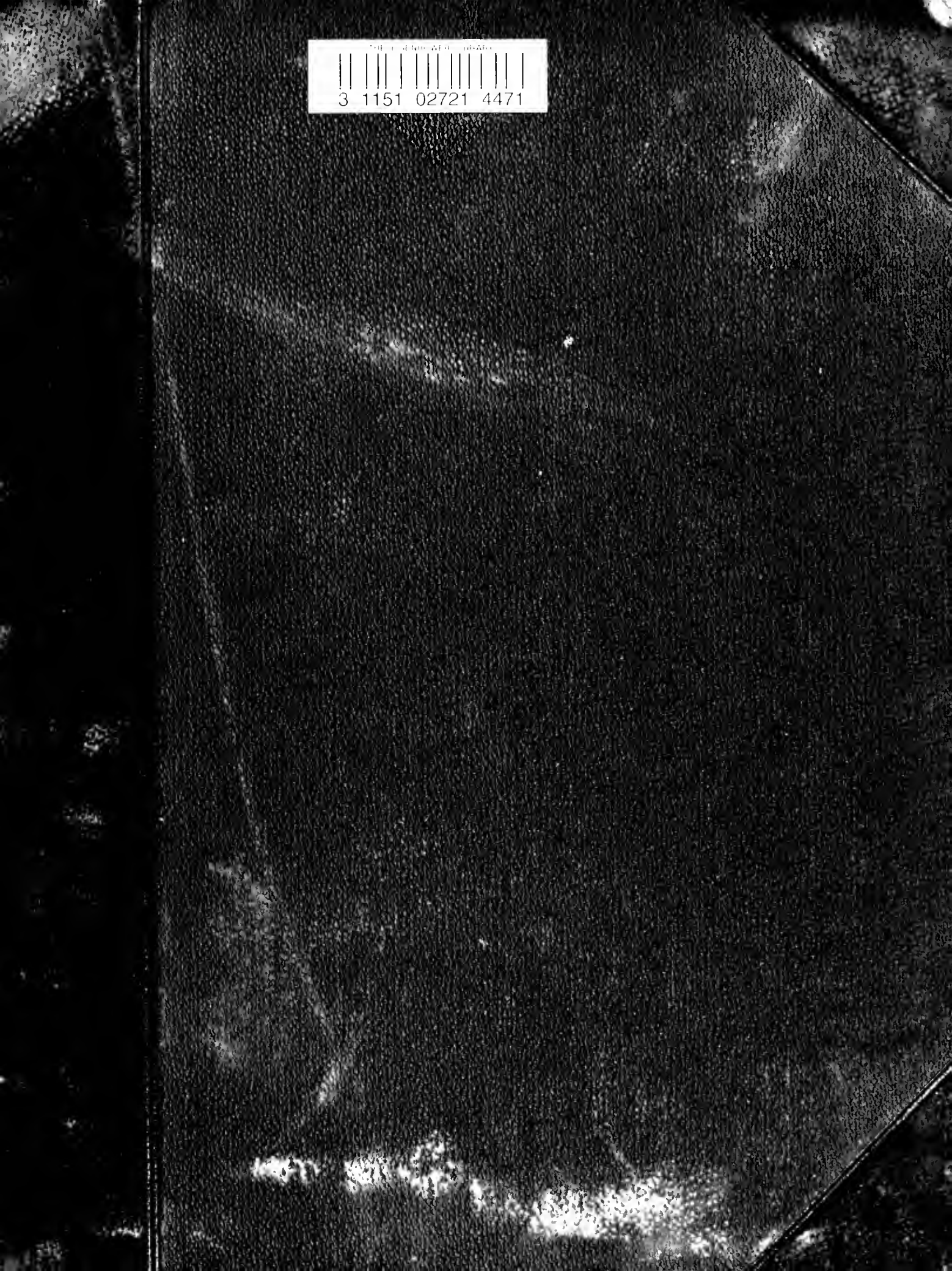

THE TEMPERATURE RELATIONS OF GROWTH IN CERTAIN PARASITIC FUNGI (1)

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INTRODUCTION

It is commonly recognized that, of the many different and varying conditions that affect life processes, temperature is one of the most important. The range of temperature at which certain important physiological processes may occur at all is relatively narrow, and comparatively slight temperature changes produce marked effects upon the velocity of other processes having more extended ranges. Although many biological investigators recognized the great importance of this subject, the more detailed study of the effects of maintained temperatures on vital processes awaited the development of simple, adequate and inexpensive methods of artificial temperature control. In the earlier investigations on temperature effects upon organisms it was often impossible to maintain the desired temperatures constant throughout sufficiently long periods of time to get results that might be considered as related to maintained temperatures. In recent years a rapidly increasing number of papers reporting investigations on the effects of maintained temperatures upon different physi-

(1) Botanical contributions from the Johns Hopkins University No. _____

ological processes is an indication that more attention is now being given to this subject. There is still, however, a great lack in our knowledge in this field especially as regards plants. On certain animal processes somewhat more work appears to have been done, though even in this field much also remains to be accomplished.

It should be remembered in this connection, also, that the subject of the temperature responses in living things involves more complicated problems than those just suggested as having to do with maintained temperatures. Most organisms (aside from warm blooded animals) are never exposed, in nature, to maintained temperature for any considerable period of time ; their temperature environment is practically always in a state of flux. From this it follows that a knowledge of the relation holding between maintained temperatures and vital processes, no matter how thorough such knowledge might be, cannot be expected to be of immediate value in the interpretation of physiological processes going on under natural conditions. Before any great advance may be made in the study of temperature influences on such processes suitable methods will need to be devised for dealing also with rate of temperature change as an environment condition, aside from the degree of temperature itself. The experimental aspect of this phase of physiological and ecological temperature relations remains practically untouched as yet. It is almost unmentioned in the literature as a serious consideration, although MacDougal (2)

(2) MacDougal, D.T., The auxothermal integration of climatic complexes. Amer. Bot. 1 : 188-193, 1914.

has called attention to its great importance. It is clear, at any rate, that the problem of temperature influence upon organisms falls readily into two fundamentally related, but superficially different, portions, one dealing with maintained temperature and the other with fluctuating temperatures. Practically all of the controlled experimental work thus far published deals with the first portion of the problem and it is in this same catagory that the present investigation lies. Indeed, it seems unwise to attack the problems related to fluctuating temperature until a more thorough appreciation has been gained concerning the general principles underlying the influence of maintained temperatures upon vital processes. It was with the aim of throwing additional light on some of the principles underlying the effects of maintained temperature on the growth of certain fungi, that the investigation reported in this paper was undertaken.

Filimentous fungi were used, because they are comparatively simple organisms whose growth rate may be easily measured, because they lend themselves readily to culture in darkness, and because each cell being in direct contact with all features of its environment, their relation to their surroundings is simple and close. The four forms, Pythiacystis citrophthora Sm. and Sm., Phytophthora terrestria Sherbakoff, Phomopsis citri Fawcett, and Diplodia natalensis Evans were used, all of them being parasitic on citrus trees. These were known to grow well on certain prepared media and some evidence was at hand showing that they differed from one an-

other as regards their temperature relations.

