination tests was demonstrated,[20] and it was pointed out that the errors arise from two sources, namely, faulty technique and random sampling; the former may be reduced but the latter cannot be avoided.

Toxicity surface. When tests are performed with three variables of concentration, time, and response, *i.e.*, per cent germination, it is possible to

show the results in the form of a three-dimensional solid model, as illustrated in Fig. 144. The concept of a "toxicity surface," as is seen in the figure, was thus introduced.[21] It was further shown that the form of the toxicity surface determines the precision of a toxicity experiment. Whether comparisons of toxic agents are made on the basis of the times required for an equal percentage response, or on the basis of percentage responding in equal times is largely a matter of convenience, for at a given point on the toxicity surface both methods are capable of equal precision.

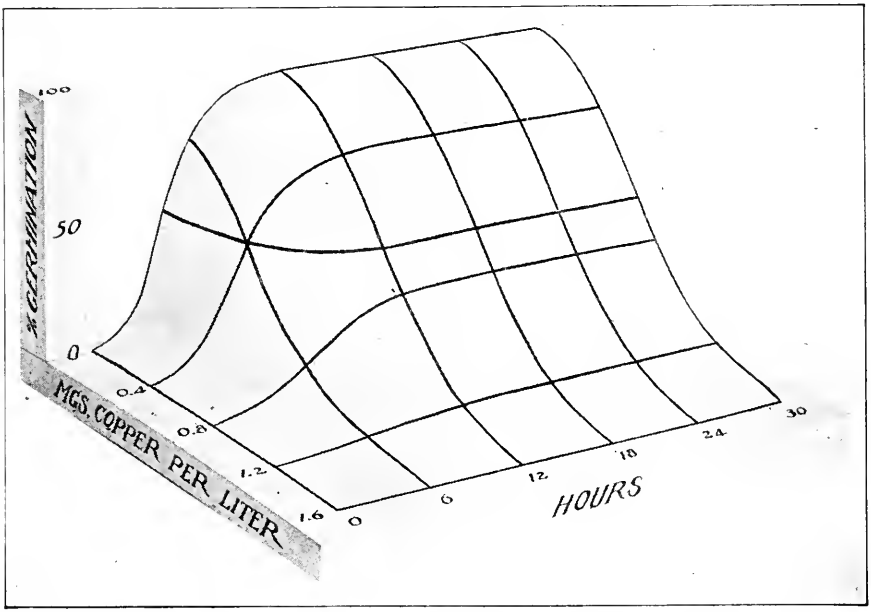

FIGURE 144. The toxicity surface for the action of copper sulfate on conidia of *Sclerotinia fructicola*.

The dosage-response curve. The germination of fungus spores in the presence of toxic chemicals was shown to be similar to that of toxicity curves in other fields and in general to give a normal distribution when plotted against the logarithm of the dose or concentration.[39] In making these calculations the newly devised "normal equivalent deviations" of Gaddum [5] were at first employed [22] and then succeeded by the later "probits" of Bliss [3] which are fundamentally similar and now more generally used. However, it was further shown [18, 39] that in most cases rapid graphic methods of calculation will suffice and for this purpose the now widely used logarithmic probability paper (as may be seen in Fig. 145) was introduced into the fungicide field. Here spore germination or toxicity curves usually plot as straight lines and comparisons may be readily made, ordinarily at the most precise midpoint of 50 per cent response, that is, the LD50.

Extensive studies have been made on the factors causing variation in

spore germination tests of fungicides.[18, 25, 26, 29] An analysis of 718 individual toxicity or dosage-response curves was undertaken.[18] Six different fungi and 20 different chemicals, both soluble and insoluble, were used. Four different types of slopes were observed on logarithmic probability paper: (*a*) orthodox simple straight lines, (*b*) double slope with left hand "break" in lower values giving a curve concave upwards, (*c*) double slope with right hand "break" in upper values or curve convex upwards, and (*d*) triple slope or sigmoid curve. The first three types are illustrated in

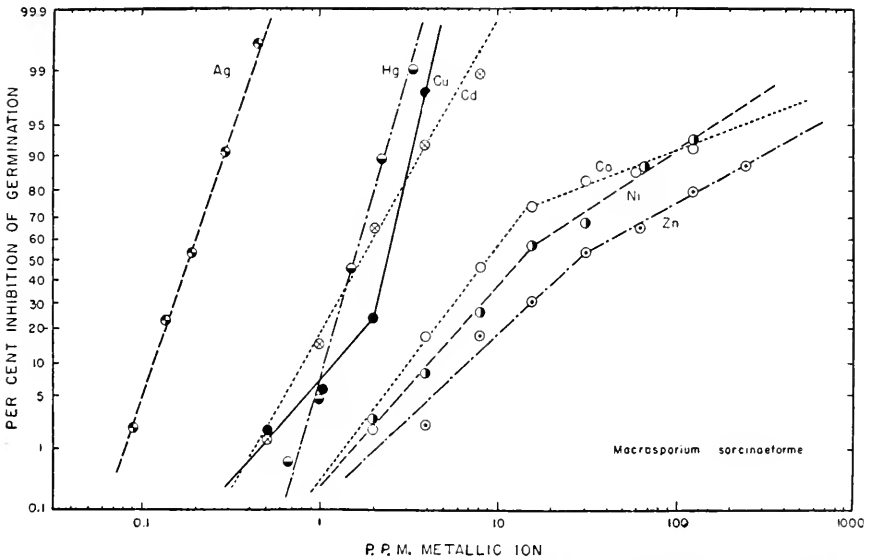

FIGURE 145. Toxicity curves of heavy metals on *Macrosporium sarcinaeforme*. Note that relative toxicity of the metals varies at the different inhibition levels due to differences in slope of the curves. Each point mean of six replicate tests.

Fig. 145. The type and steepness of slope are determined more by the chemical than by the fungus. Differences in the slope of the dosage-response curve for two fungicides will cause changes in their relative effectiveness at different levels of inhibition or control; this is especially noticeable if the curves cross each other. Since fungi vary in their sensitivity from day to day or season to season, not only in the laboratory but also in the field, commonly observed differences in the relative rating of two fungicides at different times can be expected and explained.

Error of replicate tests. It has long been observed that spores germinated at different times, though produced under seemingly standard conditions, vary widely in their response. A portion of this variation was found to be due to the presence of variable amounts of water-soluble nutrient derived from the cultures when procuring the spores.[25] If the spores are obtained by a vacuum technique or are washed and centrifuged, they may be rid of the contaminating stimulant. However, in many cases it will be necessary

to substitute a known quantity of stimulant, such as ultra-filtered orange juice,[25, 36] in order to obtain a consistent and high percentage of germination. Under these conditions fungicide tests replicated on the same day using the same lot of spores in general do not vary more than is to be expected from their internal error, whereas tests replicated at different times with different lots of spores vary considerably more than is to be expected.[18] This is believed to indicate that the replicate test variation in the main is due to the use of different lots of spores, rather than to errors of technique in applying the chemical. These results have led to the conclusion that when comparing fungicides the replicate count or internal error should not be used as the error term but rather the compound × replicate test interaction.

Time and temperature. The effect of time and temperature has been determined for the germination of spores of *Sclerotinia fructicola*, *Alternaria solani*, *Glomerella cingulata*, and *Macrosporium sarcinaeforme* in water and in the presence of various chemicals.[29] No significant difference in precision could be shown between counts made at 6, 12, 24, 48, or 96 hours. A linear relation was found to hold between the reciprocal of elapsed time and germination expressed as probits for the spores of all the fungi when germinating in water at all temperatures from 10° to 35° C (50° to 95° F). The results with *Sclerotinia fructicola* are shown in Fig. 146A. A similar relation held for germination in the presence of a given concentration of chemical, provided that concentration permitted germination, as shown in Fig. 146B. A linear relation was also demonstrated for LD50 values when the logarithm of concentration was plotted against reciprocal of elapsed time. This curve is important in the estimation of the potency of a fungistatic agent, since compounds are rated differently at various times on the same fungus as in Fig. 146C; also, fungi may differ in their relative susceptibility to a single compound, depending on the elapsed time before counts are made, as may be seen in Fig. 146D. No significant difference in LD50 values could be demonstrated at 15°, 21°, or 27° C (59°, 70°, or 81° F) but there was a temperature effect at 10° and 35° C (50° and 95° F), where compounds were not rated in the same order. This temperature effect may in part explain differences in field performance of fungicides in different seasons.

Fungicidal vs. fungistatic. The fungicidal property of a material may be defined in general terms as the ability to kill or inhibit the development of fungus spores or mycelium. This is the sense in which it is commonly used and has been employed previously in this chapter; however, in the restricted sense, "fungicidal" means the property of killing fungi, and "fungistatic" the property of inhibiting. A clarification and comparison were undertaken of the distinction between fungicidal (restricted) and fungistatic.[15] A series of water-soluble chemicals was compared as to their relative action on four different species of fungi. Fungistatic action was determined by the usual slide-germination tests where the fungus remains throughout in con-

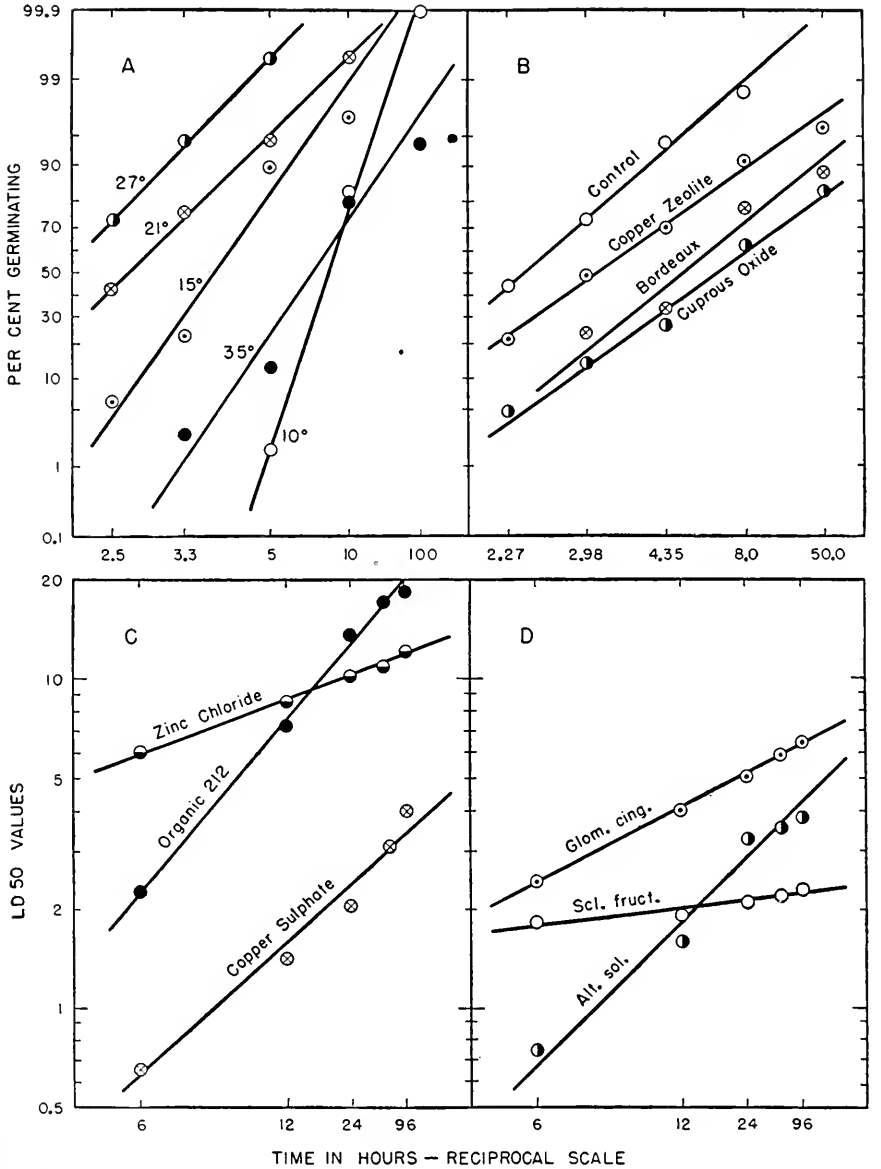

FIGURE 146. Time, temperature, and fungicide dosage curves for spore germination. A, Time-germination curves at different temperatures for *Sclerotinia fructicola* spores in water. B, As A except in presence of fungicides, dosage in micrograms Cu per sq cm, 21° C (70° F). C, Time-LD50 curves for different fungicides against *Alternaria solani* showing difference of slope. D, Time-LD50 curves for Bordeaux mixture on different fungi.

tact with the chemical. For fungicidal tests the fungus spores were allowed to remain in contact with the chemical for different periods of time, after which the chemical was removed by centrifuging and decanting and the spores were washed and allowed to germinate in water. As fungicidal activity cannot exceed fungistatic activity, three kinds of response are possible. Thus certain chemicals such as silver nitrate and copper sulfate show both high fungistatic and fungicidal activity; others exhibit high fungistatic but low fungicidal, such as formaldehyde and phenol; and still others are low in both properties. Hence the correlation between fungicidal and fungistatic properties is high except for the second group. The fungicidal dosage-response curves, like the fungistatic, tend to plot as straight lines on logarithmic probability paper. However, in many cases the fungicidal curves are decidedly flatter.

These fundamental studies on spore germination tests of fungicides, especially the dosage-response curve, have not only encouraged and advanced laboratory testing but they are also stimulating comparable studies under actual field conditions and are resulting in new concepts regarding the comparison of fungicides in the field.[6]

The standard method. The slide-germination method as now developed has been accepted as a standard by the American Phytopathological Society.[1] The method is designed to evaluate the fungistatic properties of protectant fungicides. Spores of certain fungi are placed on glass slides in water in the presence of the chemical to be tested. All conditions are rigidly controlled and, except for the chemical, are favorable for spore germination. The chemical may be applied either by the test-tube dilution technique or by the horizontal sprayer or settling-tower technique.[26] The results are expressed as concentration of chemical necessary to inhibit the germination of 50 per cent of the spores, *i.e.*, the LD50, or as the LD95; the former is more precise.

Settling tower. Prior to the development of the standard method, a special study was made of the method of applying the fungicide, and the settling tower as finally developed constitutes the most precise method of applying insoluble fungicides to glass slides. The tower is illustrated in Fig. 147.

Greenhouse Methods of Evaluating Fungicides

Recently, attention has been devoted to developing greenhouse methods of testing as an intermediate between the laboratory and field tests. This has seemed particularly desirable because of the specificity of the newer fungicides, especially among the organic chemicals.

Tomato foliage diseases. The tomato foliage diseases,[16] early and late blights, caused respectively by *Alternaria solani* and *Phytophthora infestans*, have been found particularly suitable for this purpose, in addition to representing important economic diseases caused by members of the Moniliales and Phycomycetes. The leaf spot (*Septoria lycopersici*) has also been used but has not been found as desirable. The tomato plants are sprayed with

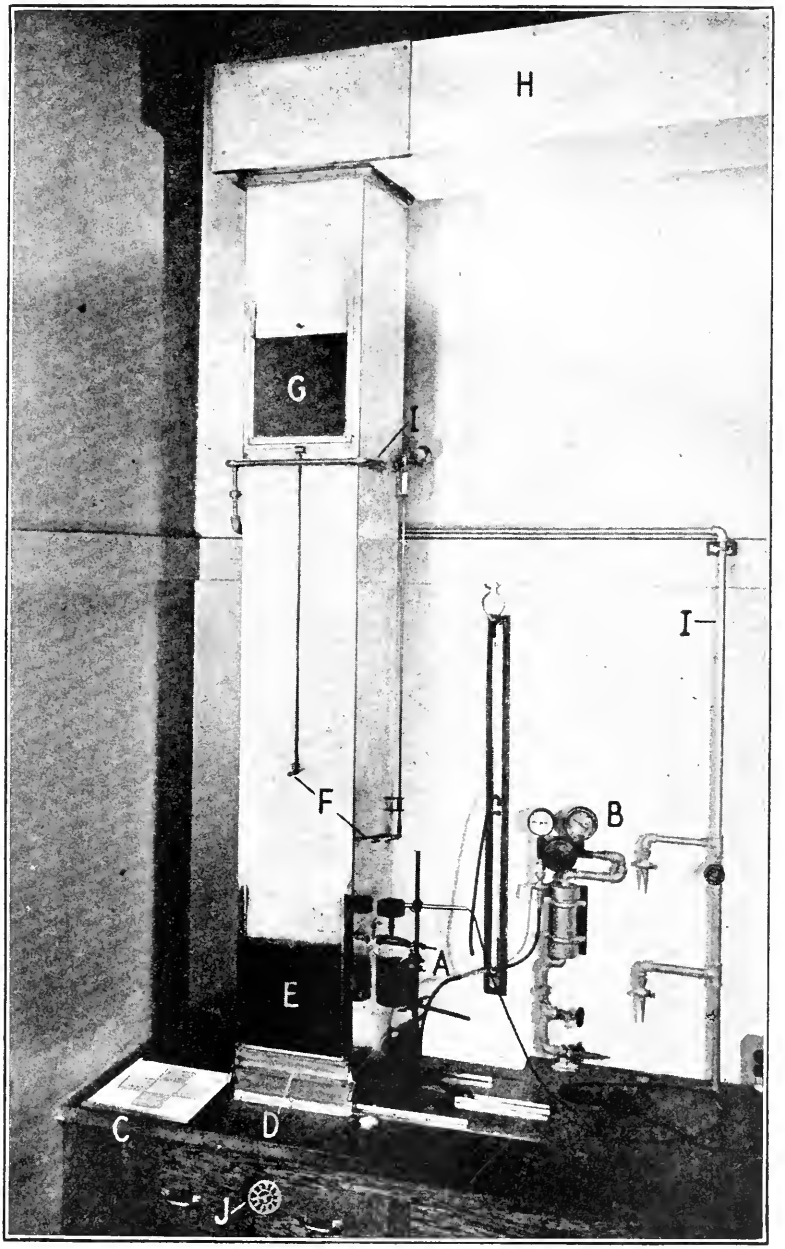

FIGURE 147. Permanently installed stainless steel settling tower for the precision application of fungicides to glass slides. A, Ringstand atomizer apparatus on steel runway; B, reducing valve on air pressure line; C, tray of slides; D, self-closing door through which tray is inserted; E, sliding glass window for draft; F, levers for opening and shutting top of settling chamber; G, upper window for adjustments on top, sprinkler nozzles, etc.; H, laboratory exhaust duct; I, water line to sprinkler nozzles for washing out; J, drain valve.

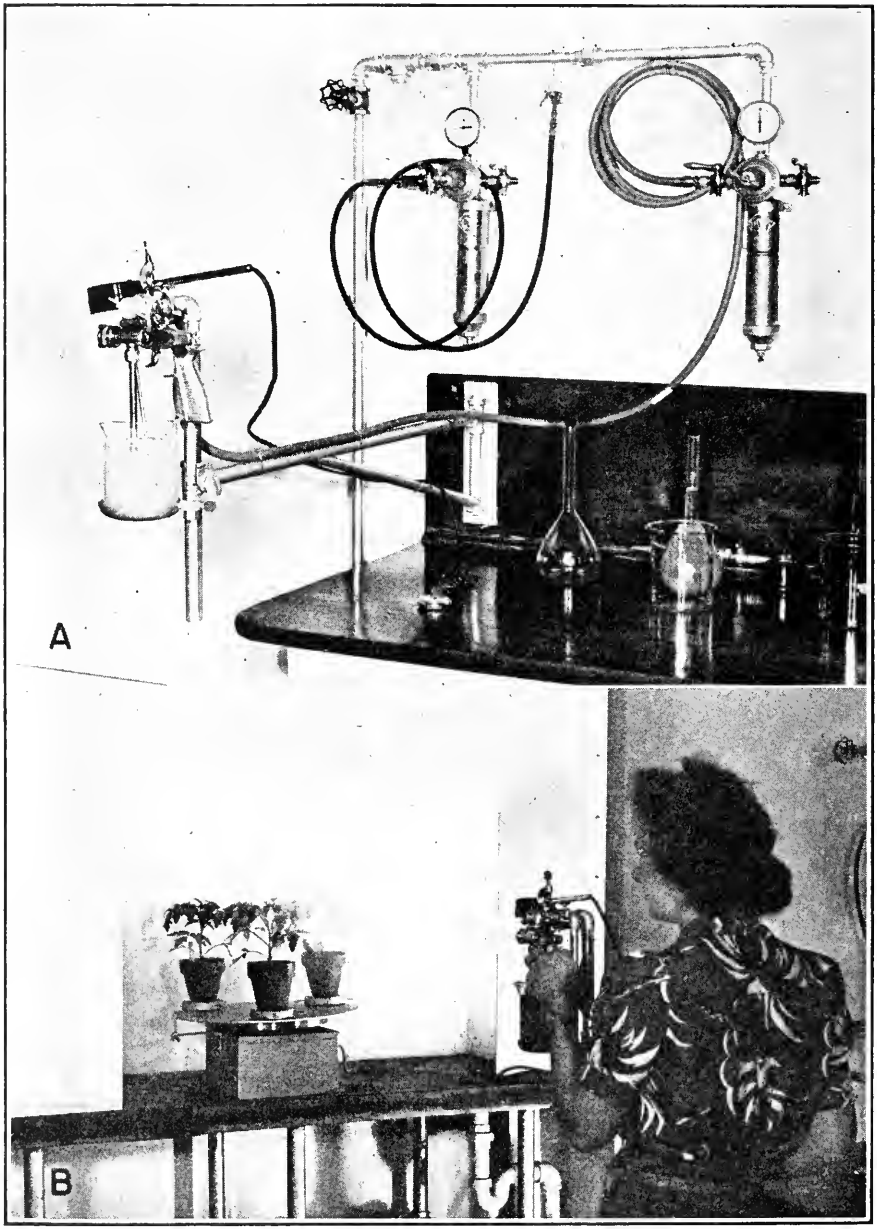

FIGURE 148. A, Spraying systems. *Upper left:* low pressure for inoculation; *upper right:* high pressure for applying fungicide. Note swinging arm mounting spray gun, height adjustment, attached stirrer, and rimmed plate holding beaker containing fungicide. B, Spraying tomato plants on compound turntable in hood, with fungicide at 40 lbs pressure.

the chemical under controlled conditions by means of a paint spray gun, as shown in Fig. 148. After drying they are inoculated with a known concentration of fungus spores produced under standard conditions and placed in high humidity infection chambers at controlled temperatures (see Fig. 149) for 24 hours, then removed to the greenhouse. Necrotic lesions develop in

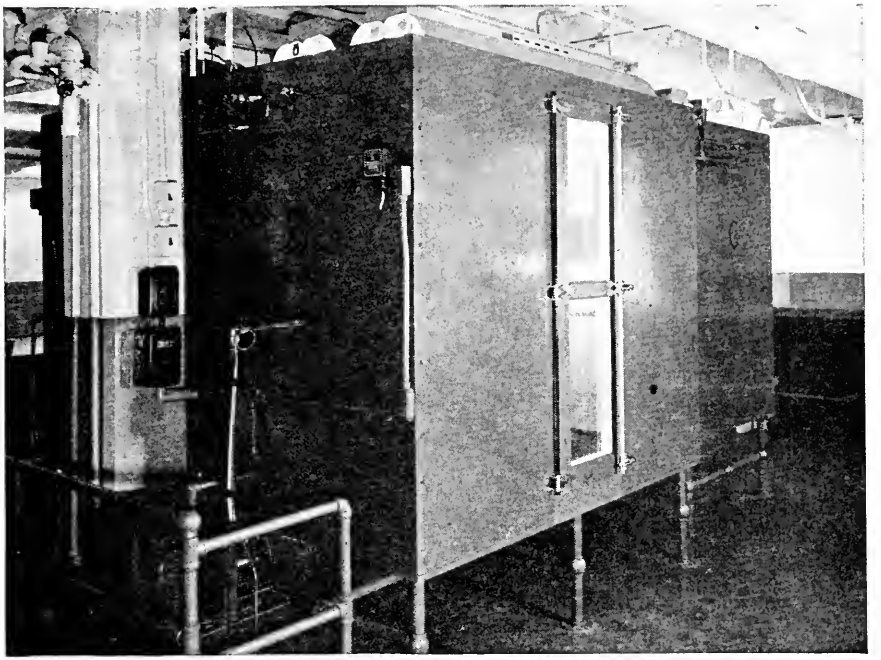

FIGURE 149. Insulated, temperature-controlled humidity infection chambers. Atomizing jet nozzles with water and air pressure line for maintaining high humidity enter at upper sides of each chamber, two may be seen above the chamber letters B and C. Refrigeration machinery for B *at lower left*. The chambers are fitted with a movable shelf in center, plate glass false ceiling below nozzles to prevent direct water spray on plants, and fluorescent lights on top.

several days, are counted, and expressed as per cent of the number of lesions on the control plants. Prior to inoculation, the plants may be subjected to several cycles of growth, high humidity, and laboratory "rain" in order to determine the tenacity of the chemical being tested.[32] The details of this method are well worked out and for a greenhouse procedure are considered precise. Special attention is required in order to ensure an adequate supply of pathogenic *Alternaria solani* spores; this is accomplished by scraping the Petri dish cultures and irradiating for 20 seconds under ultraviolet lamps transmitting to about 250 mμ.[11]

When the number of lesions is expressed as a per cent of the check, the dosage results may be plotted on logarithmic probability paper and, like the spore germination curves, they tend to give straight lines; however, the former are much flatter. Perhaps this may indicate that when fungi-

cides are applied to the foliage of plants they act in a fungicidal rather than a fungistatic manner. For the first time an absolute and direct comparison was made of the action of the same fungicides on the same organism, *Alternaria solani*, at the same spore concentration on glass slides and on the leaves of a growing plant.[16] The results of this interesting comparison are shown in Fig. 150. It will be seen that the LD50 levels are about equal, but as noted above, the greenhouse or leaf curves are much flatter; however, the same fungicide Thiosan (tetramethylthiuram disulfide) was the more toxic in both methods.