Another reason for selecting these four citrus parasites, was the suggestion that their pathogenic activities might be influenced by climatic temperature conditions. It was thus possible that a study of their temperature relations might throw light upon methods of combatting them. General observations in connection with many diseases due to plant parasites have indicated that temperature is a very important factor in their prevalence in any given season or in any given region. ... E. Stevens(3) has shown that the rate of increase in diameter of chestnut blight cankers is closely related to temperature. Edgerton(4) has emphasized the apparent relation of temperature conditions on the occurance of certain plant diseases in subtropical climates. He is convinced that the absence of anthracnose in beans grown at certain seasons in Louisiana is due to the fact that the average temperatures for the seasons when the disease is ~~so~~sende are too far above the optimum for the growth of the pathogenic fungus. The writer(5) has previously referred to the limited geographical distribution of melanose due to Phomopsis citri, one of the fungi here studied, and has suggested that temperature conditions may be among the important

(3) Stevens, N. E. The influence of temperature on the growth of Endothia parasitica. Amer. Jour. Bot. 4 : 112-118, 1917.

(4) Edgerton, C. W. Effect of temperature on Glomerella. Phytopathology 5 : 247-259, 1915.

(5) Fawcett, H. S. The geogrophical distribution of the citrus diseases, melanose and stem-end rot. Johns Hopkins University Circular, March, 1917, p. 388-391.

factors limiting its distribution. Tisdale(6), W. H., has shown, for Fusarium wilt of flax, that the temperature at which the host is most injured by the disease corresponds to that for maximum growth of the parasite in cultures. For many parasitic organisms it is probable that the temperature range within which serious infection of their hosts may occur naturally, is comparatively small. This, as well as differences in moisture conditions, may largely account for many of the striking differences observed in the occurrence of many plant diseases from season to season and from one region to another. Many other observations aside from those given above might be mentioned in this connection, but it seems to be clear enough that the pathological or agricultural point of view demands much more thorough going knowledge than we yet have, concerning the temperature relations of parasitic fungi. It was thought that the results obtained in the present study might ultimately be of value in pathological work.

Considering the limited time available for this study, it appeared better to confine the experimentation to the four forms mentioned above, and to subject the results to a critical study than to include a larger number of forms, with the accompanying necessity of treating the results in a more superficial manner. Our knowledge regarding the physiology of fungi, as well as that regarding plant temperature relations, should be increased first by intensive studies of a

(6) Tisdale, W. H. Relation of temperature to the growth and infecting power of Fusarium Lini. Phytopathology 7 : 356-360, 1917.

few forms. After the main principles have been worked out for these selected forms it may become largely a matter of routine to compare a large number of organisms with respect to the principles previously worked out. The four fungi here to be considered offered opportunities for intensive study, and they also furnished valuable material for comparisons.

As naturally follows from the general concept of conditional control of physiological processes(7), the relation of the process studied to any given condition or set of conditions are themselves determined by the remaining conditions. For example, if the temperature relations of a given organism are to be dealt with they must necessarily be stated along with as definite a description as possible of the non-temperature conditions that are supposed to be effective. To state that the mycelial mat of a given fungus was observed to enlarge more rapidly at one temperature than at another means little unless it is also stated just what sort of medium was employed in the test, just what was the length of the time period, just what relation this time period had to the beginning of the test, just what the radiation conditions were, etc. By altering the non-temperature conditions, the relation of a given process to different maintained temperatures may be profoundly altered. To illustrate still more concrete-

(7) Verworn, Max. Kausale und Konditionale Weltanschauung. Jena. 1912.

ly Lehenbauer[3] found that the optimum temperature for elongation of maize seedlings in his experiment was 30° C when the exposure period was 3 hours, while the corresponding optimum temperature for an exposure period of 12 hours was 32° C. If Lehenbauer's twelve hour period of exposure be divided into four periods of three hours each, and the optimum temperature calculated from his data for each of these four successive periods separately, they are found to be 30, 31, 31 and 32° C respectively. Obviously, any physiological process must be regarded as controlled by all the effective conditions acting together. Now the conditions that influence the rate of growth of a fungus in a culture may be roughly classified into five groups as follows :

(1) The nature of the fungus, which implies its internal conditions, everything that goes to make it that particular organism which it is. This set of internal conditions is vaguely and partially indicated by the name of the fungus, with an implied morphological description of its form and development, to which the name refers. But it is well known that the same species of fungus may develop quite different complexes of internal characteristics under different sets of environmental conditions. For this reason it is commonly stated that a knowledge of the recent history of the organism is necessary, as well as information regarding its taxonomic status. The mere morphological description

(3) Lehenbauer, P. A., Growth of maize seedlings in relation to temperature. Physiol. Res. 1 : 247-288, 1914.

of taxonomy is of so little value physiologically, that most students have long realized the great importance of giving definite information concerning the previous history of their experimental organisms.

(2) The nature of the medium, implying all the physical and chemical properties of the space about the hyphae, their environment. If the fungus is partly in the air and partly in a liquid or gel medium this set of conditions requires separation into two corresponding groups. For the most part, the conditions of the medium (aside from temperature and radiation) involve the concentration of numerous chemical substances such as oxygen, carbon dioxide, starches, sugars, acids, inorganic salts, etc.

(3) Temperature conditions. Since the temperature of the hyphae follows closely that of the medium, and since the latter follows the temperature of the more distant surroundings of the culture, it is conventional to consider the temperature of these surroundings as constituting a condition in itself. After all, it is the temperature of the fungus hypha that directly influences its rate of growth, not that of the medium, culture dish or chamber about the dish, etc. But since the temperature of these spaces are all practically the same this last distinction has generally been ignored. The temperature condition for two cultures may differ in several ways. If they are maintained temperatures they may differ in degree or intensity alone, and we may express them in terms of degrees on some thermometer scale. If they are

not maintained temperatures they may differ (a) as to the particular temperatures with which the cultures were started, (b) as to the direction of variation during a given period (whether the temperature became higher or lower with time), and (c) as to the time rate of temperature variation. It is clear that this rate of change in temperature may itself be constant, or may vary throughout a given time period. When only maintained temperatures are to be considered as in the present study, the only difference to be dealt with between any two cultures are those of degree or intensity as measured in terms of centegrade, etc., degrees.

(4) Radiation conditions, involving the various groups of wave-lengths of radiation and the relative and absolute intensities of each group. Up to the present time most biological discussion has ignored most of the wave-lengths of radiation excepting the small group commonly designated as light. Since the cultures of the present study were uniformly carried out in darkness and in chambers around which a mass of water was continuously rotating, radiation conditions will not require attention here.

(5) The duration condition, implying the length of time during which the organism is subjected to the other conditions. From one point of view every condition has a duration factor, but when most of the conditions are maintained, or practically so, the duration factor is common to all, and we may regard it as a separate condition. Moreover, as far as the present investigation is concerned this

duration condition may be divided into two parts, each one of which may be considered as a separate condition : (a) the actual length of any interval of time being considered, and (b) the location of this time interval serially in the entire culture period reckoned from its beginning. If the time period is always reckoned from the beginning then the second part of this duration condition may be neglected and only the length need be considered, as is done in the first part of the discussion of the present investigation. When, however, changes in rate of growth with time are being studied, the location of the time interval as well as its length come to be important, and these may be regarded as two different conditions and this is done in the latter part of the discussion. To illustrate these further, a certain fungus, Pythiacystis, (condition 1), is surrounded by nutrient agar (condition 2) and subjected to a maintained temperature of 23° C (condition 3) in darkness (condition 4) and it exhibits a growth rate of 7.8 mm. per day for a period of two days (condition 5) when this period is, in relation to the initial moment of exposure, the first (condition 6). If this experiment is repeated with everything the same excepting that condition 5 (the length of the observation period) is the first 3 day period instead of the first two day period, then the mean growth rate per day is 8.0 mm. which is markedly different from that shown in the first case.

In the examples just given the observation periods, though of different lengths, had the same relation to the

beginning of the culture period, that is, they began from the moment of inoculation. An observation period, however, need not begin with the beginning of the culture period and may not be continued to the end of the culture period. Thus two observation periods may be numerically alike, say 2 days, but they may still have entirely different relations to the beginning of the culture period, so as to constitute, in a sense, distinct duration conditions. For example, all other environmental conditions being supposed to be maintained, we may determine the growth-rate for the 2-day period, beginning with the second day after inoculation and also for the 2-day period beginning with the fifth day after inoculation. The mean daily rates derived from these two observation periods are very apt to be markedly different. Of course this state of affairs is to be related to changes that go on within the organism, with the lapse of time, even though all physical and chemical environmental conditions are assumed to be maintained without alteration. The organism is not exactly the same at the moment of inoculation of a culture as it is a day later, four days later, etc. This consideration introduces one of the most perplexing features of the whole study of maintained temperatures as related to vital processes, and considerable attention will be devoted to it in the later sections of this paper.

From the points mentioned in the preceding paragraphs it is, of course, clear that no very definite knowledge of the various environmental influences, as they act to control the

physiological processes of any organism, may be expected from biological tests in which any of the effective conditions are allowed either to vary or to differ in unknown ways. As long as the conditions differ only in known ways from one culture to another, or as long as they vary only in known ways in the same culture, there is hope of advancing our knowledge of environmental influences. The present study was planned so as to approach as far as possible the fulfillment of these general needs, as will be brought out in the next succeeding paragraphs.

In order that each fungus should always have approximately the same internal characteristic at the beginning of all the experimental cultures, the four species were kept in stock tube cultures with ordinary corn-meal agar, and at a temperature of about 16-18° C. From these primary stock cultures inoculations were made, at frequent intervals, on agar plates kept at a temperature of about 18° C. These formed the secondary stock cultures. The marginal region of the mycelial disk of a secondary stock culture (about 5 days from inoculation) furnished material for inoculating the experimental cultures of that fungus. From various lines of evidence (especially that of experiments repeated after long intervals) it may safely be stated that the inoculation material for any one of the four fungi was always practically the same, as to internal characters (tone, vigor, etc.) when the experimental cultures were started. Practically the same amount of inoculation material was always

transferred to each experimental culture.

It is consequently safe to suppose that all experimental cultures of the same fungus were practically alike at the beginning no matter when they were made.

The four fungi used furnish for the whole study four different sets of initial complexes of internal conditions. Progressive variation in the internal conditions of the fungus is one of the features taken in consideration, and will receive attention in a later section.

Turning to the environment, while several different media were employed in certain aspects of the experimentation, only one (corn-meal agar) will be considered in the present paper. Special precautions were taken to have this medium always the same at the beginning of all cultures, no matter at what time they were started. The consistancy of repetition showed that this aim was practically attained. It was also shown (by special evidence to be brought forward later) that the unoccupied medium did not considerably alter during the period of any single culture. It, therefore, seems safe to assume, not only that the medium was always the same at the beginning of all cultures, but also that it was practically unaltered during the progress of the cultures at least until it was reached and passed by the enlarging weft of hyphae. That the part of the medium immediately occupied by the developing mycelial disk, was altered by the influence of the fungus is, of course, highly probable, and if such changes had any influence on the subsequent advance of

the outer margin of the mycelial mats in this study they could not be separated from what appeared to be internal changes in the fungus itself. This consideration will become clearer in the sequal.

Since the elongating hypae of the fungi lie largely near the aerial surface of the agar plate, while some are partially in contact with the air-space above, it is necessary to consider the aerial environmental conditions, as well as those within the agar medium itself. Aside from temperature, the air conditions in the culture dishes above the agar were sensibly the same in all cultures at the start, excepting that the vapor tension of the water was, of course, different for cultures exposed to different temperatures. The air-space of the culture dish was always practically saturated with water vapor. But the culture dishes could not be hermetically sealed because it was desired to maintain practically unchanged the original oxygen and carbon dioxide concentrations in and about the agar. This, of course, allowed to take place a slow escape of water vapor from the dishes and consequently a slow evaporation from the agar surfaces during the culture period, the rate being somewhat greater at higher than at lower temperatures. The consideration just mentioned shows that the conditions of the medium were not <u>strictly</u> maintained throughout the culture period, but that different maintained temperatures were automatically accompanied by different rates of variation in the water content of the yet unoccupied medium. Such variation,

however, may be neglected in this case since it was shown by special tests that variations, even larger than those which actually occurred in the experimental cultures had no appreciable influence on the rate of growth of the fungi.

Two other aerial conditions standing in direct relation to the effective conditions within the medium are oxygen concentration and carbon-dioxide concentration. The growing fungus tends to decrease the oxygen content of the medium and to increase the carbon-dioxide content. At the same time gas exchange between the agar and the air-space above it tends to offset these two effects of the fungus upon the medium, since oxygen would tend to enter the medium from the air space while carbon dioxide would tend to escape from the medium into the air. The air of the culture dish being in direct connection with the external atmosphere it is improbable that it became markedly different from the latter in regard to these gases in the short time employed. It was not, however, experimentally considered in the present work. Whatever variation in the oxygen and carbon-dioxide concentration of the medium that may have occurred in the various cultures, are here neglected as far as the analysis of environmental conditions is concerned. Such variations, if effective, appear in the results as evidence of internal variations in the organism. Looked at in this way, if the growing fungus alters its own chemical surroundings (and it may alter the medium with respect to many other substances besides oxygen and carbon-dioxide) this effect may, for the

present be considered as an internal variation, just as though enlargement of mycelial disks were accompanied by a progressive increase or decrease in enzyme content of the terminal cells at the margin of the disk ; such variation in the cell itself would be a clear case of internal variation, change in the nature of the fungus itself.

As has been said, the temperature conditions were always artificially maintained, with a very small degree of fluctuation throughout any given culture period. They differed, of course, from experiment to experiment, but they did not vary appreciably in any case. Thus the temperature conditions for any culture are clearly and definitely stated by giving merely the temperature index as read on the thermometer scale.

Radiation conditions are regarded as non-existent in these tests. Light (and radiation of still shorter wave lengths) was always excluded, and the stirring apparatus operated to prevent any one-sided action of long-wave radiation upon the cultures.

The duration condition offers no particular difficulty in such work as this. If the culture period or observation period for any culture is different from that for another this fact is quantitatively shown by the records of times of inoculation and times of observation and measurement. Therefore differences in this condition are easily taken into quantitative account in the interpretation of growth differences between different cultures, in terms of their length of

observation period and the time location of this observation in relation to the beginning of the experiment considered to be zero time.

Since the experimental cultures are all regarded as alike at the time of inoculation, the duration conditions may be regarded as beginning to operate from the beginning of this culture period, the time of inoculation being considered zero time. If either the length of the culture period or the time between observations for any culture is different from that for another, this fact is of course quantitatively shown by the records of times of inoculation and times of observation and measurement. Therefore differences in these conditions are taken into account in the interpretation of growth differences in terms of length of observation periods and also in relation to the time location of this observation period to the time of inoculation regarded here as zero time.

From the preceding discussion it will be observed (1) that the research at hand was so planned as to involve the actual or assumed maintenance, during the respective culture periods of all the groups of effective conditions excepting internal ones, and (2) that the only conditions considered as effectively different from culture to culture are (1) nature of the fungus used (initial internal conditions), (2) rate and direction of internal variation, within the organism, and (3) maintained temperature.