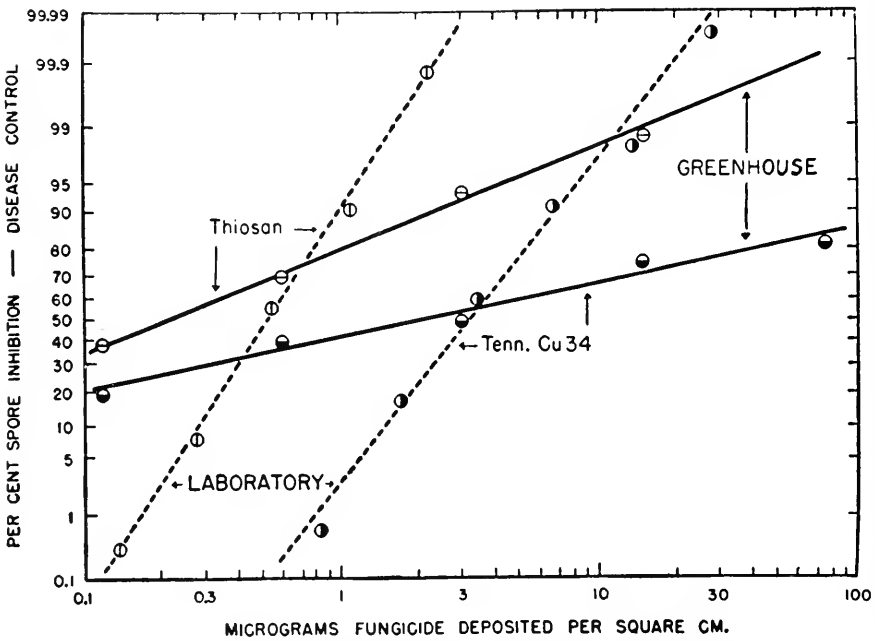

FIGURE 150. Absolute comparison of laboratory and greenhouse dosage-response curves; identical fungus and fungicides. Tennessee Copper "34" and Thiosan against early blight (*Alternaria solani* Maine strain). Spore concentration constant, 70 per sq cm.

It was found with these greenhouse toxicity curves that the most precise point of comparison was not at the LD50. From a study [9] of 431 pairs of replicate tomato plants infected with early or late blight lesions, a linear regression was obtained between the logarithm of the weight of per cent disease in replicate plants and the logarithm of the mean per cent disease. By means of the z^2 transformation, empirical probit weights were obtained. The maximum range of precision for comparison was thus found to be within the range LD80 to 95; accordingly the LD95 or 95 per cent disease control is recommended for comparison of fungicides by this method.

Snapdragon rust. Recently the snapdragon rust caused by *Puccinia antirrhini* has been developed as a method representing the rust fungi.[10] The method is fundamentally similar to that for the tomato diseases, even to the LD95 point for comparison. However, in this case the disease is suitable for evaluating organic and sulfur fungicides but is resistant to copper compounds, which are effective against the tomato diseases.

Bunt of wheat. The standard method of the American Phytopathological Society for evaluating seed treatments to control bunt of wheat [2] has been adopted for preliminary greenhouse tests.[30] This disease is of course a representative of the important seedling infection smut diseases, though somewhat easier to control than others in this group.

Pea seed decay. The somewhat commonly used method of evaluating seed treatments to control damping off fungi by means of pea seeds is erratic and has not been adequately studied. Research is now in progress in an attempt to understand this disease and its responses more fully.

Phytotoxicity. Efficient fungicides must not only control diseases but they must be non-injurious to the plant. It has been found that under greenhouse conditions snap beans, tomato, buckwheat, and tobacco are well suited for such tests of phytotoxicity.[31] Responses cannot be recorded in precise numerical terms but they can be grouped into five or seven classes. Comparisons are thus made of the injury following 1 per cent spray or the per cent of spray to give the threshold of injury.

Cumulative Error Terms

The fungicide × replicate test interaction is suggested as the error term for both laboratory and greenhouse methods of testing fungicides. With well-developed and standardized methods this error term is reasonably constant. It has been shown [17] that one handicap of small tests — that of inadequate knowledge of the standard deviation — may be overcome if it can be demonstrated that error variance is homogeneous with that of the known method. A table has been presented to facilitate this homogeneity test. Thus advantage may be taken of past experience with its cumulated error terms and larger degrees of freedom, so that smaller significant differences are required in a given test or comparison. Cumulative error terms for the methods developed and studied in this laboratory have been worked out.[17]

Correlations Between Laboratory and Greenhouse Methods of Testing Fungicides

Correlations between the slide-germination method, the three tomato diseases, and wheat smut technique have given interesting results.[30] The relative sensitivity of different fungi or diseases depends on the chemical nature of the toxicant. Among tomato foliage diseases a closer correlation exists when only nitrogen, nitrogen plus sulfur on the same carbon atom,

and chlorine compounds are included, than when the correlation includes also chromium, uranium, and copper compounds.

In most cases the correlation between laboratory slide-germination and tomato foliage disease methods was good for compounds of copper, chlorine, nitrogen, and nitrogen plus sulfur or oxygen where tautomerization is impossible. However, some uranium and chromium compounds were more effective in the laboratory than in the greenhouse, perhaps because they are fungistatic rather than fungicidal. Also, some nitrogen plus sulfur or oxygen compounds in which tautomerization could exist were markedly more effective in controlling the tomato foliage diseases than would have been predicted from the laboratory tests. This difference held even when the same fungus was used in both methods.

Wheat smut results were more highly correlated with tomato foliage disease results than with slide-germination results, though the difference was largely attributable to the same groups that gave the poor correlation between slide-germination and tomato foliage disease. Thus, with these exceptions, the three methods are reasonably well correlated.

Despite these correlations, in the search for new fungicides it is advisable to use all the available methods for screening and to select for further testing those compounds which show promise by any one of the methods.

New Fungicides

Until very recently the only agricultural fungicides of economic importance were compounds containing copper, sulfur, mercury, or formaldehyde. These compounds do not by any means fulfill all requirements and there is a pressing need for new and better fungicides.

Periodic table of the elements. In the search for new fungicides a survey was made of the toxicity of representative inorganic compounds relative to the position of the elements in the periodic table.[22] The laboratory slide-germination or fungistatic technique was employed using four different fungi.

In general, toxicity within a group increased with increasing atomic weight. Compounds of the more positive elements showed practically the same toxicity regardless of the particular compound used, but in the case of the more negative elements the toxicity varied greatly with the particular type of compound used. The volatile hydrides, so far as tested, were all highly toxic, but the most highly oxidized forms showed little or no toxicity. There was a considerable tendency for an element which is toxic to one fungus to be toxic to others also, but the correlation is by no means perfect and many exceptions may be observed. The results with one fungus, *Botrytis* sp. (*cinerea* type), are shown in Fig. 151. Compounds of silver, osmium, and mercury were most toxic. In addition to copper, toxic elements which are relatively non-hazardous to humans and which may find more extensive application as fungicides are silver, cerium, cadmium, and chromium.

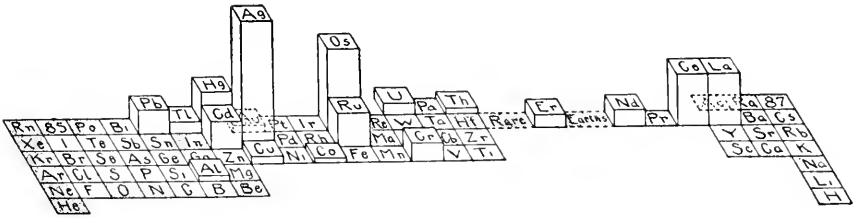

FIGURE 151. Toxicity of compounds of the elements toward conidia of *Botrytis* sp. (*cinerea* type) in relation to the position of the elements in the periodic system. The heights of the columns are the reciprocals of the LD50. Stippled elements were not tested and I, Br, Cl, F, and S can be assigned no definite value since the toxicity varies with the degree of oxidation.

Organic compounds. The organic compounds offer immense possibilities as fungicides. While it would be unquestionably a great discovery to find a "cure all" among the organic compounds, it appears more likely that these compounds will be more specific in their action than the heavy metals.

In preliminary slide-germination tests of miscellaneous organic compounds the thiocyanates and alkyl and acyl resorcinols were found to be highly fungistatic, but formaldehyde in such tests was only mildly active.[37]

In cooperation with Dr. R. H. Wellman,* an extensive investigation is being made on organic compounds as fungicides. To date, some two thousand organic or metallo-organic compounds have been tested by one or more of the above methods. Many interesting results are being obtained. Toxic compounds have been found among all classes studied. However, there is a marked specificity in the action of the various compounds, some being very toxic to certain fungi or diseases while other fungi or diseases show great resistance to the toxic action. Organic compounds have been found, the toxicity of which exceeds that of copper sulfate or Bordeaux mixture — the standard fungicide.

One of the most interesting and promising compounds is glyoxalidine or imidazoline, $HN{-}CH{=}N{-}CH_2{-}CH_2$. A large number of the glyoxalidine derivatives have been examined by one or another of the methods mentioned above. In slide-germination tests maximum fungistatic action was achieved with glyoxalidine having a straight-chain substituent containing 13 to 17 carbon atoms in the 2-position, as illustrated in Fig. 152. Substituents in the 1-position, such as hydroxyethyl, aminoethyl or butyl, do not markedly affect fungistatic action. The action of the glyoxalidine derivatives is fungistatic and not fungicidal; they are removed from solution by spores in amounts proportional to their fungistatic action. In greenhouse experiments maximum phytotoxicity is reached with the 11 to 13 carbon atom side-chains in the 2-position. Thus the greatest spread between fungistatic action and phytotoxicity, for the hydroxyethyl deriva-

* Carbide and Carbon Chemicals Corporation and Crop Protection Institute.

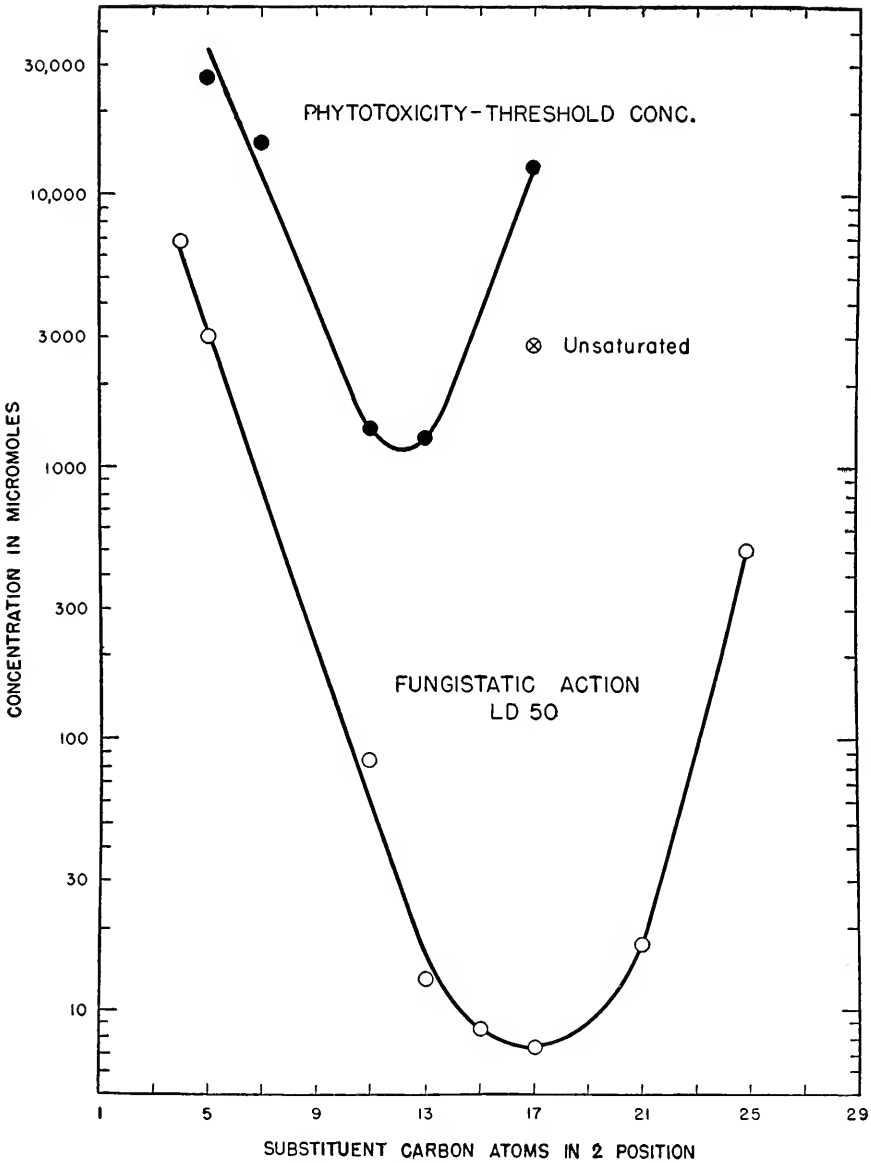

FIGURE 152. Comparison of the effect of number of carbon atoms in the substituent in the 2-position on fungistatic action and phytotoxicity for the derivatives of hydroxyethylglyoxalidine. Note that greatest spread between fungistatic action and phytotoxicity is with 17 carbon atoms. Concentration is expressed as micromols per liter. Phytotoxic values average for tobacco, buckwheat, and bush bean and fungistatic values average for *S. fructicola, A. solani, G. cingulata,* and *M. sarcinaeforme.*

tives as shown in Fig. 152, is when the substituent in the 2-position is heptadecyl. Side-chain unsaturation increases phytotoxicity, as does increasing length of chain in the 1-position. The glyoxalidine derivatives in greenhouse tests were phytotoxic to tomatoes at concentrations which would not control late blight.

Three of the substituted glyoxalidines have been tested under varied field conditions in several states for several years. Against late blight of potato, they were not effective, thus confirming the greenhouse results. Fair control of black spot of roses was obtained. However, against apple scab they gave control equal to the standard sprays, but with less injury and better color and finish to the fruit; for the control of cherry leaf spot defoliation, 2-heptadecylglyoxalidine has been found superior to all other fungicides tested.

Past and Future

When these fungicide studies were begun, it was believed that the cause of better and more efficient fungicides could be best advanced if the nature of the mechanism of fungicidal action were better understood for our important fungicides. Therefore, attempts were made along this line with the classical standards, sulfur and copper. This work has been substantiated, at least in part, and others have pursued it further.[4, 6, 28] It is believed that this is still a good policy. With new and improved techniques and fresh concepts resulting from an intensive study of the "simple" slide-germination method, this basic work could be carried much further. Especially inviting now from this angle are the new organic groups, such as the dithiocarbamates and glyoxalidine derivatives.

In the realm of new techniques of evaluation, it is probable that the outstanding disease, apple scab, should receive first consideration, so that a relatively simple and inexpensive greenhouse method would be available for handling many hundreds of new chemicals. Further studies on the improvement and standardization of techniques for the seed treatments of the staple crops of corn and cotton also seem indicated. It should not be forgotten that not all uses of fungicides are agricultural. Industrial applications, such as wood preservation and the textile and plastic fields, offer many new and interesting problems, as has been brought to our attention in the latter case by pressing war questions.

There is still room for improvement in the heavy-metal fungicides, especially by the use of metals other than copper and of molecular combinations of different metals. The organic compounds have opened up an immense field of study for fungicides with countless attractive possibilities. As yet only a beginning has been made.

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CHAPTER 12

Miscellaneous

Many shorter projects have been carried on at the Institute. Fourteen of these are described in this chapter, and several others have been cited and briefly described in connection with bigger projects. A few of the minor projects are not discussed in either.

Factors for Color in the Production of Potato Chips

A research department of a food distributing company inquired of the Institute how to store potato tubers so as to avoid sprouting and get chips of the desirable color even after many months of storage. Denny and Thornton [22] undertook to answer this question by a study of the effects of temperature and other factors in storage upon the metabolism and sprouting of potato tubers. Potato chip production in the United States is a sizable industry; it consumes fully one-tenth of the total potato production of the country — about 40,000,000 bushels a year. Because of the size of the industry, tubers for chips must be drawn from the main season crop, which demands considerable periods of storage. The researches extended over three years, or seasons, and involved the study of 25 different varieties of potatoes stored under a great range of conditions. In this necessarily brief consideration of these researches only the points of more general interest to the chip industry and facts showing the significance of storage conditions upon tuber metabolism can be presented. This means that the tables must be greatly abbreviated and many pertinent facts omitted. Those especially interested in the subject will want to read the seven original articles here cited.

Reducing Sugars Cause Browning of Potato Chips

When potatoes are held in commercial storage for a long time it is customary to hold them at a low temperature to prevent sprouting. This temperature may range anywhere from 1° to 6° C (approximately 34° to 43° F). It is a well-established principle of plant physiology that living plants at low temperatures gradually transform storage fats and starch to soluble sugars. In potatoes it is mainly starch that is transformed to sugars. It was also known that chips that are fried from tubers long in cold storage give dark brown and consequently undesirable chips because of the soluble sugars present in the tubers. Thornton [89] was the first to show

that the browning is due to reducing sugars and not to cane (sucrose) or the total sugars.

Fig. 153 shows the evidence for this early important conclusion in the solution of the potato chip problem. All the chips, including the impregnated filter paper discs, were fried under standard conditions. The temperature at the beginning was 195° C (383° F) and fell during frying to

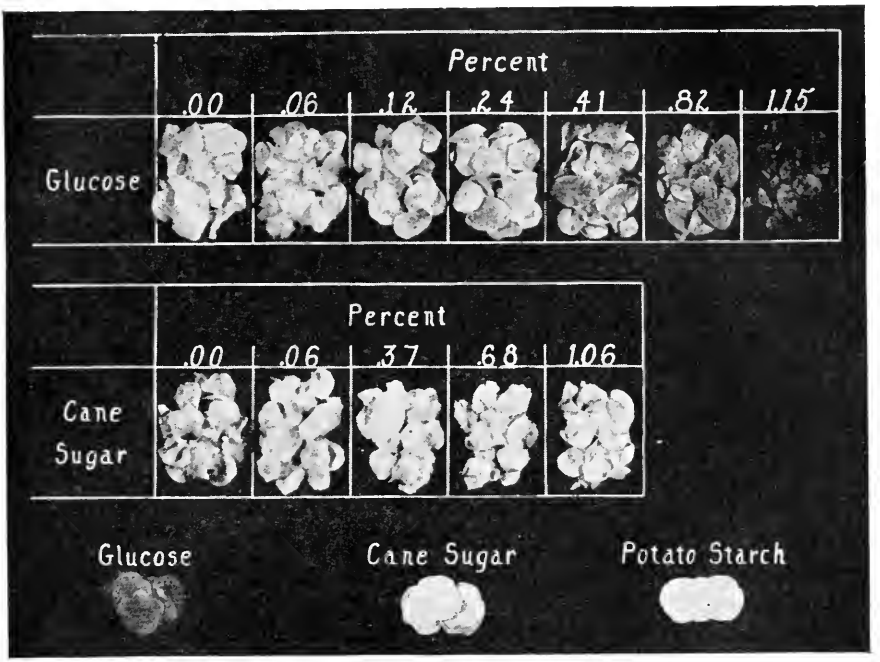

FIGURE 153. Chips fried under standard conditions. *Top row:* potato slices with increasing content of reducing sugar from left to right. *Middle row:* same for cane sugar but low in reducing sugar. *Bottom row:* filter paper discs impregnated with glucose, cane sugar, and starch. Percentages of sugars are on the fresh weight basis.

158° to 166° C (316° to 331° F). Frying was continued until all water was driven from the chips, as indicated by cessation of bubbling. The impregnated filter-paper discs contained less water and the water was more readily driven off, so they were fried for the same length of time as the chips themselves. In the upper row it will be seen that as the chips contain more glucose they become darker on frying. Those containing 1.15 per cent glucose become very dark. The investigators conclude that tubers that contain much more than 3 mg of reducing sugar to 1 cc of juice [26] are undesirable for chips. This equals approximately 0.24 per cent of moist weight of tuber. It will be noted that as the cane sugar content of the tubers rises there is no darkening of the chips, provided the reducing sugar content is low. In the bottom row are filter paper discs impregnated with glucose, cane sugar, and starch. Only the glucose-impregnated filter paper turns brown on frying.

If filter paper discs impregnated with cane sugar or chips high in cane sugar and low in glucose are fried at considerably higher temperatures, browning occurs; an initial frying temperature of 220° C (428° F) gave some browning of such chips or discs and 240° C (464° F) starting temperature gave dark brown chips. If potatoes are available with high cane sugar and low glucose, chips of any desired degree of brown can be produced by increasing the frying temperature. In impregnated filter-paper discs the monosaccharides arabinose and levulose and the disaccharide maltose acted like glucose. The disaccharide lactose (milk sugar) was more resistant to browning than cane sugar. In practice the fastest way to find out the adaptation of any batch of potatoes for chip making is not to determine the content of various sugars but actual frying of samples at standard temperatures.

Sugaring of Tubers in Low-temperature Storage

Accumulation of reducing sugar. Table 39 [89] shows the effect of three different temperatures (5°, 7°, and 8.2° C [41°, 45°, and 47° F]) of storage upon the accumulation of reducing sugars in 25 different varieties of pota-

Table 39. Reducing Sugar Values Showing Responses of Tuber of Potato Varieties to Conditions of Storage. Storage Started Oct. 25, 1940

Variety	Milligrams of reducing sugar per cc of juice				
	At harvest	At start	After 59 days at 5° C (41° F)	After 68 days at 7° C (45° F)	After 78 days at 8.2° C (47° F)
Neverblight	0.0	0.0	6.7	—	0.0
Chippewa	.0	.0	5.7	3.1	1.4
Russet Rural	.0	.0	7.4	2.3	0.0
Heavyweight	.0	.0	7.8	4.1	.0
Carman No. 3	.0	.0	7.9	2.3	.0
Blue Victor	.0	.0	9.1	1.6	—
White Rural	.0	.0	7.7	5.0	—
Rural New Yorker	.0	.0	9.2	4.5	.3
Number Nine	.0	.0	8.4	3.2	.0
Sir Walter Raleigh	.0	.0	15.4	3.5	—
Irish Cobbler	.0	.0	11.4	4.6	1.6
Early Rose	.0	—	12.9	3.5	0.7
Early Six Weeks	.0	.0	10.4	—	.0
Katahdin	.0	.0	12.9	6.7	1.9
Early Ohio	.0	.0	15.2	7.5	—
Eureka	.0	.0	16.1	5.7	2.7
Russet Burbank	.0	.0	14.3	3.7	—
Green Mountain	.0	.0	17.9	4.5	3.8
Delaware	.0	.0	14.2	6.0	3.5
Bliss Triumph	.7	.0	15.9	—	5.1
Pride of Multnomah	.0	.0	20.4	—	2.9
Spaulding Rose	.0	.0	17.3	8.9	4.9
Blue Mercer	.0	.0	16.0	—	2.3
Warba	.0	.0	9.4	10.0	—
Axtell's Bugless	.0	.0	17.6	—	5.2

toes. At harvest time none of the varieties bore a determinable amount of reducing sugar except Bliss Triumph, and it bore only 0.7 mg per gram of juice. When put into storage none of the varieties contained any reducing sugar. After 59 days of storage at 5° C (41° F), all varieties had accumulated more than 3.0 mg per gram of juice of reducing sugar. Even Chippewa with 5.7 mg per gram contained almost twice this amount, and many of them bore several times this amount. Pride of Multnomah, the highest, had nearly seven times the maximum allowable for good chips. After 68 days' storage at 7° C (45° F) many of the varieties bore less or little more than this maximum; and at 8.2° C (47° F) for 78 days' storage, few bore this maximum of reducing sugar. Bliss Triumph was the worst offender with nearly twice the maximum. It is evident that varieties vary greatly in the rate at which they accumulate reducing sugars at 5° C (41° F) and even at the other two temperatures used and that those near the top of the table belonging to the Rural group are least inclined to accumulate reducing sugars. It is also evident that the accumulation of reducing sugars falls rapidly as the storage temperature rises from 5° to 8.2° C (41° to 47° F), several varieties giving rather slow sugaring even at 7° C (45° F), and that only a few varieties accumulate considerable reducing sugar at 8.2° C (47° F). Storage temperature of 1° C (34° F) in contrast to 5° C (41° F) led to still greater accumulation of reducing sugar.[26]

Cane sugar. While the cane sugar content of potato tubers has no part in giving brown chips, it is of interest to note the cane sugar content of tubers at harvest time and the effect of storage temperatures upon its accumulation, for it is an important nutrient for the growing plant and it modifies the flavor of cooked potatoes. Table 40 shows these points. At harvest time all 25 varieties contain measurable amounts of cane sugar, unlike the situation with reducing sugar; also at the time of putting into cold storage after a period of air-temperature storage, all varieties contain measurable amounts of cane sugar. During air-temperature storage most varieties fall in cane sugar content, but some rise. Storage at 5° C (41° F) for 59 days causes a rise in cane sugar content of all varieties — a very marked rise in some. Even storage at 7° C (45° F) for 68 days causes a rise in cane sugar in all varieties, an even higher rise in some than was caused by 5° C (41° F) for a shorter period. Storage at 8.2° C (47° F) for 78 days causes relatively little change in cane sugar content, a slight fall in some, and a slight rise in others. As is the case with reducing sugars, varieties differ greatly in their power to form cane sugar at low temperature, and this power falls greatly from 5° to 8.2° C (41° to 47° F); but for some varieties there is a rise instead of a fall from 5° to 7° C (41° to 45° F).

De-sugaring at High Storage Temperatures

Reducing sugars. When potatoes have accumulated much reducing sugar due to storage at low temperatures, they de-sugar more or less readily according to variety when transferred to high-temperature storage, although

Table 40. Cane Sugar Values Showing Response of Tubers of Potato Varieties to Conditions of Storage. Storage Started Oct. 25, 1940

Variety	Milligrams of cane sugar per cc of juice				
	At harvest	At start	After 59 days at 5° C (41° F)	After 68 days at 7° C (45° F)	After 78 days at 8.2° C (47° F)
Neverblight	3.9	2.4	6.6	—	2.9
Chippewa	2.4	1.7	8.7	4.6	2.5
Russet Rural	0.9	2.1	5.5	2.3	2.4
Heavyweight	1.5	1.8	4.9	3.5	1.7
Carman No. 3	2.1	1.4	5.4	3.7	2.5
Blue Victor	1.7	0.8	3.7	6.5	—
White Rural	1.2	0.8	3.1	3.2	—
Rural New Yorker	2.5	1.0	4.4	3.3	2.3
Number Nine	4.4	1.7	5.1	6.6	2.2
Sir Walter Raleigh	2.2	1.5	4.6	2.9	—
Irish Cobbler	2.5	0.7	10.7	10.1	3.8
Early Rose	3.7	—	11.7	5.1	3.7
Early Six Weeks	2.4	2.5	7.7	—	4.2
Katahdin	2.1	1.1	5.0	4.8	3.0
Early Ohio	3.0	2.5	8.4	6.8	—
Eureka	2.6	0.2	6.8	16.9	4.2
Russet Burbank	1.5	1.2	6.5	23.4	—
Green Mountain	3.7	3.8	14.3	18.9	5.1
Delaware	3.8	4.5	15.7	12.4	4.9
Bliss Triumph	6.1	4.0	9.9	—	3.1
Pride of Multnomah	3.1	1.8	8.6	—	2.5
Spaulding Rose	2.5	2.6	5.5	5.4	4.3
Blue Mercer	1.9	1.7	6.2	—	1.6
Warba	3.2	3.0	3.9	8.6	—
Axtell's Bugless	3.9	2.0	11.6	—	4.0

it is reported by commercial producers that occasional lots fail to de-sugar properly. A favorable temperature for de-sugaring is 27° C (81° F). Table 41 shows the de-sugaring of 13 varieties after storage at 5° C (41° F) for 63, 126, and 186 days, followed by storage at 27° C (81° F) for 10, 20, and 40 days.

Let us examine the first five varieties given in this table, both as to the rate at which they accumulate reducing sugar at the low temperature and at which they de-sugar at the high temperature. Irish Cobbler and Green Mountain accumulate reducing sugar rapidly and in large amounts, the former bearing more after 126 days at 5° C (41° F) than after 186 days, and the latter more after 63 days than after either 126 or 186 days. Russet Rural accumulates only a moderate amount of reducing sugar and reaches its maximum after 63 days, showing no further rise after 126 or 186 days. Chippewa forms reducing sugar slowly in cold storage, and continues to increase up to 186 days, when the amount becomes high. All these varieties, except Chippewa, de-sugar rapidly when placed at 27° C (81° F), reaching a content low enough for chip making after 20 days for either 63- or 126-day low-temperature storage. Three of the five varieties show the same after 186

Table 41. De-sugaring: Effect upon Reducing Sugar by Transference of Tubers from Storage at 5° C (41° F.) to Storage at 27° C (81° F). Storage Started Oct. 25, 1940

Variety	Milligrams of sugar per cc of juice											
	After 63 days at 5°, put at 27° C for				After 126 days at 5°, put at 27° C for				After 186 days at 5°, put at 27° C for			
	Days				Days				Days			
	0	10	20	40	0	10	20	40	0	10	20	40
Russet Rural *	7.4	2.4	0.1	0.0	5.8	3.5	1.7	0.0	6.1	3.2	3.0	1.6
Irish Cobbler *	11.6	4.0	1.7	.0	15.2	3.5	2.1	.0	12.3	2.8	0.0	0.4
Chippewa	5.8	3.9	3.3	.0	11.9	6.8	4.3	2.4	15.6	10.1	7.0	5.0
Green Mountain	17.4	5.7	2.7	.0	15.6	5.9	2.9	2.1	13.1	7.2	5.0	2.9
Carman No. 3 *	7.8	3.0	0.0	.0	7.7	3.2	1.5	0.0	7.0	4.1	2.1	0.0
Katahdin *									15.1	7.0	2.8	2.9
Sir WalterRaleigh *									7.6	3.0	0.7	0.0
Spaulding Rose									17.0	7.1	5.7	4.6
Delaware									15.1	6.7	6.6	5.2
Early Ohio *									12.2	2.0	0.0	0.0
Rural New Yorker *									5.2	3.5	.0	.0
Heavyweight *									4.8	4.2	2.0	.0
Eureka									17.0	5.7	3.4	1.8

* All these de-sugar sufficiently in 20 days at 27° C (81° F) and are therefore well adapted to the de-sugaring treatment.

days of cold storage. After 186 days' cold storage Chippewa does not de-sugar sufficiently even after 40 days at 27° C (81° F), but Green Mountain does. All the other varieties shown in the table were transferred to 27° C (81° F) only after 186 days at 5° C (41° F). Sir Walter Raleigh, Rural New Yorker, and Heavyweight accumulate only moderate amounts of reducing sugars at 5° C (41° F) and de-sugar readily at 27° C (81° F); Katahdin, Early Ohio, and Eureka accumulate more reducing sugar at 5° C (41° F) but de-sugar readily at 27° C (81° F) with the exception of Eureka, which shows somewhat slower de-sugaring; Spaulding Rose and Delaware accumulate considerable sugar at 5° C (41° F) and de-sugar slowly at 27° C (81° F).

Chip makers often shift potatoes from low-temperature storage, which prevents sprouting, to high temperatures for de-sugaring a few days previous to chipping them. The varieties starred are rapidly de-sugared and consequently are adapted for this treatment. They need not be held at the high temperature long enough to induce undue sprouting. When stored even for 60 days at 1° C (34° F), Irish Cobbler and Green Mountain form so much reducing sugar (27 and 38 mg per cc of juice, respectively), that they do not de-sugar sufficiently for chip making after 20 days' storage at 27° C (81° F). White Rural accumulated only 11.9 mg per cc of juice after 120 days at 1° C (34° F) and de-sugared almost completely after 20 days at 27° C (81° F).

Table 42. De-sugaring: Effect upon Cane Sugar by Transference of Tubers from Storage at 5° C (41° F) to Storage at 27° C (81° F). Storage Started Oct. 25, 1940

Variety	Milligrams of sugar per cc of juice											
	After 63 days at 5°, put at 27° C for				After 126 days at 5°, put at 27° C for				After 186 days at 5°, put at 27° C for			
	Days				Days				Days			
	0	10	20	40	0	10	20	40	0	10	20	40
Russet Rural	5.5	4.6	4.3	2.2	4.7	6.4	4.9	5.8	6.5	7.9	6.4	8.2
Irish Cobbler	10.7	6.1	3.3	3.0	5.8	4.4	4.1	5.5	11.9	5.5	5.0	8.3
Chippewa	8.8	6.6	7.0	6.0	4.5	9.0	10.0	11.1	11.6	14.4	14.2	16.2
Green Mountain	14.4	7.0	6.4	5.7	8.5	6.8	7.0	8.6	9.4	7.5	7.0	11.5
Carman No. 3	5.4	4.4	3.7	3.0	5.3	4.4	6.2	5.3	7.5	6.7	6.6	6.3
Katahdin									7.8	6.6	3.4	4.6
Sir Walter Raleigh									3.2	5.5	4.9	7.6
Spaulding Rose									6.7	4.5	5.5	8.7
Delaware									11.3	8.6	8.1	13.1
Early Ohio									7.5	4.2	4.7	5.2
Rural New Yorker									3.6	4.1	3.9	6.3
Heavyweight									5.6	4.3	6.3	7.2
Eureka									9.4	5.3	5.4	7.9

Cane sugar. Table 42 shows the effect of high-temperature storage following low temperature on the cane sugar content of potato tubers. As is the case with reducing sugars, varieties differ considerably in the amount of cane sugar they accumulate during cold storage; varieties Irish Cobbler, Green Mountain, and Delaware accumulate considerable and Russet Rural, Carman No. 3, Sir Walter Raleigh, Early Ohio, and Heavyweight much less. In all varieties high-temperature following low-temperature storage does not cause marked de-sugaring so far as cane sugar is concerned.

Effect of the Period of Storage at Room Temperature on Later Sugaring at Low Temperature

Holding tubers at room temperatures for considerable periods after harvest and before storage at low temperatures decreases the rate of sugaring at the low temperature. Fig. 154 shows the relation for reducing sugar accumulation for the 1941 crop. The curves are composite curves for five different varieties (Irish Cobbler, Carman No. 3, Green Mountain, Bliss Triumph, and Katahdin) put into cold storage after four different periods of room temperature storage. The most rapid accumulation of reducing sugar occurred when the tubers were held at room temperature only 10 to 12 days before they were put at 5° C (41° F), and slowest accumulation occurred in those held at room temperature 71 to 74 days. Those held at room temperature for 108 to 111 days were second from lowest, and those held at this temperature for 131 to 134 days were next to

the highest. The results on the 1940 [24] crop showed that the longer periods of room-temperature storage previous to low-temperature storage reduced the rate of reducing sugar accumulation at 5° C (41° F) to the greatest degree. Although the two years' work differs in this respect there is no doubt that two or three months of room temperature storage just after

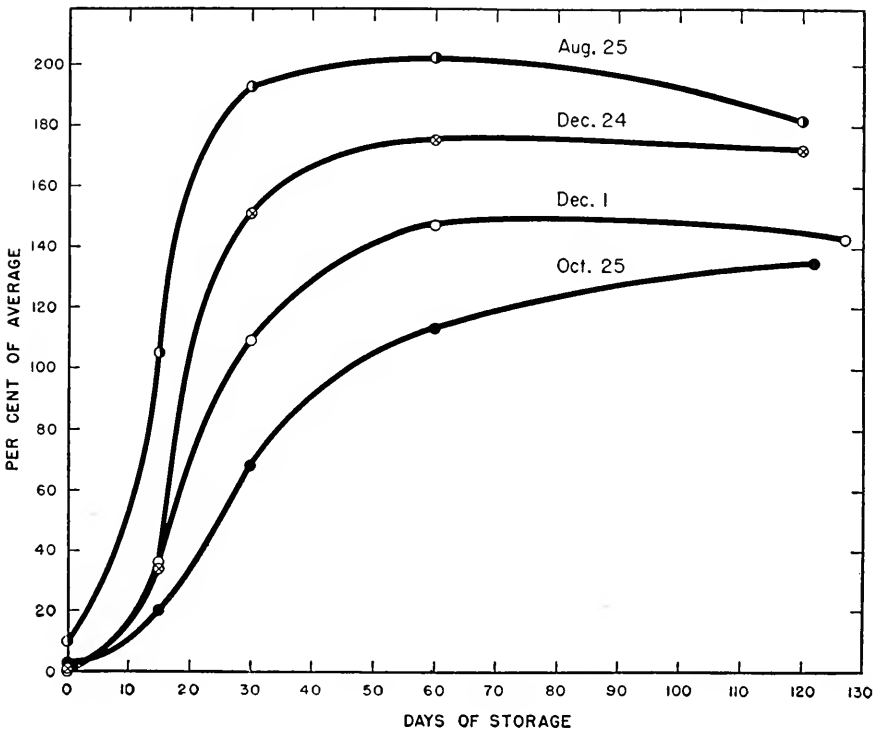

FIGURE 154. Changes in reducing sugar content of potato tubers during storage at 5° C (41° F) when the storage was started at different intervals after harvest (which was Aug. 12–15). Storage was at room temperature from harvest until dates shown.

harvest and previous to cold storage cuts down the rate of reducing sugar formation at the low temperature. Since the buds are dormant for about three months after harvest, one can gain three months with no danger of sprouting or sugar accumulation by storing the tubers at room temperature for this period immediately after harvest. To these two advantages is added that of lower sugaring power after the tubers are put at the low temperature.

The accumulation of cane sugar in cold storage was affected quite differently by various periods of room-temperature storage previous to the cold storage. The longer the tubers were held at room temperature before storage at 5° C the more rapid and more extensive was the accumulation of cane sugar in cold storage.

Critical storage temperatures for sugaring and sprouting. We have already seen (Table 39) that the formation of reducing sugar falls greatly as the storage temperature rises from 5° to 7° C (41° to 45° F), and still further as the temperature rises to 8.2° C (47° F). All but a few varieties can be stored at 8.2° C (47° F) for 78 days without accumulating enough reducing sugar to interfere with chip making. A number of varieties can be stored at 7° C (45° F) for 68 days without an interfering amount of reducing sugar accumulating. It is probable that at 9° C (48° F) few varieties will form reducing sugars in amounts sufficient to interfere with chip making, even after months of storage. Even at 7° C (45° F) so little reducing sugar accumulates that in any good de-sugaring variety a few days at 27° C (81° F) will de-sugar them to a sufficient degree.

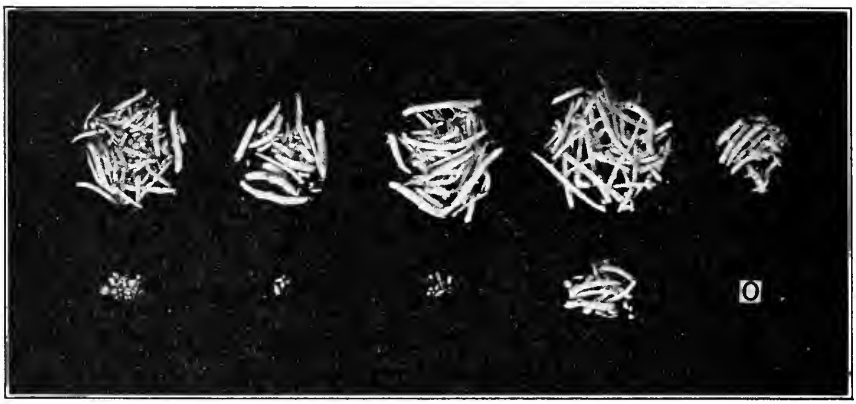

FIGURE 155. Sprouts removed from 16 tubers of each of five varieties after storage from Oct. 25, 1941, to Mar. 3, 1942, at 7° and 8° C (45° and 46° F); *top row*, 8°, *bottom row*, 7°; varieties, *left to right* are: Irish Cobbler, Carman No. 3, Green Mountain, Houma, Katahdin; the zero label (*lower right*) indicates no detachable sprouts were present.

Sprouting is long delayed and the rate of growth of the sprouts is very slow when tubers are stored at 7° C (45° F). The sprout growth is considerably faster at 8° C (46° F). Fig. 155 shows the difference in the degree of sprout growth of five different varieties stored at these two temperatures for about four and one-third months or from October 25 to March 3. These tubers were harvested about the middle of August and were held at room temperature until October 25; consequently the period from harvest until the amount of sprout growth shown had occurred was nearly seven months. Even on June 3, nearly ten months from harvest, the sprout growth for Irish Cobbler and Katahdin at 7° C (45° F) was very slight, although it was considerable for the other three varieties. On June 3 the sprout weight of the five varieties stored at 8° C (46° F) was two and one-half times that of those stored at 7° C (45° F). While it is evident that 7° C (45° F) is effective in delaying sprouting, it does lead to the accumulation of a slightly excessive amount of reducing sugar. This amount, however, is eliminated by a few days' storage at 27° C (81° F).

Effect of Carbon Dioxide on the Sugar Metabolism of Potatoes at Low Temperatures

Five per cent CO_2 with normal percentage of oxygen in the surrounding air retards the formation of reducing sugar [23, 25, 27] in tubers stored at 2°, 5°, or 7° C (36°, 41°, or 45° F). In some cases less than one-fifth as much reducing sugar forms with 5 per cent CO_2 present as with none present. Even 1.1 per cent CO_2 retards the formation of reducing sugar by 20 per

Table 43. Effect of CO_2 upon the Reducing Sugar Content of Potato Tubers Stored for Different Lengths of Time at Different Temperatures

Variety	Duration, days	Reducing sugar, mg per cc of juice								
		2° C (36° F)			5° C (41° F)			7° C (45° F)		
		% CO_2			% CO_2			% CO_2		
		0	5	20	0	5	20	0	5	20
Irish Cobbler	30	6.3	0.0	0.0	5.0	0.0	0.0	3.3	0.0	0.0
	60	20.3	4.6	0.0	9.6	0.0	4.9	2.8	0.0	6.0
	90	23.9	8.1	3.5	9.4	0.0	4.1	2.6	0.0	12.2
Green Mountain	30	6.6	1.1	1.8	7.3	2.5	1.5	4.5	1.5	1.5
	60	30.9	5.8	2.7	12.2	4.1	5.2	5.8	2.7	8.2
	90	24.3	8.5	4.2	10.4	4.4	10.8	6.1	3.6	17.4
Katahdin	30	2.3	0.0	0.0	2.7	0.0	0.0	1.1	0.0	0.0
	60	15.2	3.9	1.0	5.6	2.6	2.2	2.1	0.8	4.7
	90	17.7	9.7	2.7	5.2	6.5	4.7	2.4	2.6	9.1

Note: (*a*) The reducing sugar values at the start were: 0.0, 0.1, 0.1 for Irish Cobbler, Green Mountain, and Katahdin, respectively; (*b*) a zero value means that the sugar value was less than 0.1 mg per cc of juice by the method of sugar analysis used.

cent at 5° C (41° F). Twenty per cent CO_2 at 2° C (36° F) is even more effective than 5 per cent in retarding reducing sugar formation. At 5° C (41° F) it is much less effective than 5 per cent, and at 7° C (45° F) it increases considerably the formation of reducing sugar. Table 43 and Fig. 156 show the effect at 5 per cent and 20 per cent CO_2 on the accumulation of reducing sugar in tubers stored at 2°, 5°, and 7° C (36°, 41°, and 45° F). When the period of storage exceeds 60 days, both 5 and 20 per cent CO_2 increase greatly the formation of cane sugar in stored tubers at all three temperatures (2°, 5°, and 7° C [36°, 41°, and 45° F]). Table 44 shows the effect of 5 and 20 per cent CO_2 on cane sugar concentration in tubers stored at 2°, 5°, and 7° C (36°, 41°, and 45° F) for 30, 60, and 90 days.