For any given culture all the effective environmental conditions are assumed to remain the same throughout the culture period. For this given environmental complex, the

variations in growth-rate are here considered as due to internal variations, with time, and these are to be studied with reference to the duration factors. As to internal conditions, two cultures may differ (1) with respect to the initial status of these conditions (nature of the inoculating material), and (2) with respect to the rate and direction of internal changes going on during the development of the mycelial mat. Since the initial internal conditions of any one fungus was the same, there enter into this study only four sets of initial internal conditions, (the initial internal conditions being here understood to mean the conditions at the time of inoculation). These four correspond to the four fungus species employed.

The following scheme may serve to show the sorts of terms that enter into the interpretative comparisons that need to be made.

(Scheme)

Comparison of cultures of the same fungus

I. Internal conditions (nature of fungus)

1. Initial conditions, alike for all cultures.

2. Direction and rate of internal variation during culture period, may be different from culture to culture. This variation always to be stated as within the limits presented by (1) the initial internal conditions and (2) the external conditions.

II. External conditions (environment).

1. Initial environmental conditions except temperature considered alike for all cultures.

2. Initial temperature conditions different from culture to culture.

3. No environmental variation ; all environmental conditions assumed to be maintained at their initial values throughout culture period. Hence no difference between cultures in this regard.

Comparison of cultures of different fungi.

I Internal conditions (nature of fungus)

1. Initial internal conditions, different for four different fungi, each set roughly defined by fungus designation.

2. Direction and rate of internal variation during culture period may be different from culture to culture. This variation always to be stated as within the given limits of initial internal conditions and external conditions, as above.

II. External conditions (environment)

1. Initial environmental conditions excepting temperature considered alike for all cultures.

2. Initial temperature conditions either alike or different from culture to culture.

3. No environmental variations (see above), hence no difference between cultures in this regard.

The study here reported is thus seen to comprise five different studies. The influence of maintained temperatures on the growth rate of each of four fungi was measured, under the given non-temperature conditions which are considered as initially alike and under the given initial internal conditions, also considered as alike for all cultures of the same fungus. The fifth study comprises the comparison of the four fungi as to their temperature relations under the given set of non-temperature, external conditions.

Looked at from the mathematical viewpoint, the present investigation involves six ——————————

possible conditions as determining the observed growth rate in any case ; the growth rate is thus regarded as a function of six independent conditions , initial internal conditions (a), initial conditions of the medium (its physical and chemical properties (m), initial radiation conditions (d), the length of the observation period (p) and the location of this observation period, or time interval, serially from the moment of inoculation (n).

It may therefore be said that the growth rate (G) is a function of these six corditions a, m, t, d, p,& n, G = f (d, m, a, t, p, n). The four fungi present four different values of a. Only one value of m was encountered[2], and only one value of d was tested (almost complete absence of radiation). A number of values of t (maintained temperature) were tested. A number of values of p were considered, one, two, three, four, five and six days. Five different values of n (the first to the fifth 24 hour period inclusive) were considered. Since two of the conditions (d, m) were studied by means of only a single value of each, these two were always constant and we may say - that the growth for the medium used (m), and for the culture without radiation (d), with a time period of 24 hours (p), is a function of the kind and condition of the fungus used (a), the maintained temperature (t), and the location (n) of the time interval considered in relation to the moment of inoculation.

If G' denotes the growth rate with the given medium, with radiation excluded, we may write G' = f (a, t, p, n), it being understood in this and the following statements that

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[2] Changes in the medium during the growth of a fungus will be considered later for m is the initial condition of the medium.

the magnitude of the growth is also dependent on the constant conditions.

If we study this growth for any given fungus for any given length of period, the quantities a and p also become constant so that G" = f (t, n) where G" denotes a growth rate of fungus a with the given medium m, during a given length of period p, without radiation d. If we further study the growth rate during a period with a given relation to the moment of inoculation, n also becomes constant so that G"' = f (t) where G"' denotes the average growth rate of fungus a with the given medium, without radiation, during a given length of period when this period holds a given relation in time to the initial inoculation moment.

If all the possible combinations of these six quantities (a, m, t, p, d, and n were to be considered in a similar manner it would require 64 different statements to express the different relations. Considering two of them, (d, m) as always constant, (as was true for this study) and four of them (a, t, p, and n) as variables or constants as the case may be, the different relations could be represented by the following 16 generalized statements three examples of which have been given above.

(1) G = constant, when d, m, a, t, p, n, are constant.

(2) G = f (a), when d, m, p, t, n, are constant.

(3) G = f (t), " d, m, p, a, n, " "

(4) G = f (p), " d, m, a, t, n " "

(5) G = f (n), " d, m, p, t, a " "

(6) $G = f(a, t)$, when d, m, p, n are constant.

(7) $G = f(a, n)$, " d, m, p, t " "

(8) $G = f(a, p)$, " d, m, t, n " "

(9) $G = f(t, n)$, " d, m, p, a " "

(10) $G = f(p, n)$, " d, m, a, t " "

(11) $G = f(t, p)$, " d, m, a, n " "

(12) $G = f(a, t, n)$," d, m, p " "

(13) $G = f(a, t, p)$," d, m, n " "

(14) $G = f(a, n, p)$," d, m, t " "

(15) $G = f(p, t, n)$," d, m, a " "

(16) $G = f(a, t, p, n)$, " d, m " "

The investigation reported in the present paper was carried out during the period between October, 1916, and June, 1918, in the laboratory of Plant Physiology of the Johns Hopkins University. The author wishes to express his thanks to Dr. H. J. Webber and the University of California, for arrangements which made it possible for him to be absent from the Citrus Experiment Station during the period just named. He also wishes to record his appreciation of the privileges and facilities accorded him by the Johns Hopkins University, including a Johnston Scholarship in that Institution. Finally, he desires to acknowledge his indebtedness to Prof. B. E. Livingston and to Dr. H. E. Pulling, of the Laboratory of Plant Physiology of the Johns Hopkins University, for much valued aid and criticism in connection with the planning and carrying out of the experimental part of this study and in the interpretation and presentation of the results.

METHODS

THE CULTURE MEDIUM

The corn meal agar, employed in these experiments was prepared according to the procedure described by Shear and Wood [10] using 20 g. of corn meal and 15 g. of agar shreds for each liter of water. More water was added before the final filtering so that there was one liter of the medium for each 20 g. of corn meal originally used.

The exact chemical and physical nature of such a culture medium cannot of course be stated. It undoubtedly contains a large number of inorganic salts and a still larger number of organic compounds, all in rather low concentration. It also contains various substances in a state of suspension and, being a gel there is no tendency for these to precipitate out. Since the whole problem of definite media for fungus and bacterial cultures remains for the most part entirely untouched, especially for cases where so-called solid media (with agar, gelatine, etc.) are employed, and since the time available for this study was very limited, it was decided to make no attempt to devise a nutrient medium of known composition (which would be a very difficult undertaking in the present state of our knowledge) nor even to find out what any

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[10] Shear, C. L., and Wood, Anna K., Studies of fungus parasites belonging to the genus Glomella. Bul. 252, Bureau of Plant Ind. U. S. D. A 1912.

of the media commonly employed by mycologist may contain (perhaps a still more difficult undertaking). In order to be able to proceed immediately to the problem of temperature influence this whole matter of nutritional conditions was ignored. All that was done in this connection was to be sure that the medium employed would support what appeared to be excellent growth of all four fungi, and to take precautions so that practically the same medium might always be used throughout the entire study. Since corn-meal agar is an infusion of corn-meal and agar-agar shreds, both of them exceedingly complicated, unknown and variable materials, it was feared that different batches of this medium might be very different (especially since corn-meal is known to alter markedly with time), and an attempt was made to avoid this danger by preparing enough medium at the beginning for the entire investigation, mixing it thoroughly in a single container and then preserving it in bottles for future use. That the infusion itself might alter with time was of course possible, but various repetitions of the experiments indicated clearly that such alteration (if it occurred) was not of such nature and magnitude as to alter the growth of the fungi when other conditions were the same. It is assumed, therefore, that the medium was alike for all cultures. Indeed, the only way by which it might be determined whether two lots of media are physiologically alike is to subject them to the physiological test of cultures with all other conditions alike.