On the whole, the effect of variety, temperature, and CO_2 concentration on the accumulation of reducing and cane sugars in potato tubers is very complex. Yet with the proper selection of varieties — and there are a number of good ones — three months of storage at room temperature followed by storage at 7° C (45° F) and finally followed by a few days at

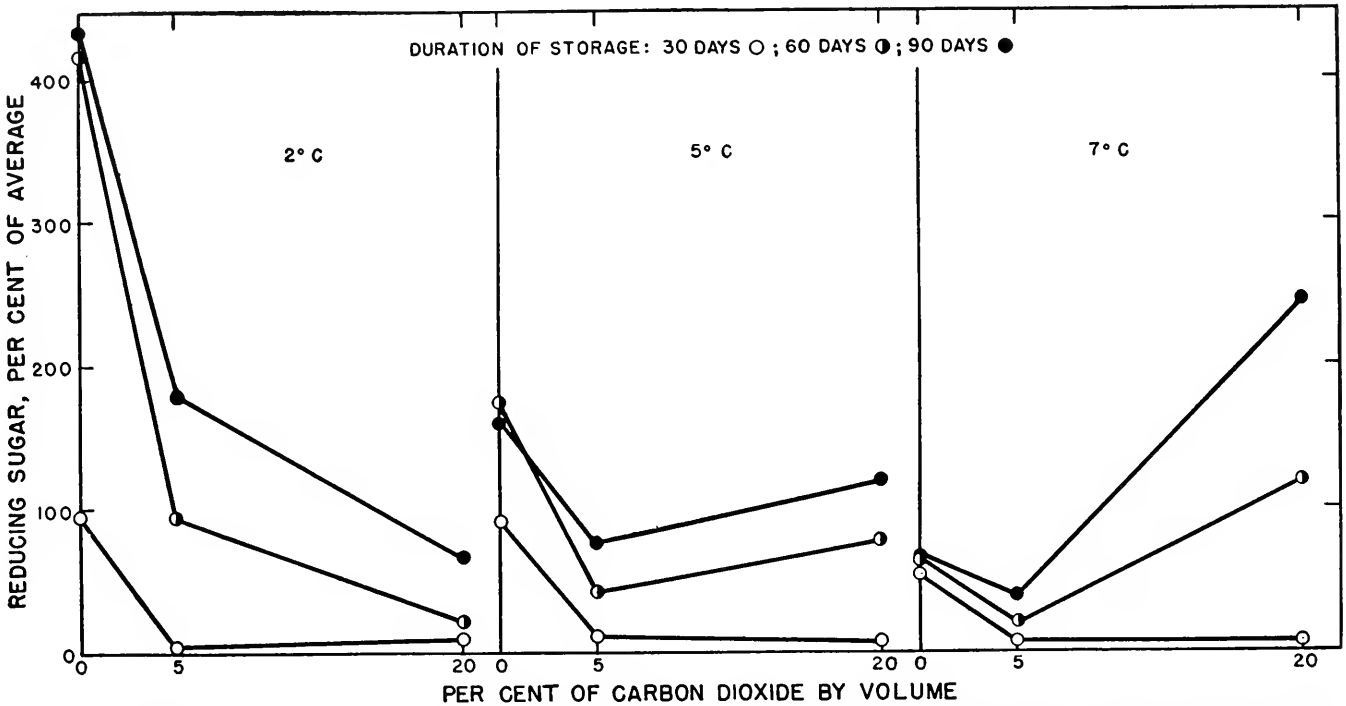

FIGURE 156. Effect of CO_2 treatment upon reducing sugar of potato tubers stored at different temperatures. Each plotted point is the average for three varieties in Table I after all of the values for each variety in the table were expressed as a percentage of the average value for that variety including the values for all temperatures, durations, and treatments.

Table 44. Effect of CO_2 upon the Cane Sugar Content of Potato Tubers Stored for Different Lengths of Time at Different Temperatures

Variety	Duration, days	Cane sugar, mg per cc of juice								
		2° C (36° F)			5° C (41° F)			7° C (45° F)		
		% CO_2			% CO_2			% CO_2		
		0	5	20	0	5	20	0	5	20
Irish Cobbler	30	29.3	24.3	10.4	8.1	28.0	26.4	5.9	13.3	30.0
	60	18.9	38.9	20.8	6.0	23.2	38.9	5.5	17.7	37.5
	90	16.4	42.8	35.2	5.8	31.3	41.1	4.9	20.0	30.9
Green Mountain	30	29.7	18.2	11.7	13.4	32.0	23.1	9.2	18.7	17.7
	60	14.2	33.9	23.2	9.9	33.1	35.8	6.0	19.0	37.2
	90	18.4	35.3	32.0	7.5	31.8	46.7	5.4	19.3	37.1
Katahdin	30	22.5	9.6	8.2	3.8	12.6	11.4	2.7	3.8	10.9
	60	12.9	26.7	14.2	2.8	13.4	15.1	3.1	5.3	25.9
	90	11.2	33.5	15.0	3.2	20.2	24.5	2.4	5.3	30.2

Note: The cane sugar values at the start were: 0.3, 3.0, 0.1 for Irish Cobbler, Green Mountain, and Katahdin, respectively.

27° C (81° F), good chips can be made even after seven or eight months of storage. This is at least a partial solution of the practical problem and the study has taught much about the effect of temperature and CO_2 on the carbohydrate metabolism of the potato tuber.

Use of a Bud Inhibitor

In the discussion above, there has been an attempt to steer between two evils in potato tuber storage, especially for chip making, merely by manipulating the storage temperature with a side glance at CO_2 effects: the evil of the accumulation of reducing sugar and the evil of sprouting. Storage temperatures that are low enough to prevent sprouting are not quite high enough to prevent the accumulation of reducing sugar. In the last chapter the fact was mentioned that the methyl ester of α-naphthaleneacetic acid, a harmless chemical, will prevent sprouting of potatoes when the chemical is used in very low dosage. Treatment with this chemical will prevent the evil of sprouting for any period desired. We have already described the talc powder method of treatment. The treatment should be made at a relatively high temperature, such as room temperature, so the chemical will have sufficient vapor pressure to reach the eyes in inhibiting amounts. After the inhibitor is applied, the tubers can be stored at a temperature that will prevent accumulation of reducing sugar in practically any variety of potatoes studied, with the possible exception of Bliss Triumph. A storage temperature of 55° F (13° C) or higher should be suitable. We have already mentioned the probable great economic signifi-

cance of the use of this inhibitor in farm and commercial storage. In farm storage there is no chance for close control of temperature, and it will not be needed if the inhibitor is used. Only the portion of the fall crop to be used after January 1 to 15 need be treated. In commercial storage there will be a saving in refrigeration, and sweetening of the potatoes can be avoided. Here, too, only the portion of the crop that is to be stored well into the winter and spring should be treated.

Physiological and Biochemical Effects of Carbon Dioxide

Thornton has made extensive studies of the effect of CO_2 in various concentrations in the atmosphere over a wide range of temperatures upon the biochemistry and physiology of various plants and plant organs. In mass storage and in nature, plant organs or the living cells of plant organs often exist in concentrations of CO_2 far above that of the atmosphere, due to respiration of the organs and the accumulation of the CO_2 about or within them. Soil air [30] sometimes has a considerable content of CO_2, and plant organs growing in water or water-logged soils may have an internal atmosphere [37] rich in CO_2. Massive organs such as fruits, tubers, fleshy roots, and tree trunks,[14] when existing individually in the air, may have an internal atmosphere rich in CO_2. In such organs the percentage of CO_2 in the internal atmosphere generally rises as the temperature rises. According to Magness,[57] the internal atmosphere of apples held individually in the air at 2° C (36° F) had 6.7 per cent CO_2 and 14.2 per cent O_2 and at 30° C (86° F) 21.4 per cent CO_2 and 3.2 per cent O_2. For carrots at 11° C (52° F) the internal atmosphere was 12.2 per cent CO_2 and 10.9 per cent O_2, and at 24° C (75° F) it was 28.6 per cent CO_2 and 5.2 per cent O_2. Bartlett pears [58] in storage in boxes at the freezing point or a little below had an internal atmosphere of about 6 per cent CO_2 and 19 per cent O_2. In similar storage at 60° F (16° C) the CO_2 was 24 to 33 per cent and the O_2 8 to 10 per cent. A Rome Beauty apple 9.1 cm. in circumference, hanging on the tree just after the middle of June, shows an average internal atmosphere [29] of 32.4 per cent CO_2 and 5 per cent O_2. At earlier and later periods the CO_2 content is lower — very much lower at the late stages of development.

Because of the facts just stated, there are two situations that ought to be kept in mind in describing Thornton's work on CO_2 effects. First, while the concentrations used in his experiments are often higher than those existing in nature or in practice, those sometimes existing in nature and practice are sufficiently high to bring about easily measured metabolic effects in plants; as a result, CO_2 may be considered as one regulator of plant metabolism. Secondly, in the experiments to be described, the concentration of CO_2 given is always that in the air surrounding the plant or plant organs being studied, and not that in the internal atmosphere of the plant organs themselves. Consequently, the actual concentration within all massive organs like fruits and tubers is considerably higher than

recorded. In the case of thin organs like leaves and petals, of course, there is not so much difference.

Effect of high concentration of CO_2 on plants in storage. The early studies [76, 77] were for the purpose of determining the concentration of CO_2 in the surrounding air that various plant organs tolerate without injury while being shipped or held in storage. This was for the purpose of determining the precautions that must be taken to prevent CO_2 from the Dry Ice as a refrigerant from reaching the fruits and vegetables in transit. In general, plant organs withstand higher concentrations of CO_2 when they are firm and solid than when they are soft and ripe; this is also true when there is no surface moisture. In general, they endure high concentrations near the freezing point better than at higher temperatures. There is one exception to the latter: the flavor of Walters variety of grapefruit is greatly impaired in 64 per cent CO_2 at 0° to 4° C (32° to 39° F), but is little impaired in the same concentration at 10° to 15° C (50° to 59° F). There is a great difference in the concentrations endured by different plant organs. At the freezing point tomato fruits will endure only 6 per cent for four days without injury, lettuce 7 per cent for seven days, Bartlett pears 10 per cent for seven days, and a number of plant organs only 15 per cent (mushrooms for four days, strawberries three days, and Talisman and Pearson rose flowers seven days). On the other hand, some plant organs endure very high concentrations of CO_2, especially at the freezing point: Delicious apples 83 per cent for seven days, carrot roots 80 per cent for five days, and rhubarb stalks 80 per cent for seven days. The other 32 fruits, vegetables, and flowers tested stand somewhere between these two groups in their resistance to CO_2 injury.

The effects of low, non-injurious concentrations of CO_2 bring about retardation of respiration and color and other ripening changes in certain fruits. Such concentrations removed astringency in green bananas and pears, and increased by four days the length of time various flowers could be kept in cold storage without injury. High, injurious concentrations impair the flavor of all fruits and some vegetables; prevent changes in color and flavor of banana, peach, and orange; kill growing tissues of asparagus and potato sprouts; promote decomposition of exposed tissues of vegetables that are high in moisture; cause internal discoloration and breakdown of apple, pear, peach, potato, and tomato; and induce external discoloration of pear, peach, and banana.

Following these more general economic investigations, Thornton made extensive studies of the effect of CO_2 in various concentrations upon the growth and metabolism of plants.

Effect on dormant seeds and buds. Recently harvested lettuce seeds require a temperature below 20° C (68° F) for germination in darkness and below 26° C (79° F) for germination in light. Treating such imbibed lettuce seeds [84] for 65 hours with 40 to 80 per cent CO_2 and 20 per cent O_2 at 35° C (95° F) causes them to germinate later at this temperature. Even

lettuce seed thrown into deep dormancy by a period in a germinator at 35° C (95° F) can be forced to germinate and produce normal plants at this temperature by 96 hours' treatment with this gaseous mixture. Even 5 to 20 per cent CO_2 with 20 per cent O_2 will force freshly harvested lettuce seeds to germinate within 17 hours in darkness at 26° C (79° F). High concentrations of CO_2 with 20 per cent O_2 force the germination of intact cocklebur seeds at unfavorable low temperatures. Kidd found that similar concentrations of CO_2 threw white mustard seeds into dormancy.

Treating dormant potato tubers [80, 83, 87] with 40 to 50 per cent CO_2 and 20 per cent O_2 hastens the germination. In this treatment 20 per cent O_2 was better than 0, 5, or 10 per cent O_2; consequently, the forcing was not due to anaerobiosis. This fact is interesting, for as was shown in Chapter 7 on "Dormancy in buds," reduced O_2 pressure without CO_2 favored germination of dormant potato buds; moreover, dormant potatoes sprout more quickly in 100 per cent CO_2 or N_2 than they do in 100 per cent O_2. The presence of high partial pressures of CO_2 raises the optimum oxygen pressure for germination. In earlier papers Thornton found that high concentrations of CO_2 with normal O_2 induced various metabolic changes in the living potato tubers: it increased the O_2 uptake, the alkalinity of the juice, the reducing property of the juice for methylene blue, the iodine absorption, the catalase activity, the content of reducing and cane sugar, the permeability of the protoplasm, and even the glutathione content some time after treatment. Several of these changes were worked out more fully in later researches and they will be discussed further presently. Some of the effects of CO_2 on the accumulation of reducing and cane sugars in potato tubers are discussed in the first section of this chapter.

Carbon dioxide renders living plant tissues more alkaline. One of the most general effects of CO_2 on living plant tissue is to make it more alkaline. Up to the time this work was done it was assumed that CO_2 had the opposite effect, for CO_2 is an acid and when dissolved in water, a non-living medium, or even dead plant tissue, it renders them all more acid. This alkalizing effect of CO_2 on living plant tissue is an indirect effect, and is brought about by a pronounced modification of the metabolism of the living tissue. In the chapter on "Dormancy in buds" we have seen that ethylene chlorhydrin, a slightly acid substance, renders living plant tissue more alkaline. In that case it causes the change by inducing the protoplasm to respire citric and perhaps other organic acids and to consume nitric and sulphuric acids in the synthesis of glutathione. Ethylene chlorhydrin and CO_2 may induce other as yet unknown metabolic changes in living protoplasm that modify the pH in either direction. If so, the net effect of all the changes is to raise the pH of the tissues.

Table 45 shows [79] the amount of the rise in pH induced in various living tissues when they are exposed to 50 to 70 per cent CO_2 for various periods at 25° C (77° F). In a number of the less acid tissues the pH shift was considerable, while in the four acid fruits and some other tissues the shift

Table 45. Effect of an Atmosphere Containing 50 to 70 per cent CO_2 and 20 per cent O_2 on the pH of Living Tissue of Various Plants. Determinations Made on Extracted Juice by Means of Glass Electrode and Quinhydrone Apparatus. Temperature, 25° C (77° F)

Tissue	Hours of exposure	pH rise
Tobacco plants in soil	2	0.77
Tomato plants in soil	5	.76
Beet roots	22–96	.74
Carrot roots	24–115	.72
Asparagus sprouts	8–24	.59
Onion bulbs	92–116	.59
Potato tubers	41–168	.53
Green lima beans	17–22	.35
Tulip bulbs	66–69	.27
Apple fruits	120	.25
Strawberry fruits	37–41	.18
Peach fruits	48	.15
Orange fruits	26–120	.15

was slight to moderate. The shift was rapid in leafy tissues where large surface per volume exists, as in the tomato and tobacco plants, and slow where the tissue was massive as in fruits, tubers, bulbs, and fleshy roots.

Asparagus shoots showed significant changes in pH with 15 minutes of exposure and potato tubers only after 12 or more hours. When tissues were placed back in air they gradually recovered the original pH. This requires 20 to 24 hours for asparagus shoots and 48 to 72 hours for potato tubers. Cut pieces as well as the whole tubers of potatoes show the change. In untreated tubers the tissue near the surface is more acid than the deeper tissue, but CO_2 treatment reverses this. The pH of potato tubers is lower when they are stored at low temperatures than at high; but CO_2 increases the pH even at 2° C (36° F), although more slowly than at 25° C (77° F). The increased alkalinity caused by high concentrations of CO_2 seems to depend upon aerobic respiration, for in absence of O_2 in the atmosphere high concentrations of CO_2 cause potato tubers to become more acid.

The researches on the effect of CO_2 on the pH of the hyphae of *Sclerotinia fructicola* in culture [81] are of especial interest because they involve a a wide range of concentration of CO_2 and of temperature, and the pH shift is large. The pH of the hyphae and culture medium was determined colorimetrically by indicators and by the quinhydrone apparatus; in addition, the glass electrode was used for the hyphae. Table 46 shows the shift in the pH after 24 hours in four different concentrations of CO_2 and at six different temperatures. The total shift in pH is induced by four hours' treatment, and no additional change occurs even up to 120 hours' exposure. It will be noted here that the shift in pH occurred at all temperatures, being somewhat higher at 2° and 5° C (36° and 41° F) than at higher temperatures. It will also be noted that even 10 per cent CO_2 induces a marked

Table 46. pH of the Hyphae of *Sclerotinia fructicola* Held at Various Temperatures during Exposure to Various Concentrations of Carbon Dioxide for 24 Hours

Treatment	Temperature of treatment					
	2° C (36° F)	5° C (41° F)	10° C (50° F)	15° C (59° F)	22° C (72° F)	28° C (82° F)
Control	5.6	5.6	5.6	5.6	5.6	5.6
10% CO_2	6.4	6.4	6.2	5.8	6.0	6.2
20% CO_2	7.4	6.8	6.6	6.2	6.4	6.4
40% CO_2	7.4	7.2	6.8	6.8	6.8	6.8
60% CO_2	7.6	7.6	7.0	7.2	7.2	7.0

change, with 20 per cent considerably more effective. The pH rises slightly as the concentration of CO_2 rises to 40 and finally to 60 per cent. Concentrations higher than 60 per cent give no additional rise in pH. The greatest shift shown was 2 pH, which means 100-fold rise in the alkalinity and a corresponding fall in acidity.

While CO_2 in the surrounding air renders the living hyphae more alkaline, it makes the culture medium and the dead hyphae more acid. These changes are shown in Fig. 157.

Thornton mentions that several workers have found 10 to 30 per cent CO_2 in the atmosphere effective in reducing fungus growth. Such concentrations of CO_2 have also been found to suppress fungus growth on fruits,

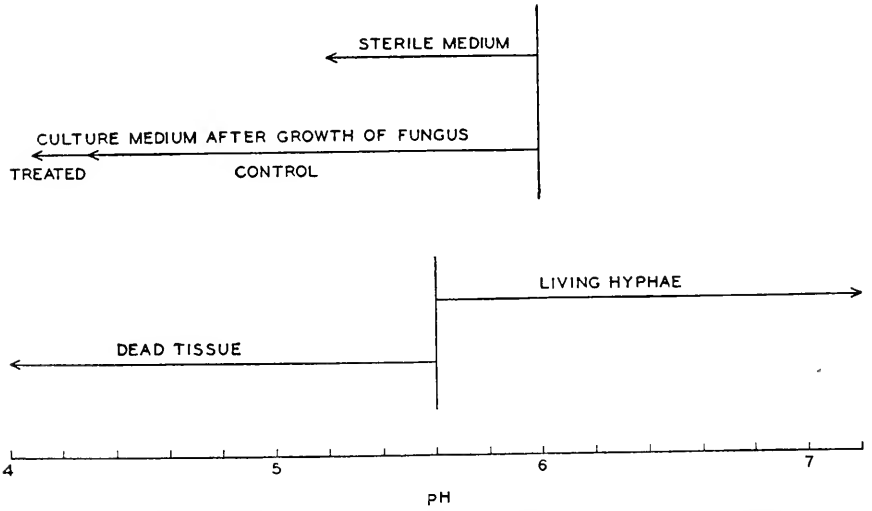

FIGURE 157. The effect of 60 per cent of CO_2 on the pH of living and dead hyphae of *Sclerotinia fructicola* and on the sterile and inoculated culture medium during 24 hours of storage at 22° C (72° F). The pH of the living hypha and the culture medium at the beginning of the treatment is shown by the vertical lines. The arrow indicating a pH of 4.3 on the line of the inoculated culture medium represents the pH of the medium developed during 6 days' growth of the fungus. The arrow at pH 4.1 represents the additional change brought about by the CO_2 treatment.

FIGURE 158. Changing the color of iris flowers by treatment with CO_2. *Left:* control, pH of petal 6.46; *right:* treated with 30 per cent CO_2, pH 6.90; *center* (*below*)*:* treated with 60 per cent CO_2, pH 7.20.

vegetables, and meats in cold storage. *Sclerotinia*, as do many fungi, grows best in a slightly acid medium and a shift of the culture medium in the alkaline direction is more unfavorable than a similar shift toward the acid. On the basis of his studies, Thornton suggests that CO_2 in the atmosphere inhibits or "stales" the growth of fungi by rendering the protoplasm more alkaline rather than by acidulating the culture medium.

Thornton's work [82] on the effect of various concentrations of CO_2 in the atmosphere on the pH and the change in color of various flowers is most interesting, for the flowers studied bore anthocyanins that indicate the more alkaline cell sap in the presence of CO_2 by a shift of color from reddish toward the blue. The flowers were placed in 20, 30 to 35, 50 to 60, and 80 per cent CO_2 with 20 per cent O_2, and the following flowers were used: rose (Templar and Briarcliff), *Verbena phlogiflora*, pink peony, and four varieties of Japanese iris (violet to purple). The change in pH as shown by the change in color of the flower was checked by extracting and determining the pH of the juice electrometrically. The shift toward the alkaline amounts in some cases to 0.8 of a pH. While the exposures were for 18 hours at 22° C (72° F) the change in color, as would be expected in these thin organs, occurred in much shorter time. When the flowers are removed from the CO_2 the petals return to their original color within a few hours, if they are not injured by too high concentration of CO_2 or too long an exposure to it. Very old and injured petals did not show the shift toward alkalinity. The change is brought about by metabolic activity of living protoplasm and probably involves both synthetic and catabolic changes.

The four irises selected for the study proved especially favorable for showing the change in flower color. The pH of the cell sap of the flower segments in these in air was 6.46, 6.47, 6.34, and 6.41 respectively, and in 50 to 60 per cent CO_2 it shifted to 7.20, 7.32, 6.96, and 7.18 respectively. Except for one, the shift was from acid to alkaline and that one became practically neutral. Furthermore, the anthocyanins in the flower segments shifted from violet to blue very near the neutral point as the sap became more alkaline. Violet flowers in air soon became blue flowers in 50 to 60 per cent CO_2. Fig. 158 shows the colors of the flowers of one variety in air, in 30 to 35 per cent CO_2, and in 50 to 60 per cent CO_2. In the flowers studied other than iris, the cell sap in air showed a pH range from 5.17 to 5.54; consequently the shift in pH even in 80 per cent CO_2 was not sufficient to reach the neutral point. In fact, the greatest shift in these was in the petals of Briarcliff rose which was from 5.21 to 5.88 pH, or about 0.7 pH.

Effect of carbon dioxide on the respiration rate of plant tissue. Since CO_2 is an end product of respiration, perhaps it is sometimes assumed that its accumulation about the plant in considerable concentration reduces the respiration rate. Since respiration is a complex process involving many enzymes and chemical reactions, such an assumption is hardly justified. Thornton's studies [78, 83] show that with some plant organs under

certain conditions CO_2 in the atmosphere surrounding the organ increases the oxygen absorption markedly, whereas with other organs it decreases this phase of respiration under a wide range of concentrations and other conditions, and finally that it does not affect measurably the rate of oxygen uptake by still other organs. A careful examination of all of Thornton's data [78] indicates that plant organs with normally low respiration (potato

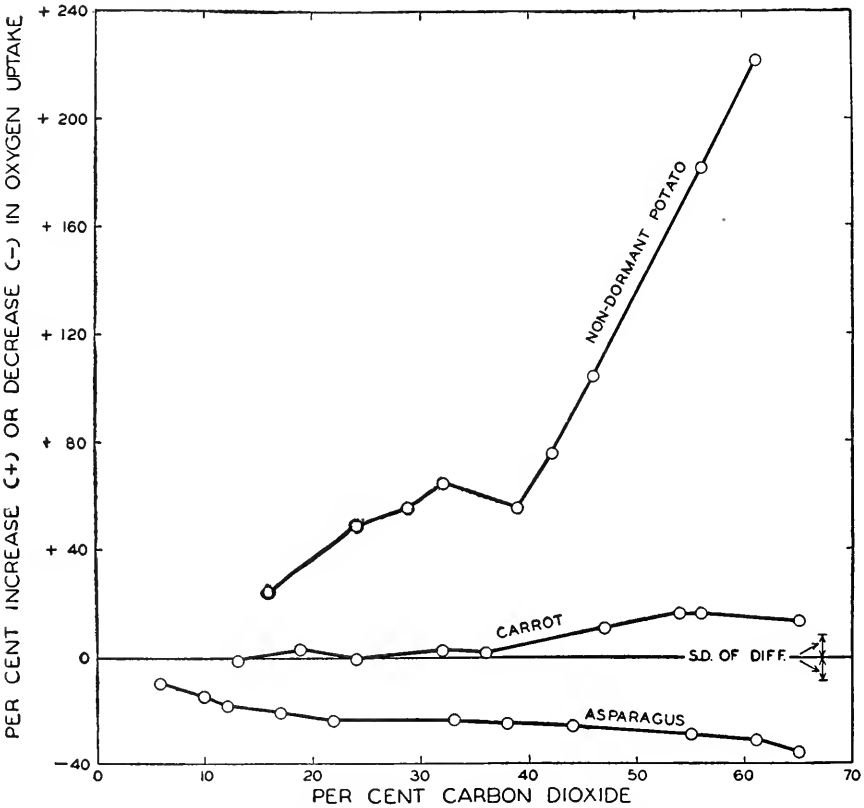

FIGURE 159. Effect of carbon dioxide upon the oxygen uptake by various tissues. The controls are plotted at zero. The curves show percentage increases or decreases in oxygen uptake by tissues treated with carbon dioxide at 25° C (77° F). Potatoes at 92, carrots at 62, and asparagus at the 20-hour period of CO_2 storage.

tubers) have oxygen absorption increased by CO_2 and those normally high (asparagus shoots) have the oxygen uptake decreased by CO_2. Those organs with moderate respiratory rates are less or not at all affected. Even old non-dormant potato tubers with a low respiration rate were increased more in their oxygen absorption by CO_2 than freshly harvested tubers with high normal respiration.

Fig. 159 shows the effect of different concentrations of CO_2 with 20 per cent O_2 on the oxygen uptake rate of non-dormant potato tubers, carrot roots, and asparagus shoots. In the non-dormant potatoes the rate of

oxygen absorption is taken after 92 hours in the various concentrations. At longer periods of exposure (as long as 8 days) the acceleration of oxygen uptake in 60 per cent CO_2 exceeded 400 per cent instead of 230 per cent, as at the end of the 92-hour period, and at shorter periods of exposure the acceleration falls off as the period of exposure is shortened. In the concentration of CO_2, 10 to 40 per cent, the acceleration of oxygen absorption is relatively slight, but rises rapidly as the concentration rises from 40 to 60 per cent. In the carrot the rate of oxygen absorption is plotted after 62 hours of exposure. At this time the rate had reached its maximum, and there is relatively slight acceleration even at the highest concentration of CO_2. For asparagus shoots, the respiration rate was plotted after 20 hours of exposure, at which time the rate had reached its minimum. All concentrations of CO_2 above 10 per cent give significant fall in the oxygen absorption rate; but even the highest concentrations used give less than 40 per cent fall.

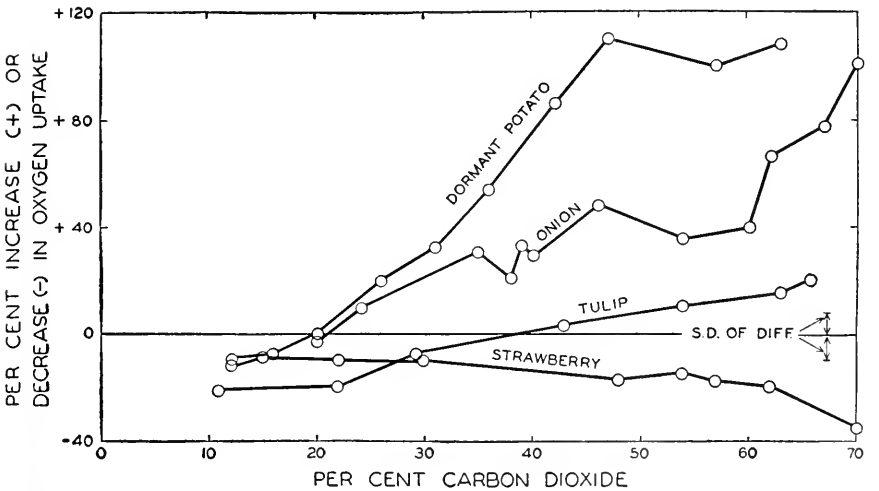

FIGURE 160. Effect of carbon dioxide upon the oxygen uptake by various tissues. The controls are plotted at zero. The curves show percentage increases or decreases in oxygen uptake by tissues treated with CO_2 at 25° C (77° F). Periods of exposure: potato, 73; onion, 90; tulip, 68; and strawberry, 22 hours.

Fig. 160 shows the effect of different concentrations of CO_2 with 20 per cent O_2 upon the oxygen uptake of dormant potato tubers, onion bulbs, tulip corms, and strawberry fruits after the following periods of exposures: 73, 90, 68, and 22 hours respectively. In the dormant potatoes and onions, concentrations of 20 per cent or lower have little effect on the oxygen absorption rate. At higher concentrations the rate rises as the concentration of CO_2 rises. In tulip corms the lower concentrations of CO_2 depress oxygen absorption slightly and higher concentrations accelerate it slightly. The oxygen absorption of strawberries is depressed slightly, if at all sig-

nificantly, at low concentrations and relatively slightly but significantly at higher concentrations.

Even in the potato tubers, in which high concentrations of CO_2 raise the respiration rate enormously if the exposure period is sufficiently long, there is a depression in oxygen absorption during the first 20 to 24 hours of exposure. Fig. 161 shows this situation when 56 per cent CO_2 is used.

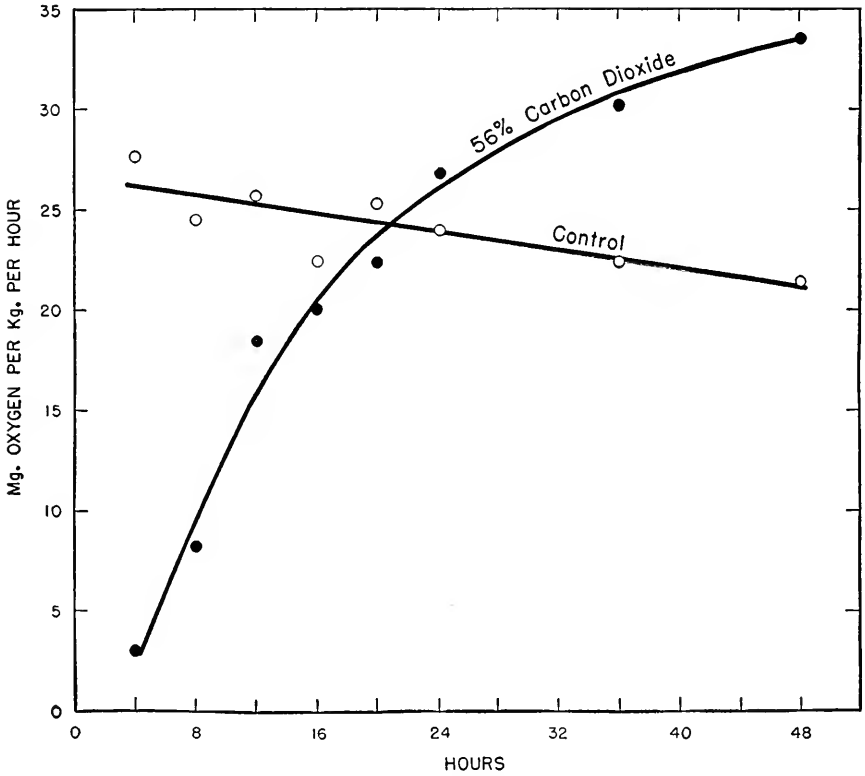

FIGURE 161. Influence of CO_2 on the rate of oxygen uptake by dormant Irish Cobbler potatoes at 25° C (77° F).

Effect of carbon dioxide in the air on the ascorbic acid (vitamin C) content of fruits and vegetables. Carbon dioxide in the surrounding air induces a great fall in ascorbic acid in some fruits and vegetables. Fig. 162 shows the effect of various percentages of CO_2 in the atmosphere at 22° C (72° F) for 24 hours on the ascorbic acid content of asparagus sprouts.[85] The loss of ascorbic acid rises as the concentration of CO_2 rises until in 60 per cent CO_2 about one-half the ascorbic acid is destroyed. It will also be noted that the pH of the tissue rises from about 6.2 to about 7.1 pH as the CO_2 concentration rises from 0 to 60 per cent. There probably is a causal relation between rise in pH and fall in ascorbic acid, since the latter is protected by acids and is readily oxidized in basic solutions. Upon

removal to air the sprouts return to the original pH, but there is no increase in the ascorbic acid content. The CO_2 has a similar effect at all temperatures from 2° to 27° C (36° to 81° F) and the fall is greater in the buds than in the sprouts. Since, as shown by the curve, even low concentrations of CO_2 cause a marked destruction of ascorbic acid, no doubt the accumulation of CO_2 due to respiration in the packages during shipping and marketing leads to a great loss in the vitamin C content of the sprouts by the time

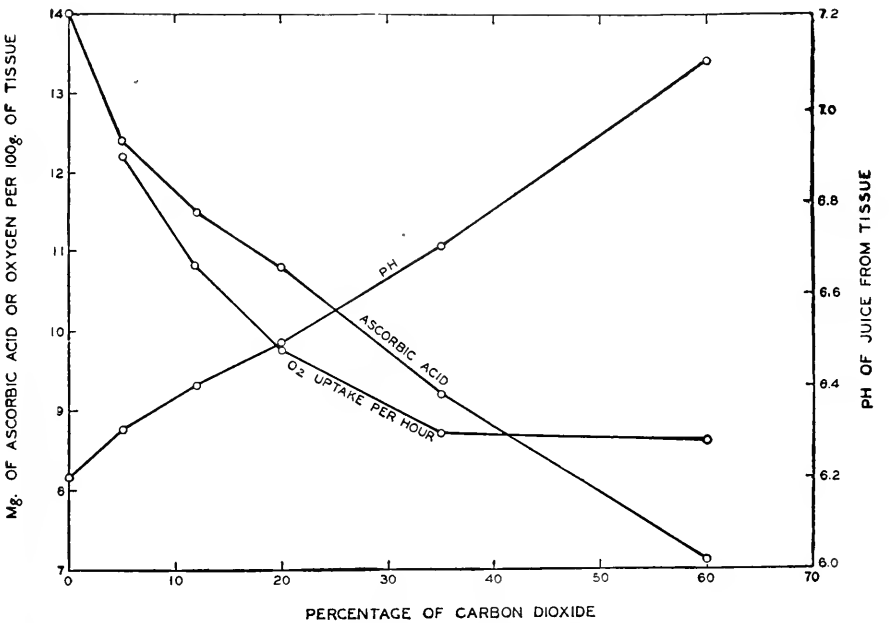

FIGURE 162. The effect of CO_2 on the ascorbic acid content, rate of respiration, and pH of asparagus tissue during storage of 24 hours at 22° C (72° F). The oxygen uptake is mg of O_2 per 100 g of tissue.

they are sold. This curve shows that the oxygen absorption rate (mg of O_2 per 100 g of tissue) of asparagus sprouts is reduced by CO_2, a fact mentioned above.

Carbon dioxide [90] causes marked reduction in the ascorbic acid content of green bananas with four days' storage at 19° C (66° F); 60 per cent CO_2 gives 66 to 85 per cent reduction, even 16, 24, and 40 per cent give great reductions and concentrations of 3 to 5 per cent cause 10 to 20 per cent reduction. Carbon dioxide has little effect on the ascorbic acid content of fruit in the yellow ripe stage. At any stage of ripening where CO_2 causes a reduction, the ascorbic acid rises back to normal some time after the fruit is removed from the CO_2; also fruit kept in CO_2 from the green stage to complete ripening ended up with about the same ascorbic acid content as fruit brought to the same stage of maturity in air bearing no CO_2. Carbon dioxide caused the juice of bananas to become more alkaline by 0.2

to 0.5 of a pH, but the juice is always acid, pH 5.9 being the highest recorded after four days in 60 per cent CO_2. Ethylene, which is a ripening hormone produced by bananas during maturity and is also sometimes added to the air to hasten ripening, did not modify the ascorbic acid content. Carbon dioxide [86] does not modify the ascorbic acid content of apples or green snap beans but does reduce the ascorbic acid somewhat in freshly harvested potatoes rich in this acid and in green pod peas, but has no effect on the ascorbic acid in potatoes after a long period of storage.

It is interesting to find CO_2, a naturally existing chemical environment of plants, having such marked effects upon certain metabolic processes here studied. It is likely that CO_2 modifies many other metabolic processes not included in these investigations. The effects produced by CO_2 are strikingly like those produced by ethylene chlorhydrin which are discussed in Chapter 7.

Diurnal and Autumn Changes in Leaves of Deciduous Plants

Diurnal changes. Denny [17, 18, 20] made a study of the diurnal changes in leaves and examined critically the several methods that might be used for accurately determining these changes. Offhand, so far as methods are concerned, the problem looks simple. If one wants to find out what changes occur in leaves during the night he can take a sample of leaves at dusk, a similar sample at dawn, and analyze the two for the various organic constituents making up the two samples. For the day changes, the first sample could be taken at dawn and the second one at dusk. The main problem is to make sure that the two samples to be compared are comparable except for the changes the 12 hours of darkness or light bring about. Denny's work shows that getting comparable samples and a correct basis for calculation of the results are far from simple. He describes four different methods that give reliable data, which, of course, give agreement in the changes obtained by the four methods.

He perfected the twin-leaf or, in compound leaves, twin-leaflet method. His later studies with this method were made on mature leaves or leaflets in order to avoid changes in weight involved in growth. In applying this method, kinds of plants were selected in which the opposite leaves are of approximately the same size. A sufficient number of the leaves are used for each sample to give a low error due to possible variation in size (25 to 300 per sample), and care was taken that on the average the two leaves of the pair received equal light exposure. One leaf of each pair was used for the first sample and the other was collected at a later hour for determining the changes brought about by the day or night exposure. Since, as the data show, the two samples were practically identical except for the changes caused by the day or night exposure, the changes in each constituent are reported in amount for the whole sample without reference to any measurement that might vary with exposure, such as wet or dry weight, or area.

The twin-leaf method — as do the other three methods, as we shall see later — shows that the main changes in mature leaves with night and day are changes in assimilable carbohydrates, soluble sugars, and starch (determined by the acid hydrolysis method). The acid hydrolysis undoubtedly breaks down some other polysaccharides besides starch. During the day photosynthesis leads to the accumulation of starch and sugars in the leaves, which increases the dry weight of the leaves. At night the starch is partly or wholly digested and the sugars and starch in part used up by respiration of the leaf and in part transported to other regions of the plant. Denny's data show that the total nitrogen of the leaf did not change measurably from day to night.

He finds that a modification of Sachs' half-leaf method gives results that check with the twin-leaf method. Sachs' method consisted in cutting a given area from one-half of many leaves for the first sample and a like surface from the other half of the leaves for the later comparable sample. Two main criticisms of this method have been offered: the mutilation of the leaf due to removal of the first sample modifies the processes going on in the other half of the leaf, and the two halves of the leaves are not symmetrical. Consequently, equivalent tissue is not used in the two cases. Another error in this method has been pointed out: the water content of the leaf generally increases during the night and with it the area of the leaf; consequently, the morning sample, while it has the same area, has less tissue. Denny's modified half-leaf method consists in selecting plants that have symmetrical leaves and cutting off one-half of the several leaves close to the midrib for the first sample and using the other half, discarding the midrib, for the later comparable sample. In sampling, the right and left halves of the leaves were taken alternately for the first and, of course, the remaining halves for the later sample. Here, as in the twin-leaf method, the several constituents were determined on the total amounts in the samples and need not be related to surface or to wet or dry weight, all of which are variables. Unlike the twin-leaf method, this method can be used on plants with alternate as well as opposite leaves. Denny concludes that when the half-leaf method is used with proper precautions it gives reliable results. Apparently the disturbance due to mutilation is not as great as has been assumed.

As stated above, the dry and wet weights, the assimilable carbohydrates, and the total leaf surface in mature leaves vary with day and night. There are, however, two fractions that remain constant in mature leaves during day and night, according to Denny's findings: residual dry weight (the total dry weight minus the assimilable carbohydrates) and the total nitrogen. Mason and Maskell [59] had already used the residual dry weight as a basis for calculating changes; and Denny confirms the constancy of this fraction and justifies its use as a basis for calculating leaf changes. While Denny finds in the leaves that he studied that the total nitrogen is a constant, he warns that workers ought to determine that it is a constant in

the particular leaves being studied before they use it as a basis for calculating leaf changes. If one has complete analyses of two similar samples of leaves taken, say at dusk and dawn, he can calculate the percentage change in any constituent during the night on the basis of either of these fractions and get a correct picture. In this case the absolute amount of change in like samples will not be compared, but rather the percentage change based on one or the other of the two constants. In using these two methods of calculation one is not limited to twin-leaf or half-leaf sampling, but can compare any two similar samples of leaves taken at dusk and at dawn or for any other period studied.

Table 47. Comparison of Different Methods of Computing Changes

Plant	Period	Per cent loss					
		Twin-leaf basis		Residual-dry-weight basis		Total nitrogen basis	
		Total carbohydr.	Total sugar	Total carbohydr.	Total sugar	Total carbohydr.	Total sugar
Stringless bean	5:40 P.M. to 9:20 P.M.	23.0	15.1	22.9	15.1	23.9	16.1
	6:30 P.M. to 2:00 A.M.	43.3	14.3	42.7	13.8	42.8	13.6
	7:20 P.M. to 5:45 A.M.	58.3	30.2	57.5	28.5	56.9	27.6
Cutshort bean	5:50 P.M. to 9:30 P.M.	24.8	22.5	26.6	24.2	24.3	21.6
	6:40 P.M. to 2:20 A.M.	49.7	19.6	50.3	20.5	50.7	21.2
	7:30 P.M. to 6:00 A.M.	57.2	44.6	58.8	45.6	58.8	45.4
Peanut	6:00 P.M. to 9:40 P.M.	6.3	13.9	6.3	13.7	5.6	12.9
	6:50 P.M. to 2:40 A.M.	12.4	14.0	13.1	14.6	12.7	14.0
	7:40 P.M. to 6:40 A.M.	17.2	15.8	18.0	16.7	18.0	16.3

We have already said that the twin- and half-leaf methods give comparable results. Table 47 shows that the twin-leaf, the residual dry weight, and the total nitrogen methods give comparable results. These analyses are made on the basis of the twin-leaf method, and the results are then calculated on the basis of residual dry weight and the total nitrogen. For each of the three kinds of leaves reported in the table, three different com-

parable pairs of samples were taken: one to show the change taking place from evening until early in the night; another to show the change taking place from evening until late in the night; and a third to show the changes occurring during the whole night. In the first column under each method of calculation is shown the percentage loss in total carbohydrate (starch by acid hydrolysis method plus soluble sugars) and in the second column the loss in soluble sugars. One will see by following along the several lines that the three methods of calculation give comparable results both as to the total carbohydrate and soluble sugars. Following down the columns for each kind of plant makes it evident that the percentage of loss in both total carbohydrates and sugars increases as the night progresses. If one compares the two beans with the peanut, he will see that in the beans a much larger percentage of both constituents is lost during the night than in the peanut. An examination of the original analytical data, not here given, shows that the starch in the several leaves in the evening exceeds by five- to ten-fold the amount of soluble sugars, and that peanut leaves are richer in both starch and sugar than the bean leaves. All these leaves are starch storers. In leaves that do not store starch, as is the case with onion, the change, of course, is in the sugars.

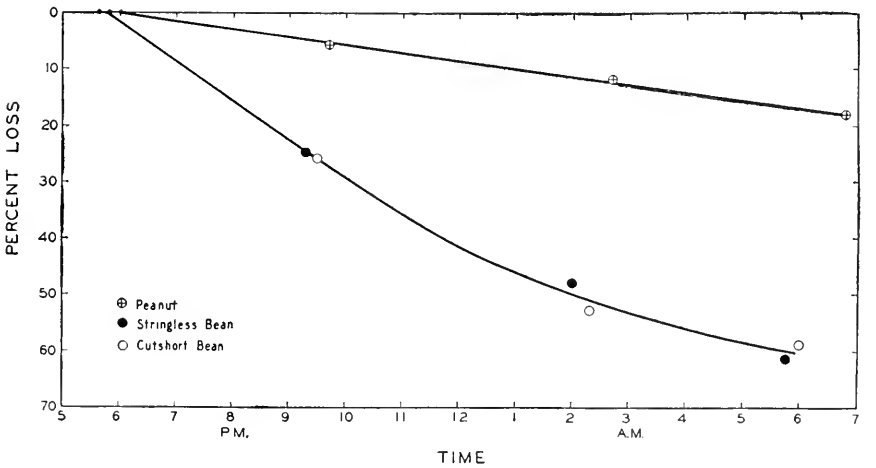

FIGURE 163. Per cent loss of polysaccharides (alcohol insoluble acid hydrolyzable substances, including starch) of peanuts and beans at intervals from night to morning; percentage computed on the basis of the amount present in the initial sample taken in the evening.

Fig. 163 shows the percentage loss in the polysaccharides in the bean and the peanut leaves during the three periods of the night, and Fig. 164 shows the amount of total carbohydrates in the same leaves at various times during the night calculated on the basis of residual dry weight and total nitrogen.

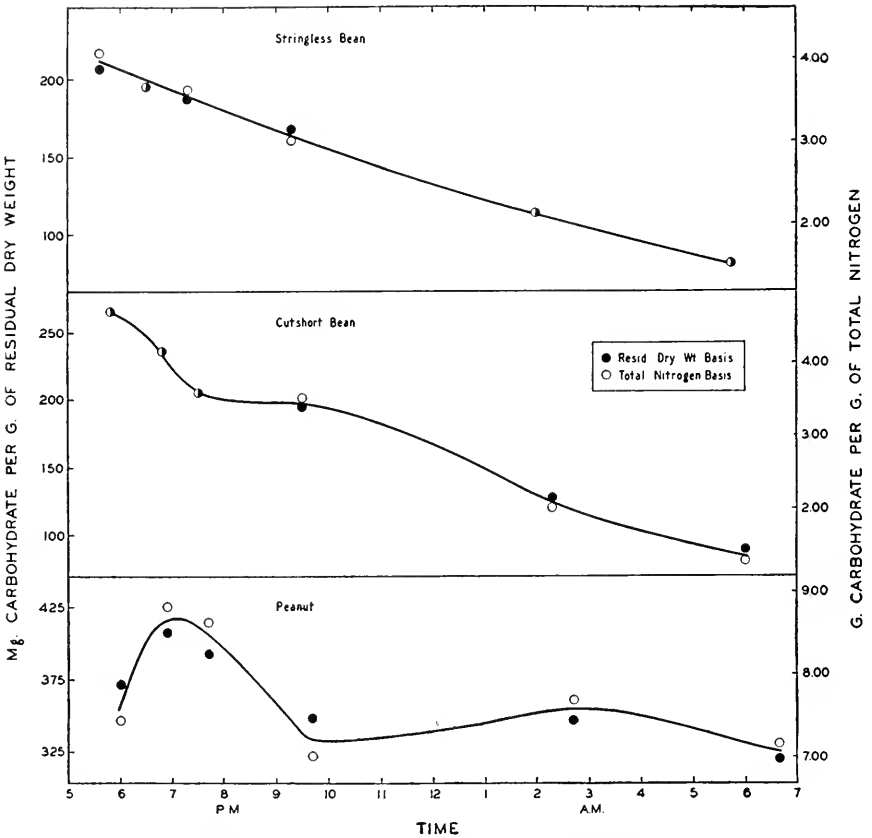

FIGURE 164. Amounts of total carbohydrates found in peanut and bean leaves at intervals during the night. Comparison of residual dry weight and total nitrogen as bases for calculating the change in total carbohydrates.