Since the amount of medium necessary for the entire study could not well be made in a single day, about eight liters were prepared at a time, until about 48 liters were ready. The entire amount was then liquified, placed in a 20 gallon earthen-ware vessel and thoroughly mixed, after which it was poured into liter bottles. The mouths of the bottles were then plugged with cotton in the usual way and the bottled medium immediately subjected to a temperature of 115°C for 15 minutes. This heating was also repeated on the two following days after which the tops of the cotton plugs were flamed and covered with several thicknesses of paraffined paper, tied tightly around the bottle neck. The bottles of medium were stored in darkness at a temperature of about 18° C. When a lot of cultures were to be made, the required number of bottles of medium were removed from storage, brought into the liquid condition by heating in the autoclave, and used in the ordinary way for pouring the plates. About 15 cc. of medium was used in each culture dish. It was found by test that this amount might be increased or diminished by as much as 3 cc. or more without perceptibly influencing the growth of the fungi. The bottoms of the dishes used had not been planed, and slight irregularities in the glass produced corresponding differences in the thickness of the plate of medium.

STOCK CULTURES

The original sources of the fungus materials were as follows : -- Pythiacystis citrophthora isolated from the diseased bark of a lemon tree suffering from gummosis, at

Whittier, California, in August, 1915, Phytophthora terrestria isolated from the diseased bark of a citrus tree suffering from mal di gomma at Palmetto, Florida, in January, 1914, Diplodia natalensis. isolated by Mr. J. M. Rogers, from a citrus tree in the Isle of Pines, W. I., and received by the writer in the fall of 1913, and Phomopsis citri received from H. E. Stevens from Florida, in October, 1915. All four fungi had been cultivated in tubes of corn-meal agar in darkness at 15 to 20° C. since the original material was received by the author. During that time transfers to new tubes of media had been made at intervals of about six or eight weeks. These cultures will be known as the primary stock cultures.

THE EXPERIMENTAL CULTURES

Approximately five days before the starting of each series of experiments several secondary stock cultures of each fungus were started by transferring small bits of medium containing mycelium from a primary stock culture to the center of the new agar plate. These secondary stock cultures were kept in darkness with a temperature of about 20°C for about five days previous to the making of the experimental cultures. Little plugs or disks were cut out of the agar plate just back of the advancing margin of the circular growth area of one of these five-day secondary stock cultures. The disks were 2.5 mm. in diameter and about 1.5 mm. thick. They were cut out by means of a cylindrical platinum cutting device like that described by Keitt[1]. Each disk was lifted on the

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[1] Keitt, G. W., Simple technique for isolating single spore strains of certain types of fungi. Phytopathology. 5, 266-269. 1915.

flattened end of a platinum needle and was then inverted and placed centrally upon the surface of a new agar plate. The petri dishes containing the agar were 10 cm. in diameter and 1 cm. deep; each contained approximately 15 cc. of corn-meal agar which had been poured hot and allowed to solidify before the transfers were made. After inoculation, the experimental cultures were divided into seven like groups, and one of these groups were placed in each of the seven chambers of the temperature-control apparatus. The cultures of any given species always occupied the same relative position in all the chambers and in all the series. This precaution was observed so that any possible difference in the temperatures between the upper and lower portions of the chamber would not render the measurements of the different lots of the same species incomparable. But such differences in temperature of different parts of any one of the seven chambers proved to be slight (less than .5° C. between the top and bottom of a chamber). The cultures of Phthiacystis citrophthora and Phytophthora terrestria occupied a position on the rack in a chamber, at nearly the same level as the bulb of the thermometer from which the temperature records were taken. The cultures of Phomopsis citri were about 15 cm. below and those of Diplodia natalensis about 15 cm. above the thermometer bulb, in each case.

OBSERVATIONS ON GROWTH.

As the hyphae grew out in all directions from the center of the plate, a rounded mat or mycelial disk was formed

on or near the surface of the medium. This disk remained practically circular, as it enlarged, for both Pythiacystis citrophthora and Phomopsis citri, forming a nearly perfect circle at all stages of enlargement. The mycelial disks of Diplodia natalensis and Phytophthora terrestria were often slightly irregular in form or evenly lobed, especially at the higher temperatures used. No irregularities in growth such as bring about zonation in mycelial mats of many fungi were observed in any of the maintained termperatures. In special tests, however, in which the fungi were grown for a certain time in one temperature, and then transferred and grown in a markedly different temperature, zonation was marked.

Observations were made at daily intervals for a culture period of from four to six days on the enlargement of each mycelial disks. The chief matter of observation was the mean diameter of the disk, which was obtained by averaging two measurements of different diameters, selected to represent the disk as a whole. When the margin of the enlarging disk was clear and definite, these measurements were made by means of a thin millimeter scale applied on the bottom of the petri disk, outside. In other cases the petri dishes were inverted under a low power of a microscope and the length of the mycelial outgrowth measured by means of an ocular micrometer. Measurements with a millimeter scale were read to within .5 mm. This was deemed sufficiently precise for the purpose since the differences between the rates of enlargement so measured for cultures supposed to be alike were often greater than this amount.

Since none of these fungi produces anything but vegetative hyphae during the culture periods employed, the growth activities being therefore not complicated by the formation of any reproductive bodies, these measurements of the mycelial disks, and the daily increments of disk enlargement derived from them, appear to furnish as satisfactory a criterion of physiological activity in general as might be found. The only other criterion for such comparisons as these, that has been generally used by physiological workers, is the rate of production of mycelium measured on the basis of dry weight, and the employment of this criterion offers great practical difficulty when agar medium and short culture periods are used. The diameter measurements were accompanied by simple visual observations as to the general appearance of the mycelial mat (its shape if not circular, its texture, etc.) and by microscopic observations as to any peculairities in hyphal form that might be evident. These observations were recorded, along with the diameter measurements, for each culture at each time of observation.

At the time of observation, each chamber was opened for a fraction of a minute, to remove just one group of cultures, all alike. These dishes were immediately wrapped in cotton batting, to exclude light and prevent very rapid temperature changes. Each dish was removed from the wrapping for a minute or less while the usual observations were made and was then returned to the wrapping. After all cultures of the group had been observed the entire group was replaced in its temperature chamber and another group was taken out for observation. The time required for the entire operations

of removing, measuring and replacing a group of 10 cultures averaged less than 10 minutes.

The opening of the chambers for the removing and replacing of the groups of cultures had very little effect upon the temperature of the chamber itself. The thermographs in the chambers usually showed the occurrence of this series of momentary openings by a slight rise or fall of the pen tracing, producing short vertical lines each representing a degree or less of momentary alteration in the temperature of the chamber.