Fig. 165 shows the amount of soluble sugars found in the bean and peanut leaves at various periods during the night. All these are plotted on the basis of data given in Table 47.

The diurnal changes in leaves of several other plants (tobacco, salvia, sunflower, hawthorn, redbud, lilac, Virginia creeper, peach, soybean, cotton, grape, etc.) are reported using the twin- or half-leaf method of determination. In general, herbaceous plants show much greater diurnal changes than woody forms. The night changes in lilac are not measurable, those in hawthorn small, those in Virginia creeper and grape considerably larger, while in some herbaceous plants, as the data reported on the two kinds of beans show, more than 50 per cent of the starch and sugars disappears from the leaf during the night. In the peanut under similar conditions the loss in these two constituents is less than 20 per cent.

Autumn or pre-leaf fall changes in deciduous leaves. Denny [21] used the twin-leaf method of sampling to study the chemical changes occurring in

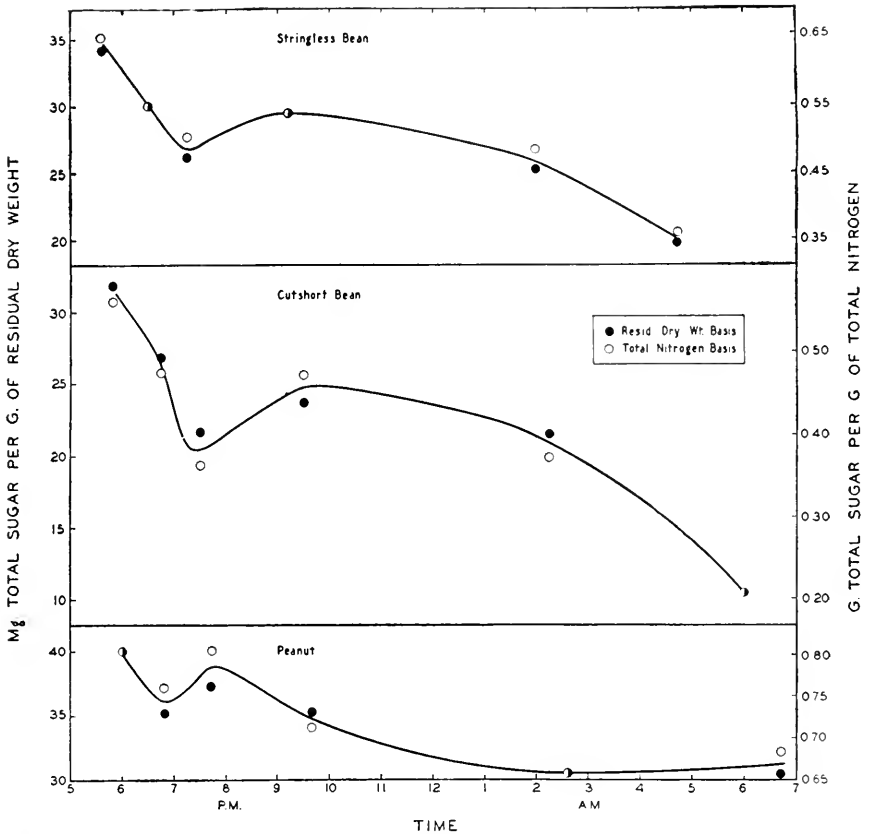

FIGURE 165. Amounts of total sugar found in peanut and bean leaves at intervals during the night. Comparison of residual dry weight and total nitrogen as bases for calculating the change in total sugars.

deciduous leaves during a period of 41 days previous to autumn leaf fall. His methods, results, and conclusions are stated by him as follows: [p.311]

"Samples of 50 pairs of opposite leaves of *Viburnum dentatum* and *Syringa vulgaris* were selected to measure the changes in leaves at intervals of three to five days from September 24 to November 4, at which time the experiment was ended because of frost. One leaf of each pair was taken at the beginning and the other was left on the plant until the end of each interval, there being ten such intervals during the experimental period.

"Dry weight, sugars, polysaccharides (alcohol-insoluble substances hydrolyzable with dilute acid), and nitrogen were determined, and calculations were made upon three bases: per cent of the dry weight, per cent of the residual dry weight (obtained by subtracting from the dry weight the sum of the carbohydrates and 6.25 times the nitrogen), and the total amounts of constituents in 50 leaves.

"The dry weight of the leaves was nearly constant throughout the period

of sampling, and no important change was observed in total carbohydrate (sum of sugar and polysaccharide). Previous reports of extensive losses of substances from leaves during the interval preceding frost (autumnal migration) were confirmed only for the nitrogenous substances in these species, and, even in this case, only the *Viburnum* results should be emphasized, as the nitrogen losses from lilac were small and of doubtful significance."

Concerning the Determination of the Isoelectric Point of Protoplasm

Attempts had been made to determine the isoelectric point of living plant tissue by placing pieces of the tissue in buffer solutions of low concentration but of various H-ion concentrations. The buffer solution in which the tissue did not change the pH was interpreted as the isoelectric point of the tissue. It was assumed that in this pH the amphoteric substances (especially proteins) of the protoplasm did not react differentially with either basic or acid ions of the buffer solution and that consequently the pH of the buffer solution was not changed. It was assumed, on the other hand, that if the buffer solution had either a higher or lower pH than the living tissue the amphoteric substances reacted differentially with basic ions in the first case and acid ions in the second, moving the pH of the buffer toward that of the tissue.

Youden and Denny [101] find that it is the substances that leach out of plant tissues when soaked in the buffer solutions (phosphate, phthalate, and borate) that mainly determine the change in the pH of the solutions, and not the ion absorption from the solutions by the insoluble amphoteric substances of the protoplasm. Tissue soaked in distilled water gave solutions of the same pH as the buffer solution that was not changed in pH when the tissue was soaked in it. The substances that leach from the tissue and mainly determine the pH of water or buffer solutions are heat-stable, diffuse through collodion membranes, and are soluble in acid alcohol. This shows that proteins or other colloidal substances do not play an important role in causing the change in pH. Fig. 166 shows how several plant tissues soaked in buffer solutions of a considerable range of pH values change the pH of the solutions in the direction of the pH of water in which the tissue has been soaked for the same length of time. One exception is mentioned in the description of the figure.

The authors make the following statements: [101, p.752, or p.287 in C. B. T. I.] "Most of the effect upon the buffer solution was not due to absorption of ions from the buffer by the tissue, but was caused by substances leaching out of the tissue into the buffer. On the acid side of the isoelectric point only about 5 per cent of the change in reaction undergone by the buffer was caused by the tissue itself; on the alkaline side the tissue was more effective, causing about 25 per cent of the change." [p.751–752, or p.286–287 in C. B. T. I.] "It is not our purpose to claim that plant tissue does not contain

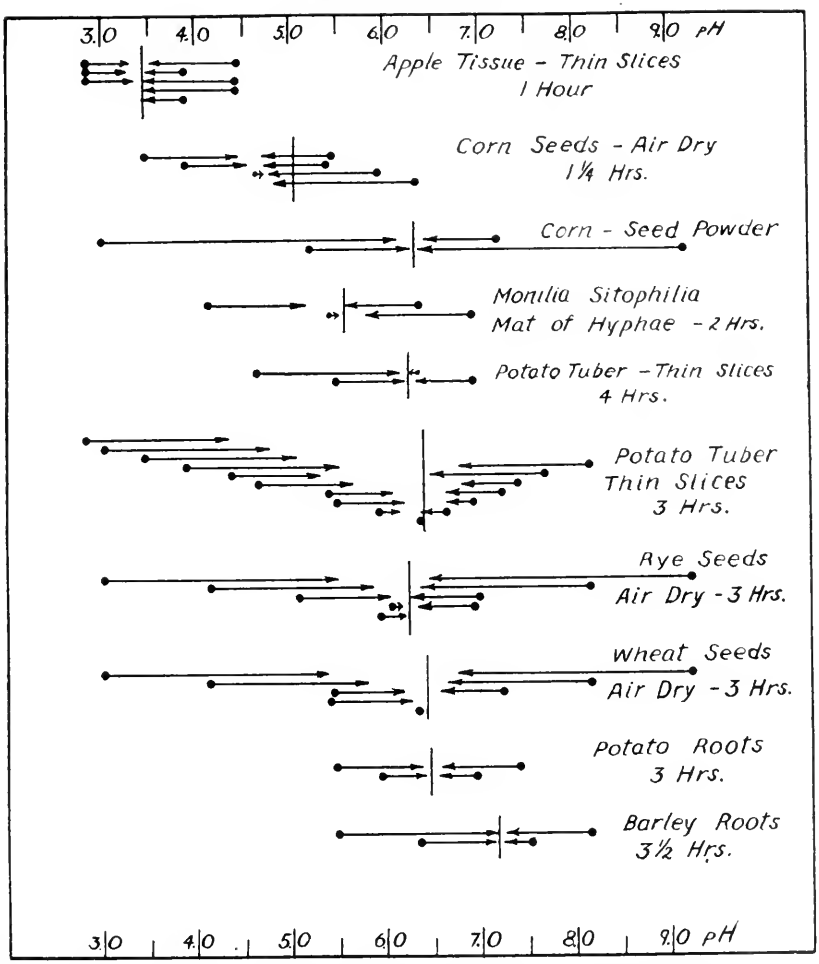

FIGURE 166. The relation between the pH equilibrium of tissues in a series of buffers and the pH of the water extracts of these tissues. The arrows show the pH of the buffer in which the sample of tissue was placed, the direction of change in pH, and the final pH attained. The vertical lines show the pH of the water extracts of the tissues. It is seen that the isoelectric point of the tissue as determined by this method coincides with the pH of the water extract in all tissues except in the case of corn seeds, air-dry whole seeds.

substances with isoelectric points, nor that these substances are not of great importance in the life processes of plants. Nor do we claim to have shown that the tissue itself does not have an isoelectric point. Robbins and his co-workers have brought evidence by other methods of experimentation (water absorption, toxicity of ions, staining of tissues, etc.) regarding the existence of such a point.

"Our objection is mainly to the method of determining tissue isoelectric points by immersing the tissue in a series of buffers and assuming that the

pH value at which no change in reaction is shown is the isoelectric point of the tissue.

"Furthermore, it is unlikely that the equilibrium point of a tissue in a series of buffers represents the isoelectric point of the proteins of the tissue. Thus the equilibrium point for potato tissue is about pH 6.4; but the isoelectric point for tuberin, the principal protein of potato, is about pH 4.0 according to Cohn, Gross, and Johnson. Pearsall and Ewing find that when the tissue is made as acid as, or more acid than, the isoelectric point of the principal protein in the tissue there is a rapid exosmosis of ions, indicating a serious injury to the tissue. The point for potato at which rapid exosmosis of chlorids took place was not at pH 6.4 but at about pH 4.4.

"Chibnall found that the pH of the cell contents and the isoelectric point of the cytoplasmic proteins are not identical in any tissue he studied, and points out the probability that any change in the reaction of the cell which brings the proteins to their isoelectric points will result in the death of the cell. The buffer capacity of the cell contents protects the cell against injury by tending to prevent the $[H^+]$ from reaching the isoelectric point of the proteins of the cytoplasm."

Rudolfs [73] finds that when any given kind of seed is soaked in solutions of various salts and organic and mineral acids, the solutions (except for very dilute solutions) all change to the same pH, which he terms the isoelectric point of the particular seed. He believes that the change is brought about by ion absorption by the amphoteric substances, mainly proteins of the living portions of the seeds, and not by substances leaching out of the seeds. In grains it is chiefly due to the embryo rich in proteins rather than to the endosperm, which is mostly carbohydrates. The pH equilibrium point for corn grains is at 3.9 to 4.1 pH, for *Phaseolus vulgaris* seed at 5.5 pH, and for *Lupinus albus* seed 4.7 pH. Scott [74] finds similar results by soaking living mycelium of *Fusarium lycopersici* in unbuffered and buffered salt solutions with the equilibrium point at 5.4 pH. Dead mycelium did not give a definite equilibrium point. He interprets this as the isoelectric point of the main proteins of the mycelium.

Denny and Youden [28] placed various plant tissues (thin slices of potato tubers, carrot roots, and apple fruit, whole seeds of corn, rye, and wheat, and corn seed-powder) in solutions of various salts ranging in concentrations from $0.1M$ to $0.001M$. Samples of the salt solutions were removed after various periods of soaking and the pH determined. In all cases where a change in pH occurs, the change is in the acid direction. Salts of the monovalent cations, sodium and potassium, give slight or no shift in acidity. Salts of the bivalent cations, calcium, magnesium, and strontium, give decided shifts toward the acid; those of the bivalent cation, zinc, still greater shifts; and finally those of the bivalent cation, copper, and of the trivalent cation, lanthanum, give very large shifts toward the acid. The amount of acidulation increases with the concentration of the solutions, especially in the lower ranges of concentration. The final pH equilibrium

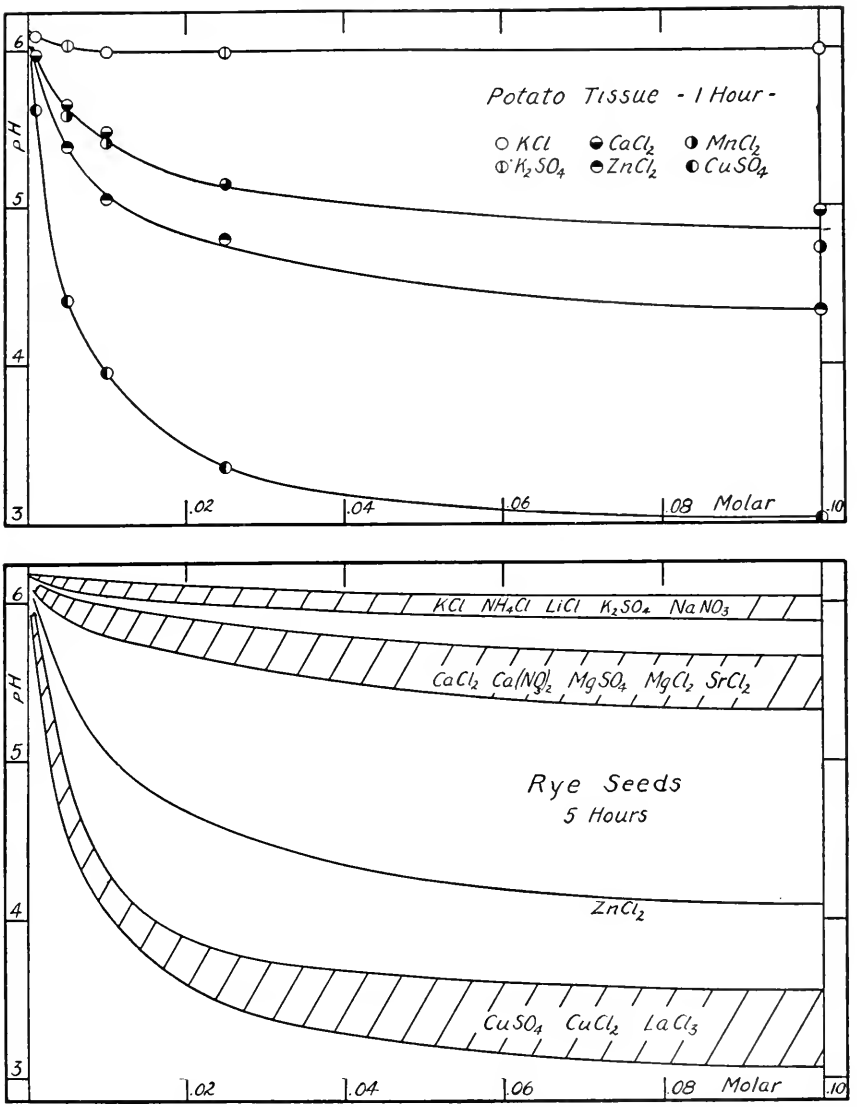

FIGURE 167. Ordinates show the pH values of the external solution when 25 grams of tissue were immersed in 25 cc of various salt solutions of the concentrations shown as abscissae. In the graph for rye seeds the salts listed in the shaded areas gave similar results, all the data for these salts falling within the limits shown. Note that each tissue did not bring the external solution to definite pH values, but that the values obtained varied with the salt used, with the concentration of the salt, and especially with the nature of the cation.

reached varies with both the concentration of the solution and the nature of the cation, which means that pH was not constant and could not represent the isoelectric points of the living cells. All these findings are shown by Fig. 167 for potato tissue and rye seeds. It will be noted that high

concentrations of copper salts for potato tissue and of copper salts and $LaCl_3$ for rye seeds reduced the pH to 3, far below any value that is claimed for the plant proteins of these plant organs.

Salt solutions are similarly acidified when water extracts of plant tissue are added to them; and this occurs even when the extracts are boiled or dialyzed to precipitate proteins and remove all colloids from the extracts. When salts were added to organic acid solutions (malice, oxalic, succinic, aspartic, etc.) there is an increase in hydrogen-ion. Additions of $CaCl_2$ to salts of organic acids increased the acidity in all pH values from 2 to 7.5. The increase in hydrogen-ion appears whether the salts of the organic acid were or were not precipitated. Additions of $CaCl_2$ to phosphates and pectin increased the hydrogen-ion. The addition of $CaCl_2$ to that portion of potato extract containing the protein tuberin shows that the change in hydrogen-ion is not analogous to the change caused by addition of potato tissues.

From a consideration of the results of all their experiments, the authors draw the following conclusions: 28, p.414, or p.328 in C. B. T. I.:

"Although our results do not show that the tissue itself or the proteins take no part whatever in these changes in pH, they indicate that the soluble, non-protein, non-colloidal substances which diffuse out of the tissue into the salt solution and which then react with it are important factors in the acidifications that are produced in the external solution.

"The observed changes in hydrogen-ion concentration, therefore, can not be interpreted as indicating an isoelectric point for the tissue as a whole, nor furnish proof that reaction has occurred between the ions of the salt solution and proteins with characteristic isoelectric points."

Growth Substances and Vitamin B_1 for Seed Treatment

One commercial firm has been vigorously promoting the use of plant hormones for seed treatment, claiming that the treatment greatly increases crop yield. Investigators at this Institute and most, though not all, of many investigators of the subject elsewhere have found no advantage in hormone treatment of seeds.

Barton [9] soaked non-dormant seeds of twenty-nine different species and varieties of farm, garden, and flowering plants and grasses in a wide range of concentrations (320.0 to 1.2 mg per liter of water) of three different growth substances (β-indoleacetic, β-indolebutyric, and α-naphthaleneacetic acids) and grew them to maturity in soil. Except for higher concentrations which in some cases inhibit growth or cause malformations, the treatments had no effects, either on germination or final yield. She also treated seeds with several proprietary plant hormone preparations, mainly talc dust mixtures, with similar results.

Dormant American elm seeds [10] soaked in various concentrations of potassium α-naphthaleneacetate showed some improvement in germina-

tion, but this treatment was ineffective compared with low-temperature stratification or illumination of the seeds while under water. Treatment with growth substances had no beneficial effect on the germination of dormant seeds of a variety of domestic apple and a species of crabapple. Low-temperature after-ripened seeds of *Cornus* and *Pyrus* were thrown back into dormancy by treatment with growth substances. Growth substances did not force the growth of the naturally dormant epicotyls of seeds of *Paeonia, Viburnum,* or lily.

Youden [96, p.218] describes a set of experiments in which he treated wheat and soybean seeds with growth substances. "Wheat and soybean seeds were treated in the dry state with indoleacetic acid, naphthaleneacetic acid, and indolebutyric acid, talc, and Rootone (commercial preparation) and grown in sand and soil in the greenhouse and in the field. The concentration of the organic compounds in the talc preparations, as well as the proportion of powder to seeds, was adjusted to cover the range 0.5 to 240 parts per million of active substance by weight of the seeds. In a series of ten experiments observations were made of the germination, seedling height, wet weight of tops, yield of grain, and root systems, and no significant case was found in which the germination and growth of the treated lots exceeded the controls. On the contrary the aggregate of evidence points to slightly lower values for the treated lots, and indicates that these are the result of the presence of the talc. Nineteen contrasts of talc-treated seeds with controls gave fifteen cases in which the controls were superior and one tie. On the average, the excess weight of the control plants was about 5 per cent."

An experiment was conducted to test the report that treatment of grass seeds with plant hormones stimulated the growth of lawns. Fig. 168 shows that there were no beneficial effects on any of the 14 plots tested.

In 1939, *Better Homes and Gardens* published an article announcing the remarkable stimulative effect of vitamin B_1 on green plants. It was said to produce veritable giants out of garden and house plants and to make transplanting entirely safe when plants were in full bloom. The article would lead one to think that the greatest discovery of modern horticulture had broken upon an unexpecting world.

Investigators [32, 105] at this Institute as well as most of many investigators elsewhere who have worked upon the problem have found that vitamin B_1 has no such remarkable effects upon growth of higher green plants — indeed that it has very minor, if any, effects. The B group of vitamins is an important accessory factor in the growth of various non-chlorophyllous plants, such as yeasts and fungi. If vitamin B_1 is important for the growth of higher green plants it is likely that these plants synthesize an adequate supply to meet their needs.

Zimmerman [105, p.94] says: "The preponderance of evidence from scientists is opposed to the use of vitamin B_1 for practical purposes. My own convictions, based on our experience at the Boyce Thompson Institute and

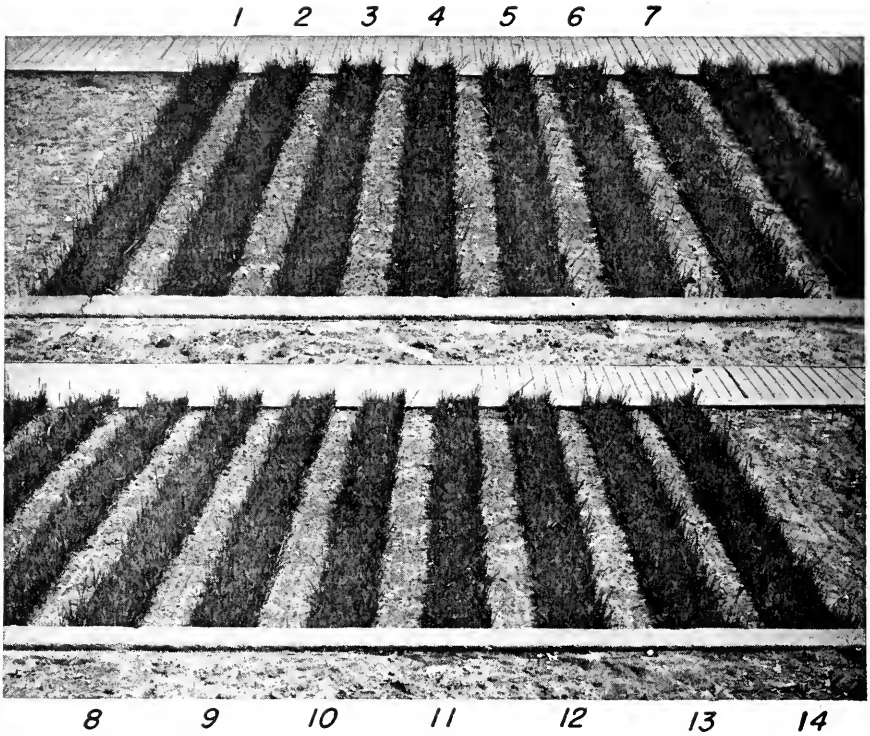

FIGURE 168. Seed (Scott's lawn mixture) treatment involving three of the most effective synthetic "plant hormones," a commercial preparation (Rootone), and talc. The chemicals were mixed with talcum powder and the seed dusted with the preparations. The concentrations were at the rate of 1, 2, and 3 mg of the substance per gram of talcum powder. *Top rows:* 1–3, three different concentrations of indoleacetic acid; 4, talc control; 5–7, three different concentrations of naphthaleneacetic acid. *Lower rows:* 8, non-treated control; 9–11, three concentrations of indolebutyric acid; 12, talc control; 13, "Rootone," commercial preparation; 14, non-treated control.

the published results of other scientific laboratories, are that *vitamin B_1 has been badly exploited, and that it has no value as recommended for horticultural practice.*"

IMPORTANCE OF THE MOTHER-TUBER IN THE GROWTH OF THE POTATO PLANT

Denny [15, 16] undertook to determine the importance of the mother-tuber to the development of the potato, also how long it must be retained on the vine to insure maximum growth of vine. He developed a clever technique for removing the mother-tuber from the plant at any stage of development without seriously disturbing the soil or interfering with the root system that had been established up to the time of removal. This method is shown in Fig. 169.