Several tests were carried out to determine whether the daily disturbance of the maintained temperature, caused by removing the cultures for observation might exert any appreciable influence on the growth of the fungi. These tests showed that the amount of growth observed after several days was practically the same whether the cultures had been left in the chamber for the whole period or had been removed for daily observation in the regular way. These daily disturbances of the maintained temperature are considered negligible in the present study.

THE MAINTAINED TEMPERATURE CHAMBERS

The various temperatures employed in the experiments herein considered were maintained by means of the apparatus described by Livingston and Fawcett[12]. This apparatus consisted essentially of seven experiment chambers about 33 cm. in diameter and 43 cm. deep, each one surrounded below and at

[12] Livingston, B. E., and Fawcett, G. N., A battery of chambers with different automatically maintained temperatures.

the sides by a large mass of water. Light was excluded. The air of the chamber and the water around it, were kept in constant rotation by mechanical stirrers. The seven chambers were built in a row with the water jacket of one in contact with that of the next, excepting for a sheet-iron partition which kept the several masses of water entirely distinct. A tank of mechanically stirred water having automatic temperature control was added at either end of the series of experiment chambers, and the entire apparatus was well insulated from the surroundings. The two ends of the series were adjusted for any two desired temperatures. Between these, after equilibrium had been established, lay the maintained temperatures to be studied, each of which differed from the next by a certain amount depending on the position of the chamber assuming it, in the series. The daily fluctuations were only rarely more than .5C. Access to the chambers was had from above, and of course, the maintained temperature of the cultures was more or less disturbed when the chambers were opened for an instant to take out a group of them for measurement.

STRUCTURAL DIFFERENCES RELATED TO TEMPERATURE

Microscopic observation of the fungus hyphae near the margin of the mycelial disk was made occasionally at the time the measurements were carried out. No spores were produced in any of the experimental cultures, so that vegetative growth alone can be considered. The only structural differences observed between different cultures of the same fungus consisted in more or less marked peculiarities in cultures that had

been exposed to very high or very low temperatures. Within a range of maintained temperatures extending about 13 degrees or 15 degrees centegrade below a temperature slightly above the optimum temperature for enlargement, no influence of temperature on structure was noticeable.

Cultures grown with temperatures that approached the maximum temperature for enlargement showed very different structures from those grown within the temperature range just mentioned. Within this range favorable for enlargement the hyphae were of regular and simple form, mostly cylindrical, and the branching was regular. With temperatures near the maximum temperature for enlargement the outgrowing hyphae were of irregular shape, coarse and twisted, with occasional swellings and usually with apical enlargements. The hyphal diameter was usually much larger than with more favorable temperatures for enlargement and these thicker, irregular hyphae showed contents appeared dark-colored and granular, in contrast to the smooth, clear appearance of the cell contents for cultures with the more favorable temperatures. The granulation was frequently pronounced and refraction was such as to give the whole hypha a very dense appearance.

Typical visible differences between hyphae grown with temperatures near the maximum and those grown with temperatures within the optimal and sub-optimal range above mentioned, are shown in figures 8 to 10.

The drawings of figure 8 represent Phytophthora hyphae near the disk margin. The regularly branched filament (a) represents a culture grown 4 days with a maintained temperature of 33° C. The densely granular and irregular filament (c) represents a culture grown with a maintained temperature of

36°C for the same period. Similar illustrations for Diplodia after 3 days are shown in figure 10. The larger hyphae (a) with uniform diameter and regular branching was grown with a temperature of 23°C., while the other, (b), with irregular branching, peculiar swellings and densely granular contents, was grown with a maintained temperature of 36°C. Some of the cells formed with the higher temperature appeared to be nearly or quite devoid of protoplasm. The drawings of figure 10, (b) also represents the usual appearance of Diplodia hyphae after one day at 45°, and after two days at 40°C. Growth was discontinued after these periods with these temperatures. Cultures of the other two fungi for maintained temperatures of 32° and 34° C showed much the same irregular and densely granular filaments as those illustrated for Phytophthora and Diplodia with higher temperatures.

Turning to the structural characteristics of cultures grown with very low temperatures, which approached the temperature minimum for enlargement, with the lowest maintained temperature tested for Pythiacystis and Phytophthora (7.5°C) the hyphal diameter was much greater than with temperatures within the favorable temperature range. The hyphal contents for these low temperature cultures was only slightly granular. Sample filiments representing 4 day culture of Pythiacystis with maintained temperatures of 23° and 7.5° C. are shown at a and c, figure 8, and the same comparison for 4 day cultures of Phytophthora may be made by means of drawings shown at a and c, figure 9. The low temperature filiments of Pythiacystis are much swollen and divide into many short, thick club-shaped branches, and those of Pythophthora show a series of swollen

joints having somewhat the appearance of budding yeast. These low temperature cultures of Phomopsis showed filaments of somewhat smaller diameter, with less frequent branching, than those grown with favorable temperatures for rapid enlargement. For Diplodia the only observed difference between filaments produced at a temperature of 7.5°C. and those grown within the favorable range for enlargement, lay simply in this, that the former had diameters somewhat smaller than the latter.

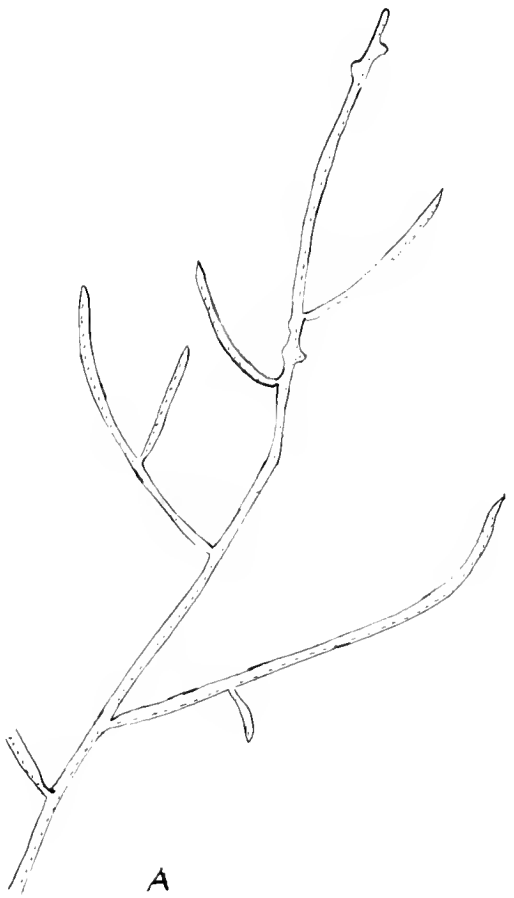


Fig. 8. Representative hypnae of Pylaisiastis grown 4 days from inoculation. a, with maintained temperature of 15°, c, 7.5°C.

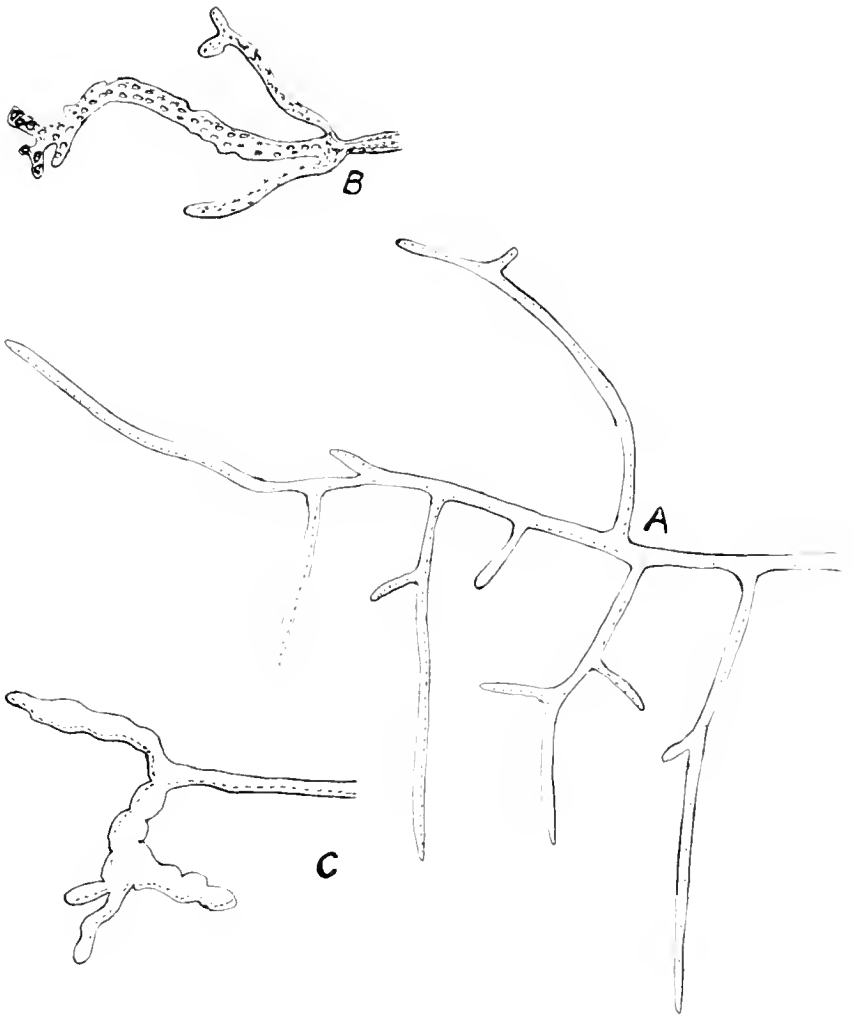


Fig. 9. Representative hyphae of Phytophthora from 3 days from inoculation, a, with maintained temperature of 25°, b, 36° , c, 7.5° .

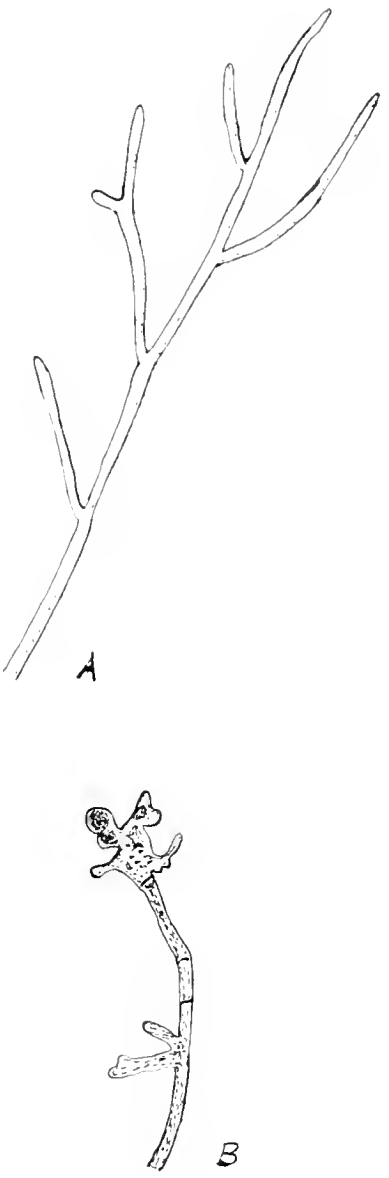


Fig. 10. Representative hyphae of [illegible] grown 3 days from inoculation. a. with maintained temperature at 60°, , 36° c.

THE EXPERIMENTAL DATA

As previously noted, two diameters of each mycelial disk were measured at 24 hourly intervals during each experiment. The average of these two measurements are taken to represent the average diameter for a given culture at the time of measurement. From this average diameter at the end of the first 24 hour period, the diameter of the transplanted cutting (2.5 mm.) was substracted, and the remainder was taken as the value for the increment of enlargement for this first observation period. The difference between the average measurement for the end of the first and that for the end of the second 24 hour period represented the increment of enlargement for the second 24 hour period. Increments for the subsequent periods were obtained in a similar way. All of the culture averages for a group of like cultures in the same chamber (each average derived from two measurements as just described) were finally averaged to give the group mean. These group means were taken as relative measures of the radial enlargement for the different fungi, different time periods and different maintained temperatures. Such means are usually derived from a group of from 6 to 10 duplicate cultures. Examples showing the derivation of the group means from averages of diameter increments of individual cultures are given in tables I and II. The first column in these two tables, gives the maintained temperature, the second gives the number of the individual cultures in each group. In the following four columns are given the averages from which the group means are to be derived, each of these values being the average of two

diameter increments for the first, second and third 24 hour periods and for the first 3 day period after inoculation. These two tables may suffice to show the sort of individual differences encountered in the culture averages and the way in which the group means have been derived from them.

In Tables III - VI are presented the group means for the four fungi, for the different maintained temperatures and for the different 24 hour observation periods as well as for the first 2 day and first 3 day periods.

In cases where considerable number of data are at hand for culture periods longer than three-days the group means are given for the first 4 day, first 5 day, etc., period after inoculation. The number of cultures employed in the derivation of these group means is also indicated in each case, in parenthesis. The notation of these four tables is self-explanatory, but it may be noted that the means for observation periods other than of 24 hours are all given at the right in the second part of the table. In this connection it should be borne in mind that the first 24 hour period is equivalent to the first 1-day period, so that the series of values for different lengths of time after inoculation begins with the value given in column 2 and continues with the values given in the second part, in each case.

TABLE

Average 24 Hourly Diameter Increments for Individual Mycelial disks of *Pythiacystis citrophthora*.

Temperature	Culture No.	First 24 hr. period	Second 24 hr. period	Third 24 hr. period	First 3-day period
deg., C.		m m.	m m.	m m.	m m.
	1	3.0	4.0	6.5	4.5
	2	2.5	3.5	5.0	3.7
	3	2.0	5.5	6.5	4.3
15.5	4	2.0	4.3	5.5	3.9
	5	2.0	4.5	5.0	3.8
	6	2.5	4.3	6.2	4.3
	7	2.0	4.5	5.5	4.0
	8	2.5	5.5	6.0	4.7
	Group mean	2.3	4.5	5.7	4.16
	Culture No.	First 24 hr. period	Second 24 hr. period	Third 24 hr. period	First 3-day period
		m m.	m m.	m m.	m m.
	1	7.5	7.5	7.0	7.3
	2	7.0	7.7	6.5	7.1
	3	6.5	7.5	7.5	7.5
30	4	6.0	8.0	8.0	7.3
	5	7.5	7.5	8.5	7.9
	6	7.5	8.5	7.5	7.5
	7	7.0	8.5	8.0	7.8
	8	7.0	8.3	7.0	7.4
	9	7.0	9.0	8.5	8.2
	10	6.8	8.5	7.2	7.5
	Group mean	7.0	8.1	7.6	7.56

TABLE II

Average 24 Hourly Diameter Increments for Individual Mycelial Disks of *Diplodia natalensis*