The mother-tuber was removed at four different stages of development

FIGURE 169. Method of amputating mother-tuber from sprout. *Left:* plant grown in pot buried in soil; *right:* plant inverted, placed upon board with slot from edge to center used for support; pot then discarded. Note mother tissue being removed piece by piece with scalpel; plant placed in soil after removal of mother-tuber.

of the plant: (stage 1) when the plant was just emerging from the soil; (stage 2) when the germination was complete and the leaves were expanded; (stage 3) when the plant was 10 inches above ground and tubers were set; and (stage 4) when the plants were at nearly maximum height and flowering. The various stages of development when the mother-tubers were removed are shown in Fig. 170.

Removing the mother-tubers at stages 1 and 2 reduces the yield of both Irish Cobbler and Bliss Triumph plants, and the reduction is greater with 56- than with 14-gram seed-pieces. Removing mother-tubers of Irish Cobbler at stage 3 reduces the yield when 56-gram seed-pieces are used by 14 per cent, but not significantly when 14-gram seed-pieces are used. Bliss Triumph plants are not affected significantly when the mother-tubers are removed at this stage whether the seed-piece is 56 or 14 grams. Removal of mother-tuber at stage 4 did not reduce the yield of either variety for either size of seed-piece. In fact, a slight increase is shown which Denny, in agreement with previous investigators, attributes to toxic substances from some of the seed-pieces that rot late in the season.

Chemical analyses were made of the mother-tuber seed-pieces at the beginning and at the other stages of development. Table 48 shows these

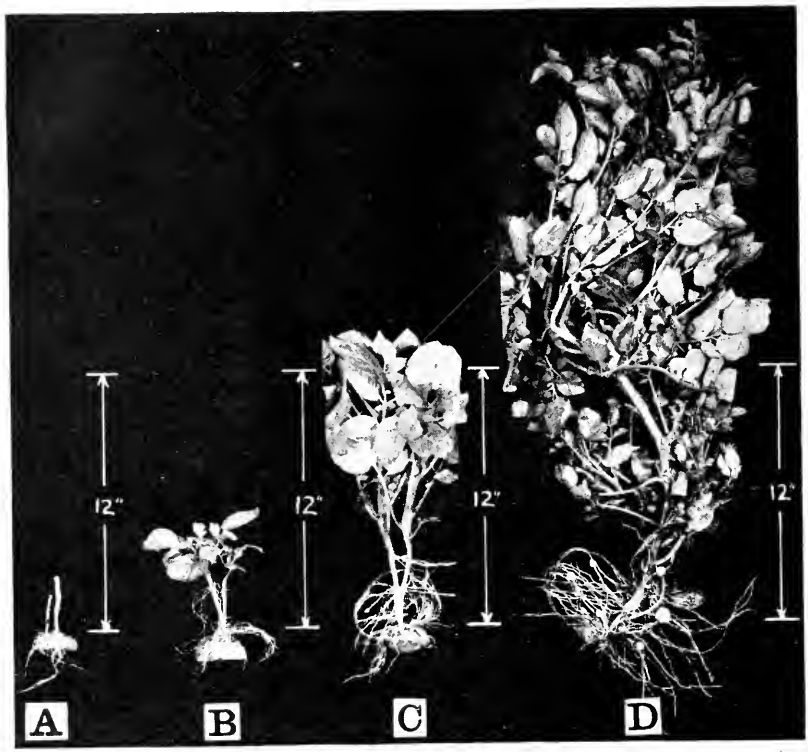

FIGURE 170. Conditions of plants at four different stages at which mother-tubers were amputated: A, stage 1; B, stage 2; C, stage 3; D, stage 4.

analyses. The data in this table are given on the basis of the percentage of the original total amount of each constituent remaining in the seed-piece at each stage of development. Large and rapid losses in dry weight, starch, and nitrogenous substances are shown. The losses are greater and earlier in Bliss Triumph than in Irish Cobbler, also greater in 14-gram seed-pieces than in 56-gram seed-pieces. Since starch constitutes a large per cent of the original dry weight, a large loss of starch — 89 per cent in Cobbler and 97 per cent in Triumph — means a large loss in dry weight, 75 and 90 per cent respectively. Moisture and soluble sugars increase in Cobbler mother-tubers throughout the growth period; but with Triumph, increase in the early season is followed by a later decrease.

What does the mother-tuber furnish the growing plant that is necessary for maximum yield? As is seen from the chemical data in Table 48, the mother-tuber ceases to be of importance about the time the main nutrients, especially starch, are exhausted. In Irish Cobbler mother-tuber, the starch is exhausted later than in Bliss Triumph; also in the former the mother-tuber has a beneficial effect on the growth of the plant for a longer period. This at least indicates that the mother-tuber may be of importance as a source of nutrients. Appleman [2] thinks growth-promoting substances

Table 48. Changes in Amounts of Substances in Mother-Tubers

Variety	Approx. size of seed pieces (grams)	Stage of development	Per cent of amount originally present				
			H_2O	Dry matter	Starch	Total sugar	Total nitrogen
Cobbler	14	Start	100	100	100	100	100
	14	Stage 2	126	59	53	167	71
	14	Stage 3	131	30	12	181	43
	14	Stage 4	143	18	4	114	29
Cobbler	56	Start	100	100	100	100	100
	56	Stage 2	118	74	80	118	79
	56	Stage 3	125	39	27	169	41
	56	Stage 4	139	25	11	187	24
Triumph	14	Start	100	100	100	100	100
	14	Stage 2	162	43	19	200	50
	14	Stage 3	130	10	2	45	17
Triumph	56	Start	100	100	100	100	100
	56	Stage 2	135	53	37	161	67
	56	Stage 3	145	23	6	133	25
	56	Stage 4	131	9	3	50	17

from the mother-tuber are of importance in promoting the growth of the daughter plant and that the mother-tuber must be of a certain minimum size in order to furnish enough of these substances. Denny's work does not disprove this claim. It is also claimed that the mother-tuber serves as a water-storage organ. Denny observes wilting in some cases after mother-tuber removal, but in the later stages of growth this did not reduce the yield. His plants were grown with adequate water. Water storage may have some significance in dry seasons.

Flower Color of Hydrangea macrophylla

The amount of aluminum available determines the color of the flowers of the common hydrangea, *Hydrangea macrophylla* DC.

Many gardeners and a number of scientists have debated the question, Why are the flowers of our common hydrangea (*H. macrophylla*) sometimes pink and at other times blue, and how is it that some flowers on certain plants the same year are pink while others are blue?

Allen [1] gives a critical review of the investigations on this question and describes a set of experiments that appear to give the answer. He states that several explanations have been offered, but that recently the question has resolved itself into whether it is an abundant supply of aluminum or iron in the flower that makes it blue, a medium supply that produces a mauve flower, and low supply that gives a pink flower. His answer is in favor of aluminum salts.

Part of his evidence is given in Fig. 171. In the upper left corner is a

branch showing a pink corymb and a blue corymb. Both were pink at the beginning but the one at the right was changed to blue by spraying it with 0.5 per cent solution of aluminum ammonium citrate. The flower at upper right was produced when the nutrient solution for the plant bore 1.34 ppm or less of aluminum. The middle left flower (mauve) was produced on a plant receiving 13.4 ppm of aluminum in the nutrient, and the middle right on a plant furnished a nutrient bearing 134 ppm of aluminum. The plant at the bottom of the plate was grown in a pot with the root system and lower part of the stem divided with a plate of glass, so the right-hand side has a lower supply of available aluminum than the left. In this way both pink and blue flowers are produced on the same plant.

It is common knowledge that when this plant is grown in nearly neutral or alkaline soil the flowers are generally pink, while those grown in acid soils are generally blue. Neutral or alkaline soils bear little iron or aluminum in solution, while acid soils bear much more.

The following statements by the author [1, p.240–241] add further evidence for his conclusions and bring out other interesting facts about the problem:

"Blue flowers from plants grown in sand cultures had an aluminum content of more than 250 ppm; pink flowers contained less than 150 ppm, while mauve flowers varied from approximately 150 to 250 ppm. Bright blue flowers from plants growing in the field usually contained 800 to 900 ppm of aluminum, although the amount varied widely depending upon the conditions under which the plants were growing. Aluminum compounds added to the soil increased the aluminum content of the flowers.

"The iron content showed less relationship to the flower color than did the aluminum. Blue flowers from plants growing in soil had a higher iron content than pink ones, but in sand cultures where the amount of iron in the nutrient solution was controlled, some of the blue flowers contained less iron than the pink.

"Flowers produced on plants grown from cuttings in soil at pH 5.5 or below were blue. Between pH 5.5 and 6.25 the flowers were intermediate between pink and blue. Above pH 6.7 the flowers normally showed no trace of blue. Blue flowers were produced on plants growing in alkaline soil at pH 7.5 or above. There was evidence that aluminum could be accumulated in the tissue and bring about mauve or blue color when the plants were transferred to neutral or alkaline soil.

"Single plants were caused to produce flowers varying from clear blue to clear pink by dividing the root system and growing the two halves in different types of soil. Wherever there was a vascular connection between the roots growing in acid or aluminum sulphate treated soil, the flowers were blue.

"Some varieties failed to produce clear blue flowers in soil made acid by the addition of aluminum sulphate. Flowers of the same varieties turned blue when aluminum compounds were sprayed on mature sepals or absorbed through a slit in the stem."

FIGURE 171. *(See text for descriptions.)*

Soil Studies

Peats and composts. McCool [38] made a study of widely different classes of peats as substitutes for the ever-diminishing supply of manure. The peats varied in mineral content from 3.45 to 53.75 per cent, in nitrogen from 1.78 to 3.35 per cent and in pH from 3.39 to 6.85. Under storage they showed considerable but variable nitrate formation. When added in large amounts along with complete fertilizers, and lime for the more acid peats, they brought non-productive mineral soils to a high level of fertility. Use of peats [39] in composts increased nitrification and plant growth, but different peats varied considerably in effectiveness. A partly decomposed, sedimentary, fibrous peat, when composted with fertilizer salts, lime, and a small amount of manure, proved to be a valuable soil improver. It was superior to the same material without composting. Straw disintegrated readily when composted with the peat mentioned above and with proper amounts of fertilizer salts and lime; it was more effective for soil improvement than equal amounts of commercial manures. A number of factors [40] were found to modify the pH of peats. In some peats increasing the water content increased the pH by 1, while in others adding water had little effect. Oven-drying of peats had little effect upon the pH when they were again soaked, although the effect was measurable in some cases. Air-drying increased the soluble salt content of some peats and oven-drying was more effective. Very acid peats were rendered far less acid by leaching with large volumes of distilled water. The less acid peats showed slighter changes. Addition of salts to peats including fertilizers increased the acidity of peats. Salts with bivalent cations were more effective than salts of monovalent cations and less acid peats were more modified than more acid ones. It took heavy applications of super phosphate and complete fertilizers to change the pH noticeably.

A study [45] was made of various methods of composting several kinds of plant materials (leaves of oak, Norway maple and sugar maple, pine leaves, salt-marsh hay, straw and cat-tail plants) and the value and best methods of applying the several composts to soils. Additions of cyanamide greatly hastened the decomposition of leaves, pine needles, and cat-tail composts. The acidity of leaf composts decreased rapidly upon standing, and salt-marsh hay composts were alkaline. Adding the uncomposted materials to soil decreased crop growth; oak leaves and some other composts also decreased crop growth if added to soils alone, but proved very beneficial if added with complete fertilizers. A domestic peat composted with cyanamide alone proved injurious; but when composted with cyanamide plus a still greater amount of acid phosphate it was beneficial to the growth of rye.

Soil solutions. Following the suggestion of Knudson and Ginsburg that the density of juice pressed out of plant tissue varied with the amount of pressure used, McCool and Youden [53] determined the acidity of successive samples of water pressed out of ground tissue of several plant organs and

several peat soils as the pressure rose. The pressure varied from that of a screw hand press to 30,000 pounds per square inch exerted by a hydraulic press and rose in steps of 1000 or several thousand pounds per square inch. The acidity fell as the pressure to squeeze out the successive water fraction rose. In the more acid peats the acidity fell as much as 3 pH, from 3.64 pH at low pressures to 6.77 pH at high pressures. The most marked fall was from minimal pressures to 1000 to 4000 pounds, but there were slight falls above 4000 pounds. In peat soils the phosphorus content of the water fractions pressed out by increasing pressures fell markedly. In a brown sphagnum peat, phosphorus content fell from 50 to 55 ppm with slight pressure to 3 to 4 ppm with 20,000 to 30,000 pounds. The phosphorus content of expressed water continued to fall noticeably even above 4000 pounds. Even a sandy loam showed similar changes in phosphorus content of water fractions as the pressure increased. The supply of acids and phosphorus was not exhausted by the pressure; for on rewetting these materials give fractions similar to those initially observed as far as the hydrogen-ion concentration and phosphorus content are concerned. The authors suggest that high pressures may form semi-permeable membranes in the materials and mention Bouyoucos' suggestion that the small capillary spaces may contain more dilute solutions. They feel, however, that the real explanation of the phenomenon is still to be learned.

Insoluble organic sources of nitrogen. In mixed fertilizers insoluble organic nitrogen sources that gradually become available during the crop season are supposed by some to have an advantage over nitrates and other soluble compounds which are subject to leaching in light soils under heavy rainfall. Such nitrogen sources also add to organic content of soil. McCool [44] has made a study of several insoluble organic nitrogen compounds as to rate of ammonification and nitrification in soils and as to their relative values as nitrogen sources for crop growth.

A patented mixture of fermented molasses concentrate, after distilling off alcohol, and calcium cyanamide, bearing about 55 per cent soluble nitrogen, was compared as such and after leaching out the soluble nitrogen with two grades of tankage. While there was considerable variation in the relative rate of ammonification of the four materials in Gloucester loam and Norfolk fine sand the two tankages early showed most rapid nitrification in both soils; but later the nitrification of tankage was no more rapid in Gloucester loam than that of the molasses mixture. The molasses mixture was equal to high-grade tankage for production of snap beans, tobacco, and corn, and superior to tankage for cotton, rye grass, and millet. Also the slower rate of nitrification of the molasses mixture should lead to lower rate of loss of nitrogen by leaching in soils. McCool also made a similar study [47] of the du Pont Urea-Ammonia Liquor-37 (UAL–37) which, when mixed with acid phosphate, potash, and other materials, forms an insoluble nitrogenous material. This proved a little less effective for millet growth in Norfolk fine sandy loam than the insoluble nitrogen of

cottonseed meal. It was, however, as valuable as cottonseed meal for the growth of millet, corn, and tomatoes in Gloucester loam. It was also superior to processed tankage. The residual (or carry-over) effect of insoluble nitrogen in UAL–37 base was greater than it was in cottonseed meal, as evidenced by the yield of a second crop of millet grown in Gloucester loam. A ground plastic molding resin bearing nitrogen proved of little value as a N-source for crops, while the nitrogen in a resin scrap proved effective. The nitrogen in garbage tankage had low value. The readiness [48] of nitrification of three organic sources of nitrogen in Sassafras sandy loam was in the order named: urea, cottonseed meal, and UAL–37. Cottonseed meal and UAL–37 base gave greater crop yields if added to the soil 68 days before planting rather than at planting time.

In greenhouse tests ground samples of kitchen wastes or garbage [50] were less effective in crop growth than tankages with equal nitrogen content but superior to cow manure and shredded stockyard manure on the same basis. The residual, or second crop, effects were greater than the manures or tankage. Incubating the wastes at 40° to 55° C (104° to 131° F) did not increase the availability of the nitrogen but it did improve the texture and disposed of obnoxious volatile materials. In field tests mixed kitchen waste proved superior to stockyard manure but both showed similar residual effects. Placing the ground wastes in the soil 30 days before planting the crop increased their value for the early growth of the crop. Kitchen wastes [49] were improved somewhat by liming, inoculating with active cultures of decomposing bacteria, and incubation at 40° C (104° F) but incubation should be discontinued when nitrogen reaches the maximum percentage and before it begins to fall.

It is evident that with proper treatment and usage a number of insoluble organic nitrogen compounds can be used both as nitrogen and as a source of organic material for soils.

Sulfur dioxide from cities. Attempts [51, 52] were made to determine the effect of SO_2 produced in burning coal in our cities on the soils and plants about the cities. St. Louis, Mo. was selected as a city producing much SO_2 due to burning high-sulfur soft coals and Philadelphia, Pa. as a city producing much less due to burning low-sulfur hard coals. Samples of soil and of various species of plants were taken from each of several soil types. The stations from which the soil and plant samples were taken ranged from the centers of the cities to localities 30 to 40 miles away. For the soils, the pH values, titratable acidity, base exchange capacity, replaceable calcium and magnesium, and sulfur content were determined and for the plants the total sulfur, sulfate, and nitrogen content. The authors conclude that soils about centers of considerable SO_2 production, like St. Louis, have not been noticeably altered in spite of many years of exposure, and that the acidulation, if any, is much less than that due to natural processes in the soil. It can be overcome by slight increases in the rate of lime application. With few exceptions the total sulfur, sulfate,

and nitrogen content of the leaves of plants did not decrease significantly with distances from the source of SO_2.

Manganese injury. Certain steam-sterilized soils[42] used at the Institute proved toxic to plants because of the high content of soluble manganese salts. Soils steamed at 240° F (116° C) showed a manganese content ranging from 384 to 22.9 ppm. High organic content seemed to increase the amount of manganese rendered soluble. Other factors that affect the solubility of manganese in soils were studied and the data obtained were discussed in relation to previous literature on the subject.

Soybeans, snap beans, and tobacco [43] were grown in untreated soil and in soil to which 400 and 600 ppm of manganese sulfate were added under four different intensities of light: full sunlight, 78, 58, and 35 per cent of full sunlight. In general, the injury to the leaves by manganese salts, as shown by brown-spotting, chlorosis, and the amount of manganese salt absorbed by the leaves, decreased as the light intensity decreased. The salt decreased the yield of plants in all light intensities, except perhaps tobacco under heaviest shading. The amount of manganese absorbed by roots and stems was much less affected by light intensity than was that of the leaves.

Colloidal phosphate. Florida pond or colloidal phosphates[46] were no more effective for crop production than finely ground Tennessee brown rock phosphate.

Fungi for determining soil fertility. Mehlich[60] (joint contribution of Boyce Thompson Institute and Tennessee Agricultural Experiment Station) extended the earlier research by himself and associates and by other investigators on the use of fungi to determine deficiency of potash, nitrogen, and phosphorus in soils for the growth of crops. Use of these organisms requires much less space and less time than the growth of crop plants. Mehlich considers *Aspergillus niger* to be well adapted to determine potash deficiency, and within limits *Cunninghamella blakesleeana* is of value in detecting phosphorus and nitrogen deficiencies.

Thallium effects. It is established that use of thallium sulfate for rodent control leaves patches of soil where it is applied incapable of producing plants for a considerable period. McCool[41] carried out a series of researches to put the knowledge in this field on a quantitative basis. He found that it was not possible to render thallium sulfate-treated soils capable of growing plants even by leaching with large quantities of water, partly because of the base exchange of thallium with calcium, aluminum, and other bases. Commercial fertilizers and lime did not lower measurably the toxicity of thallium in the soil. Crop plants were badly injured by concentrations of thallium in the soil that were too low to affect nitrification; hence it is more toxic to green plants than to nitrifying microorganisms. The nature of the soil determines to a degree the amount of thallium salt that must be added to injure plants; sandy loam requires less than silt loam and silt loam less than fibrous sedimentary peat. Very low concen-

trations of the salt in the soil injure plants growing in it; 2.1 ppm of the sulfate in the sandy loam slightly retarded the growth of roots and tops of soybean, wheat, alfalfa, buckwheat, rye grass, corn, tobacco, and tomato, while 8.5 ppm was very injurious to these plants. The first dosage would amount to 4.2 pounds per acre, considering plow depth of soil; the latter would be 17 pounds per acre, considering the thallous sulfate, and much less considering only the thallium. Wax beans were somewhat more resistant. The type of injury also varied with the kind of plant. In corn the tissue between veins of the leaves was killed; in soybeans, wax beans, and buckwheat tissue along the veins was damaged; in rye and wheat this was true all over the plant; and in alfalfa it was limited to basal tissue.

Studies on Lilium, Gladiolus, and Dahlia

Storage of pollen and hybridization. In breeding work it is often desirable to keep pollen in a fully viable condition for considerable periods in order that crosses may be made between plants flowering at different times. It is also sometimes desirable to ship pollen to distant points for breeding work. Following the earlier experiments on the effect of humidity and temperature on the life span of pollen, Pfeiffer [63, 64, 65] in order to facilitate breeding experiments on *Lilium*, hybrid *Amaryllis*, and *Gladiolus* attempted to determine good conditions for pollen storage in these genera. She later made a study of *Cinchona* pollen.[68]

The viability of the pollen was tested on a synthetic medium when possible and in some cases on stigmas of living plants. In the latter cases the development of fruits and set of seeds were the criterion of viability. The pollen was stored at 10° C (50° F) with various constant humidities of the atmosphere, at sub-freezing temperatures in gelatin capsules, and in gelatin capsules wrapped in paraffined paper without humidity control. Also the pollen was sealed in evacuated tubes and stored at various temperatures. Humidity controls were obtained by storing in desiccators over saturated solutions of various salts, as well as by using various concentrations of H_2SO_4 as well as $CaCl_2$. The following are the saturated solutions used with the relative humidities produced by each:

Magnesium chloride: 35 per cent humidity at 10° C (50° F); 33 per cent at 20° C (68° F).
Calcium chloride: 38 per cent humidity at 10° C (50° F); 32.3 per cent at 20° C (68° F).
Potassium carbonate: 44 per cent humidity at 18.5° C (65° F); 43 per cent at 24.5° C (76° F).
Potassium thiocyanate: 47 per cent humidity at 20° C (68° F).
Sodium dichromate: 52 per cent humidity at 20° C (68° F).
Magnesium nitrate: 56 per cent humidity at 18.5° C (65° F).
Ammonium nitrate: ca. 68 per cent humidity at 10° C (50° F); 64 per cent at 20° C (68° F).

For all pollens studied, thorough desiccation over concentrated H_2SO_4 or $CaCl_2$ was injurious. In general, proper humidity of the atmosphere in the 10° C (50° F) storage greatly lengthened the longevity of the pollen.

Cinchona pollen at 10° C (50° F) lived a year with relative humidity of 35 to 50, gave 3 per cent germination in 3 months with 65 per cent R. H., and 2 to 3 per cent after 6 weeks with 25 per cent R. H. *Gladiolus* pollen kept best at 10° C (50° F) with 40 to 50 per cent R. H., but degenerated rapidly in open air storage at room temperatures. Pollen of most varieties used kept 8 to 10 weeks under this condition and pollen of a few varieties showed live grains after 102 days. *Lilium* pollen kept as long as 7 months when stored at 10° C (50° F) with controlled humidity; *L. auratum* best at 35 per cent R. H.; *L. speciosum* best at 50 per cent R. H.; and *L. longiflorum* at 65 per cent R. H. Under the same conditions the *Amaryllis* hybrid pollen lived for 5 months. Under similar conditions *Lilium* pollen in gelatin capsules wrapped in paraffin paper at −10° or −11° C (14° or 12° F) kept well. Sealed vacuum storage at 5° or −5° C (41° or 23° F) was unfavorable for *Cinchona* but favorable for *Lilium* pollen. As with seeds, the longevity of pollen can be increased greatly by improving storage conditions. Constant humidity is important in both, but in many seeds much lower humidities are favorable. Likewise, lowering the temperature is beneficial in both. Absence of oxygen is beneficial in dry seeds. The evidence for this on pollen is not clear. It would be interesting to know the optimum of all three of these factors for several pollens and to learn how much the life span could be increased by putting all these factors at the optimum.

In hybridization several species of lilies show maternal characters. This is true of *L. superbum* L. and *L. auratum* Lindley. In spite of this, Pfeiffer [66] in her lily hybridization work got some hybrids with these two species as maternal parents that combined the characteristics of both parents. This was true of *L. auratum* ♀ with both *L. rubellum* Baker and *L. japonicum* Thunberg as pollen sources. The same was true of *L. superbum* ♀ with *L. canadense* as the pollen source. Intermediate forms [67] were also obtained with *L. sulphureum* Baker as seed parent and *L. Henryi* Baker as the pollen source.

McLean and McLean [54] got reciprocal crosses between *Lilium tigrinum* Ker-Gawl., noted for its sterility, and two different lily hybrids, *L.* × *umbellatum* and *L.* × *elegans*. The new hybrids showed characteristics of both parents and produced viable seeds. McLean [55] produced a bigeneric hybrid by using the pollen of the garden hybrid gladiolus "Byron L. Smith" on *Antholyza revoluta* Burm., the Rood Kapje of South Africa. The hybrids showed strongly the vegetative and flowering characters of the pollen parent, but the seed parent habit of winter growth during short daily illumination and low temperatures. The hybrids produced no viable pollen and did not produce seed when pollinated from either South African or American gladioli. McLean [56] produced a scented hybrid gladiolus approaching in stature and flower size our summer flowering gladioli. He designates it as Z–36 and describes it as "being derived on the one hand from a garden hybrid gladiolus, 'Gretchen Zang,' as pollen parent, and

from two clones of wild species, *Gladiolus tristis* L. concolor I and *G. recurvus* L. bronze as great grandparents on the other."

Dormancy in Easter lily bulbs. Thornton and Imle [88, 91] point out that for many years commercial growers of Easter lilies have been faced with the problem of dwarfed plants, or even no growth whatever, from bulbs which at the time of planting appeared to be perfect specimens free from mosaic. Immaturity at time of harvest or unfavorable storage conditions before forcing may induce this bulb dormancy. Bulbs harvested before the flowers open remained alive and later produced roots but no tops. Those harvested just after the flowers had withered produced about 50 per cent stand in the forcing house, while the other half remained dormant but sound. Bulbs harvested when fully mature (when the plants were almost dead) gave full stands when forced after storage. Storing the bulbs in conditions that led to the exhaustion of oxygen or to the accumulation of high concentrations of carbon dioxide also induced dormancy. These injurious conditions were especially detrimental on immature bulbs. A short drying period after harvest and low-temperature storage up to forcing time were necessary to avoid dormancy, the latter especially in the case of immature bulbs.

Pfeiffer [62] studied the effect of varying the cold storage period and time of planting the two lilies (*Lilium longiflorum* Thumb. var. *eximium* Nichols [*L. eximium* Courtois] produced in Bermuda, and var. *giganteum* Hort. produced in Japan) formerly used in United States for forced Easter lilies upon the date and rate of development of flower primordia. In the first kind the purely vegetative stage seen in August persisted until the middle of October in cold storage at 10° to 13° C (50° to 55° F). If continued in cold storage until November 26 there was a broadening of the apex and slow elongation of the axis indicating the pre-differentiation stage of the floral axis. In bulbs of the second kind, first obtained in early December, there was already a broadening of the apex and in cold storage at 3° C (37° F) there was slow elongation of the axis. Potting the bulbs even without change of temperature hastened the development of flower primordia. The order of development of the floral organs was acropetal.

Lily diseases and pests. Because of lack of knowledge of lily diseases and pests, a fellowship was established in the fall of 1927 by The Horticultural Society of New York, New York Botanical Garden, Cornell University, and Boyce Thompson Institute. This fellowship was continued for fourteen years, or up to 1941. The laboratory work was done mainly at the last two institutions mentioned above, and during the last five years the fellowship was supported entirely by these institutions. The fellowship was held in succession by the following plant pathologists, C. E. F. Guterman, 1927 to 1930, Keith O'Leary, 1931 to 1937, and E. P. Imle, 1937 to 1941. The work was done in close cooperation with commercial and private growers and with lily breeders in eastern United States.

In 1930 Guterman [31] summarized his work and that of other investigators

on lily troubles. The author describes and suggests methods for control of two virus diseases (mosaic and yellow flat); five fungal diseases or groups of diseases (Botrytis blight, bulb rots, rust, stump rot, and foot rot); four physiological troubles (limber neck, frost injury, non-infectious chloroses, and brown-tip of leaves); and one insect pest (bulb mite). He also mentions several other minor diseases described in the literature.

Because it is the most destructive disease of lilies, except for species and varieties that are tolerant, mosaic deserves special mention. When it attacks lily plants outside it causes complete degeneration in a year or two in some species. The disease is transmitted from diseased to healthy plants by *Aphis gossypii* Glover and spreads rapidly under conditions favorable for this insect. It can also be transmitted by intergrafting between diseased and healthy plants and with some difficulty by scratching juice from diseased plants into tissue of healthy plants. Once the disease enters a plant, there is no cure for it since it is carried by the bulb. The main symptoms are yellow mottling of younger leaves, showing three types in different species, deformation and stunting of flowers and more or less stunting of the whole plant. The main remedy is roguing out and destruction of all plants showing the disease. This must be done as soon as the disease appears on a plant in order to prevent spreading by the vector. Also the foliage takes on a deeper color when the plant sets flowers or when the temperature is high, thereby masking the mottling. Control of the insect vector by sprays of course slows the spread of the disease. It spreads faster in some species than in others, because the insect prefers these as food. It is not carried by seeds; consequently seedlings are disease-free until infected from an external source.

Although of less economic importance than the mosaic, the yellow flat or lily rosette disease of *L. longiflorum* is of interest. It is carried by the same vector as mosaic but cannot be transmitted mechanically. It causes general chlorosis of the foliage rather than mottling. It also causes marked stunting of the whole plant and rolling or twisting of the outer portions of the leaves, reduces or prevents flower set, and distorts the flower and modifies the shape and size of the bulb. Later work [13] indicates that this disease is of considerable economic importance in Easter lilies and can be transmitted by the vector to several species and varieties. The symptoms of the disease vary with the species, which makes it difficult to diagnose in some cases.

Botrytis blight is a very destructive disease, sometimes under favorable conditions ruining an entire crop in a few days. There is considerable variation between species and varieties as to susceptibility to the disease. It may attack the top of the young plant and stop further growth, and even in some cases destroy the bulb. It may merely spot the leaves. The spots may increase in size in more susceptible forms under favorable conditions until the whole leaf is destroyed and plants are without live foliage. When the disease attacks the plants in late stages, the flowers are spotted,

deformed, and may rot completely. The best remedial measure for stopping spread of the disease is removal and burning of all diseased portions of plants. In greenhouses disease-free soil should be used and plants grown at low humidities and without over-watering. Sulfur fungicides, copper lime dust, and Bordeaux are effective if the plants are sprayed before the disease starts and frequently enough thereafter to maintain a protective cover.

Besides the lily pests mentioned in Guterman's summary, the following were later studied and reported upon under this fellowship: *Penicillium* rot of lily bulbs,[61] a cecidomyid larva of lily stems,[34] bulb rots of lilies,[33] and some insect pests of lilies.[35] Some other work done has not yet been published.

Dahlia mosaic. A survey showed that dahlia mosaic[11, 12] is prevalent throughout Connecticut, New York, and New Jersey. It is probably distributed widely. All members of the genus are susceptible, but there are no suspects outside the genus. Mosaic is not synonymous with "stunt" which is applied to the less tolerant varieties that are stunted by mosaic and by certain insect injuries. The symptoms of the disease on the less tolerant varieties are chlorosis, leaf distortion, shortening of internodes and flower stems, and vein necrosis. The more tolerant varieties that are not stunted by mosaic had been overlooked as carriers of the disease by the earlier American work on dahlia stunt. The mosaic is carried in the tuber and other vegetative parts of the plant but is not transmitted through the seed; also it has not been transmitted mechanically. It can be transmitted to all varieties by grafting and manifests itself with marked or slight symptoms on the various varieties in line with their tolerance.

Myzus persicae seemed to be the specific insect vector of the disease. Other insects tested did not transmit the disease. Its symptoms appear in the plants four weeks or longer after insect transmission. Since dahlia is not a preferred food of the insect, the disease spread is at the rate of 10 to 25 per cent of the crop per year. The disease is controlled by selection and isolation of disease-free plants along with control of aphids during early growth and roguing during later growth. Tolerant varieties, if grown at all, should be isolated from those showing marked symptoms of the disease. Seedlings are free from the mosaic until infected by the insect. The infected tuber, of course, carries the disease to the plant grown from it. Ring spot, yellow ring spot, and oak leaf are three other virus diseases of the dahlia studied by Brierley. The researches indicate that they too are specific dahlia diseases.

Propagation of Trailing Arbutus and Lycopodium

Abundant moisture, protection from excessive sunlight and probably the presence of a mycorrhizal fungus in the soil are necessary for rooting trailing arbutus.[6] Vigorously growing plants bore well-developed coils of the endophytic fungus in the roots; stunted plants showed little or no fungus

in the roots. Cuttings taken in August or September and including current and previous year wood rooted readily; those taken in the spring root less readily. Live sphagnum, native soil, peat moss, and mixtures of peat moss and good sand were all good rooting media.

There was found to be a great difference in the percentage of germination in seeds taken from different fruits, the germination ranging from 0.3 to 87.3 per cent. Best germination occurred when the seeds were planted soon after harvest. In open air storage the vitality of the seeds fell rapidly. Batches of seeds that gave 45 per cent germination in July gave only 2 per cent in the 5 out of 80 lots which showed any germination after storage for six and one-half months. Arbutus is dimorphous as to style and stamens and subdioecious and hand pollination increased the average set of seeds per fruit from 241 to 403. Plants of trailing arbutus thrive over a wide range of pH; plants did well in soils with pH anywhere between 4.65 and 7.67. No observations were made in lower or higher acidity ranges.

A later study [8] emphasized again the significance of the endophytic fungus in the life of trailing arbutus. This fungus appears in the roots, stems, leaves, and fruit and on the pollen, seed, and ovule. If the fungus is not present, the growth of the plant is improved by the addition of soil from around vigorously growing plants. Seedlings develop normally if fungus is present. The root tip of this plant furnishes excellent material for studying living chromosomes.

The increasing use of several species of Lycopodiums (mainly collected in the wild) for Christmas decorations threatens the depletion of these plants. Barrows [4, 5, 7] made a study of the propagation of several species of the genus, hoping that commercial growing might protect the wild stands.

Her work with the germination of spores makes it quite evident that on the basis of present knowledge the spores cannot be used as a commercial means of propagation. After 30 months with the best cultural conditions used, 15.8 per cent of the spores of *L. complanatum* var. *flabelliforme* germinated. In 28 months *L. obscurum* spores gave 20 per cent germination. In no case did the gametophyte, prothallium, develop beyond the 10-cell stage. The problem of securing more advanced development on artificial media remains unsolved. The 10-cell stage is far short of a mature prothallium with archegonia and antheridia, and finally with the sporophyte resulting from the fertilization of the egg. While apparently some previous investigators working with other species produced mature gametophytes and sporophytes from spores, most workers have met difficulties similar to or even greater than Barrows in spore germination and growth of the prothallium.

In fact, most of our knowledge of the gametophyte of *Lycopodium* species has been gained from material collected in nature. The gametophytes are not abundant in nature but they have been found repeatedly in various stages of maturity. It is a challenge to the botanist to learn how to grow a species of *Lycopodium* from spores with the same assurance he now grows

ferns from spores. This will be of scientific interest rather than interest in commercial propagation. Barrows discusses the structure and chemical composition of the spores and the ability to adapt them to such commercial uses as toilet powder and powder to prevent castings from sticking to the sand in moulding. She found an endophytic fungus generally present in gametophytes collected in nature, and agrees with Treub, Bruchmann and others on the necessity of the fungus for gametophyte growth beyond an early stage. She grew many cultures of the fungus but was unable to identify it.

The spore coats seem to offer resistance to the germination of the spores, but other unknown factors present even greater difficulties in producing mature gametophytes. Apparently gametophytes sometimes produce antheridia but no archegonia. These of course cannot produce sporophytes.

The sporophytes of *Lycopodium* can be propagated by cuttings or bulbils. Spring cuttings of *L. clavatum* L., *L. complanatum* var. *flabelliforme* Fernald, and *L. obscurum* L. did better than fall cuttings. Fall cuttings of *L. lucidulum* Michx. rooted well. *L. lucidulum* can be propagated from bulbils, two crops of which are produced annually under greenhouse conditions. Young apical growth of rhizomes roots readily; older, lignified material roots poorly. *Lycopodium* needs well-watered but well-drained soil and, except for *L. complanatum*, some protection from direct sunlight. They grow in soils that range from 5.28, or perhaps lower, to 8 pH. An endophytic fungus is found generally distributed in the roots and old parts of the rhizome of all species studied. The fungus was cultured from several species but not identified.

Two Studies on Physiology and Cytology of Fungi

After having found that when opposite sex strains of *Neurospora sitophila* (Mont.) Shear & Dodge are grown in opposite arms of U-tubes filled with nutrient agar no fusion of the two strains occurs to form perithecia until the agar dries and cracks to supply oxygen at the base of the tube. Dr. B. O. Dodge suggested to Denny [19] that he determine the minimum oxygen pressure necessary for filament fusion, perithecia formation, and growth of the mycelia. At room temperature perithecia formed in 1 to 2 per cent oxygen by volume, but reducing the oxygen to 0.5 per cent by volume inhibited perithecia formation for at least 30 days, the duration of the experiments. At 10° C (50° F) no perithecia form in any oxygen percentage after 30 days, and only an occasional one at 15° C (59° F). Perithecia are formed somewhat less readily at 31° C (88° F) than at room temperature. Growth of the mycelium occurs under much lower oxygen pressure; 0.3 per cent or higher gives rapid growth of the mycelium, but the rate of growth diminishes as the oxygen percentage falls below this. At 0.01 per cent oxygen the growth was very slow, but complete inhibition of growth occurs only when the atmosphere over the culture is held free of oxygen by alka-

line pyrogallol. In 0.3 per cent oxygen 32 per cent CO_2 retards the growth of the mycelium only slightly.

Backus [3] studied *Coccomyces hiemalis* Higgins, the cherry leaf-spot pathogen, throughout its life history in the cherry leaf, and also in artificial culture. Special emphasis was placed on the mechanism by which the ascocarp originated, whether by heterothallism or homothallism. Microconidia were found attached to trichogyne-ends but fusion was not observed, nor could microconidia be germinated. The disposition and behavior of the structures led to the conclusion that the ascocarp is homothallic. The results are fully discussed in relation to the work of many investigations of autonomous Ascomycetes, lichens, Rhodophyceae, and various fungi in which similar problems appear.

Effect of Nitrogenous and Carbohydrate Reserves on Growth of Seedlings

Reid [69, 70, 71, 72] varied the relative amount of nitrogenous and carbohydrate material supplied to growing seedlings first by selecting high and low protein and high and low carbohydrate seeds, by removing CO_2 from the air supplied to growing seedlings, or by raising the CO_2 in the air more than ten-fold to 0.4 per cent, and by withholding nitrates from or adding them to the nutrient solutions. High oil reserves in seeds were aligned with high carbohydrate reserves. In the case of wheat and corn it was possible to get high and low protein and high and low carbohydrate varieties within the same species. Other seedlings used in the studies were: sunflower, tomatoes, barley, peas, beans, soybeans, and muskmelon.

In general, when the reserve available to the growing seedling was high in carbohydrates in proportion to nitrogenous material, the roots were large in proportion to the tops; and when the nitrogenous reserves were high in proportion to the carbohydrates, the tops were large in proportion to the roots. High CO_2 concentration in the air increased the carbohydrate supply, and nitrates in the nutrient solution increased the nitrogenous reserve. Seedlings from seeds with high carbohydrate but low nitrogen reserves give more increase in growth with nitrate addition than with extra CO_2 in the air; and the reverse is true of seedlings from seeds with high nitrogen in proportion to carbohydrate reserves. Seedlings with low nitrogen reserves and without nitrates added to the nutrient solution tend to accumulate much carbohydrate and have thicker-walled xylem vessels and greater lignification of bast fibers. Contrary to other investigators, the author finds that excessive amounts of carbohydrates in seedlings limit growth and chlorophyll development unless nitrates are added to the nutrient solution.

Analytical Methods

Ashing plant tissue. Ashing plant tissue [75] in a combustion tube furnace under one pound oxygen pressure at 450° C (842° F) for 8 to 16 hours

gives higher ash content and better checking duplicates than ashing in a muffle furnace for the same time at 650° C (1202° F). This method of ashing leaves no residual carbon and volatilizes none of the chlorides except magnesium chloride, whereas the higher temperatures necessary in the muffle furnace volatilize chlorides of potassium and calcium and lead to loss of the metals themselves by sublimation. This method also eliminates the necessity of adding chemicals to further the ashing, such as sulfuric acid, which gives too high an ash value by changing the chlorides and carbonates to sulfates. The authors describe the method in detail and give the precautions necessary for avoiding explosions.

Determination of reducing sugars. The permanganate method for estimating reducing sugars is used extensively in biological work. It was considered necessary to standardize the potassium permanganate against cuprous oxide produced by a known quantity of pure glucose, since it was claimed that the use of oxalic acid, sodium oxalate, or iron as a standard gave results which were too low.

Kraybill, Youden, and Sullivan [36] standardized the permanganate with Bureau of Standards sodium oxalate in acid solutions and also with weighed samples of cuprous oxide prepared by reduction with invert sugar, and obtained excellent agreement. The amounts of copper found by the permanganate method were closely checked by direct electrolytic determinations of the copper.

Capillary glass electrode. The complex and unstable nature of many biological fluids, such as plant sap, milk, and blood, complicate the task of determining the hydrogen ion concentration of these liquids. In many cases it is a great convenience to use extremely small samples of liquid. This is true for work with insects or small localized regions in plants. The glass electrode possesses the important advantage of eliminating the possibility of altering the sample, as may happen by bubbling hydrogen through the liquid with the hydrogen electrode or adding quinhydrone for the quinhydrone electrode. The action of the glass electrode depends on separating the test solution by a thin glass membrane from a reference solution. A glass electrode [102] in the form of a fine-bore capillary tube with very thin walls was found to possess, besides the usual desirable features of the glass electrode, the further advantages of employing less than 0.01 cc of sample and exposing only a minute surface of the liquid to the air.

Experimental Planning and Statistics

The variability of biological material has always been a source of difficulty in biological experiments. In chemical and physical experimentation the materials are usually more homogeneous and the environment more easily controlled and reproduced. Various methods are open to the biologist in order to deal with this variability, which in itself is often a characteristic of considerable interest. Among these methods the first to be used was the employment of extensive replication of the samples and of the

measurements made on these samples. A second method is to take great pains to secure homogeneous material by selecting from a large supply a relatively homogeneous portion and discarding the remainder. At the same time much effort may also be expended in the control and regulation of the environment, thus providing uniform conditions in the relatively large spaces often required. Finally, in the last two decades there has been a rapid development in experimental planning and statistical methods. The essence of this approach lies in the division of the experimental material into parts, each relatively homogeneous within itself. As a consequence, the parts now differ substantially among themselves. In the simplest form each part constitutes a complete experiment, there being as many replications as there are parts. It has been found that the marked differences which exist between the parts do not contribute to the experimental error. Subject to appropriate randomization of the comparisons within the parts, statistical techniques exist which provide for a valid estimate of the precision attained. The precision attained by this technique is substantially that which would have been possible if all the material had been as homogeneous as the material *within* the parts into which the initially heterogeneous supply was divided. This procedure, and more or less complex modifications of it, has been very widely employed in recent years, and it is evident that some understanding of these developments in experimental planning is becoming of increasing importance in many fields of work.

The papers mentioned in this section, while usually concerned with a specific inquiry, also constitute examples of the application of experimental design and statistical analysis. A field trial [104] of fiber pots which could be buried and allowed to disintegrate and possibly give the seedling an added advantage over the usual practice was arranged on a somewhat complex plan. In all, there were 40 different combinations of pot type, pot size, soil, and variety of tomato. Based upon early field technique, considerable difficulties would be expected from employing so many treatments. The use of a split-plot Latin Square arrangement in the field made possible the detection of an effect of pot diameter on the yield of tomatoes which was of the order of 6 per cent. The plants in the most successful type of pot showed a large gain over the plants grown in flats.

From one point of view, a field trial may be regarded as a sampling process. The field is divided into plots which may be thought of as samples of the field and which it is hoped will be closely similar except as treatments are deliberately applied. In consequence the same statistical processes are valid in appraising a sampling technique and they provide an approach to determine the number of samples needed for a specified precision. Youden and Mehlich [103] tested the efficiency of different spacings in soil sampling and found that, for surveying large areas, intervals as small as 10 or even 100 feet between replicate samples were inefficient. For a given

number of samples larger spacings would give more information about the area.

Another example of sampling technique is discussed in a paper [92] on the determination of arsenic spray residues on apples which concludes that the sampling variation of the apples was in excess of the analytical errors of the Gutzeit analytical method.

The carrying over of the identical experimental designs used in field studies of crops to experiments in pathology is reported in a series of papers.[94, 95, 99, 100] A method for measuring the concentration of mosaic virus makes use of the appearance of local lesions on *Nicotiana glutinosa* L. leaves. In a given batch of plants the top leaves form a class which has an average susceptibility which is different from the average for the leaves in the second (or some other) position on the plant. Cutting across these classes, the leaves on a given plant form a family such that all the leaves on it are more (or less) susceptible than leaves in corresponding positions on some other plant. Thus the whole assembly of leaves can be divided into regions or parts in which a certain degree of homogeneity of response exists; by planning the experiments with this in mind a three-fold increase in the precision of comparisons was attained.[99]

A statistical study [100] of the existing literature on the relationship between virus concentration and the number of lesions produced on the leaves led to the formulation of a mathematical relationship between these quantities. Further experiments [95] undertaken with a view to getting improved data to test this equation led to a novel experimental design — a Latin Square with missing rows.[94] This arrangement (also called Youden Square) was found especially adapted to greenhouse trials,[97] since fewer replications are required than in the Latin Square design.

Two other papers illustrate applications of statistics. A statistical analysis [93] of a considerable body of data obtained in seed germination tests showed how the Chi-square test can be used to check the sources of variation in seed-testing methods. In connection with studies of the effect of SO_2 on plants, a record of the SO_2 concentration in the atmosphere was available at half-hour intervals for a year. The concentration of the SO_2 showed wide fluctuations,[98] with pronounced weekly and annual cycles. By means of a linear regression technique the SO_2 concentration was found to be related to the wind direction and velocity, the temperature, and the rainfall.

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