Temperature	Culture No.	First 24 hr. period	Second 24 hr. period	Third 24 hr. period	First 3-day period
deg. , C.		m m.	m m.	m m.	m m.
	1	9.5	12.8	14.5	12.3
	2	10.5	13.0	14.5	12.7
	3	11.5	12.5	14.0	12.7
15.5	4	11.0	14.5	14.0	13.2
	5	9.5	13.7	14.8	12.7
	6	8.7	14.5	13.7	12.3
	Group mean	10.1	13.7	14.2	38.0
	Culture No.	First 24 hr. period	Second 24 hr. period	Third 24 hr. period	First 3-day period
		m m.	m m.	m m.	m m.
	1	26.5	25.0	22.5	24.7
	2	28.5	25.5	22.0	25.3
	3	26.5	25.5	20.5	24.2
32.5	4	26.0	25.8	21.7	24.5
	5	27.5	25.0	19.0	23.8
	6	28.5	24.0	22.5	24.7
	7	29.0	24.7	20.	24.c
	Group mean	27.5	25.	21.1	24.5

FOLD OUT

FOLD OUT

FOLD OUT

TABLE VI

Mean 24 Hourly Diameter Increments, (m m.) for

Mycelial Disks *Diplodia natalensis*

Maintained Temperature	First 24-hr. period	Second 24-hr. period	Thirs 24 hr. period	First 2-day period	First 3-day period
7.5	.05 (10)	1.9 (10)	2.1 (10)	.97	1.35
13.5	8.0 (10)	11.0 (10)	10.0 (10)	9.5	9.66
15.5	10.1 (6)	13.7 (6)	14.2 (6)	11.9	12.66
18.5	13.0 (12)	17.5 (12)	18.5 (12)	15.25	16.33
19.5	15.5 (8)	20.5 (8)	18.5 (8)	18.0	18.16
21.5	17.0 (8)	21.5 (7)	23.5 (7)	19.25	20.66
23.	18.2 (22)	24. (22)	23.7 (22)	21.1	21.96
25.	23.0 (10)	27.2 (17)	26.0 (17)	25.1	25.4
27.5	25.9 (18)	31. (18)	26.1 (18)	28.45	27.66
29.5	27.7 (9)	29.3 (9)	24.5 (9)	28.5	27.16
30.	30.0 (12)	29.8 (12)	23.6 (12)	29.9	27.8
31.	29.3 (7)	25.5 (9)	21.5 (9)	27.4	25.43
32.5	27.5 (7)	25. (7)	21.1 (7)	26.25	24.53
34.	26. (8)	21.5 (8)	18.0 (8)	23.75	21.83
35.5	14.5 (10)	5.0 (10)	0 (10)	9.75	6.5
36.5	9.1 (15)	1.0 (15)	0 (15)	5.05	3.36
40.	1.5 (15)	0 (15)	0 (15)	.7	.5
41.	.7 (8)	0 (8)	0 (8)	.35	.23
45.	.2 (10)	0 (10)	0 (10)	.1	.06

The rates given in tables III - VI do not always represent single series. In many cases the same maintained temperature was tested at different times for the same fungus and all the measurements that were available for any fungus and temperature have been used in deriving the mean rate for that fungus and temperature, without reference to the particular series of tests in which any group of measurements may have occurred. Also, the data in any vertical column of these tables, representing the enlargement rates for the respective maintained temperatures indicated in the first column, do not all represent the same series.

Experimental series : General experimental series were carried out for each fungus, and the seven temperatures employed were not the same in all cases, the battery of chambers being so adjusted for each series as to give the maintained temperatures that promised to be most useful. Without any reference to experimental series, all the available data have been brought together in these tables. This procedure, by which the separate series are not kept distinct, is allowable in such work as this, where all conditions were practically controlled. In so far as the inoculating material for all cultures of any fungus was always the same, and in so far as the nutrient medium was likewise always the same, it makes no difference at what time of year any given test was made. The radiation and temperature conditions of the general surroundings, outside of the chambers, were sensibly without influence on these conditions within the chambers. Thus a test for a given maintained temperature may have been made

in July and repeated in August and the two sets of data obtained from a single series for the particular fungus and temperature in question. Many repetitions of this sort, were made involving the same maintained temperature in different experimental series, and the growth rates of similar cultures in different series, usually agreed as closely as did those of duplicate cultures of the same series. This indicates that the fungus materials and nutrient medium used did not appreciably alter during the period of the investigation.

DISCUSSION OF RESULTS.

General Considerations

The data of tables III - VI show, in the first place, that the growth rates of each of these four fungi, as here measured, differ very markedly with different maintained temperatures. As for other organisms and for other physiological processes, it appears that the rate of enlargement of these mycelial disks is very low for certain temperatures, comparatively very high for certain higher temperatures, and again very low for certain still higher temperatures.

Students of process-rates have employed the term optimal to indicate this group or range of temperatures for which the rates are greatest. For certain processes, especially many of those studied in laboratories of physics and chemistry, serious attempts have been made to determine the optimium temperature with enough precision so that it becomes practicable to consider an optimal temperature rather than an optimal temperature range, but of course, the significance of such optimal temperatures depends on the relative precision with which the determinations have been made.

For several reasons, it seems best to condider merely that there is a group or range of temperatures that give highest rates for any given process and for any given set of non-temperature conditions. This range is of course smaller in some cases than in others, and it is smaller in any particular case as determinations are made with greater precision

and as effective conditions are more precisely controlled in experimentation.

With maintained temperatures progressively either higher or lower than the optimal range these rates of fungus growth are progressively lower. The data given in tables III - VI show that the rates become zero for each fungus when a certain temperature higher than the optimal range is passed, and it is clear from other studies of plant growth rates - that a similar temperature might be found below which the rates would be zero. Thus, growth itself, the very occurrence of the process, can take place (for any given fungus and for any given set of non-temperature conditions) only within a certain range of maintained temperatures, this total temperature range being considerably larger than the corresponding optimal range and including the latter within its limits. These two temperature limits have been called the minimum and maximum temperatures, and they are readily determined, in any case, with a certain degree of precision. But, even for these, the precision of statement is not very great. On the whole, it is best to consider all three of these so-called cardinal points of temperature influence as only approximate in the few cases where attempts have been made to determine them at all, and the results of the present study must be regarded in this way.

It is obvious that the data of growth rates -- as those of all other process rates must always involve a duration factor , a process is measured in terms of the amount of work accomplished, or of product produced during a given time period. Since the change considered may go forward more or less rapidly during different partial time periods within the per-

iod considered in deriving the rate, it is always necessary to regard experimentally determined rates as averages. Thus, in the present study, all rates of enlargement are to be considered as average or mean 24-hour rates, and such are the values given in table III - VI. These mean 24-hour rates may of course be reduced to corresponding rates on the basis of any other unit of time, by employing a simple coefficient. Thus a mean 1-hour rate is obtained by dividing the given 24-hour rate by 24, a mean 2-day rate is obtained by multiplying the given 24-hour rate by 2, etc. Since only mean rates are considered -- and instantaneous rates cannot be dealt with experimentally -- it makes no difference what time unit is employed. Twenty-four hour rates of increase in the diameter of the mycelial disks are dealt with throughout this paper. It may be remarked that corresponding rates of radial increase may of course be obtained by dividing each given value by 2. As has been stated, the spatial unit employed for all these rate values is the millimeter.

Besides the time unit for which the mean rates are calculated (24 hours or 1 day, in the present instance) the characterization of any process also involves a quantitative statement of the length of time during which the process is considered as in operation ; that is, the length of the exposure period in such experiments as these. It is usually convenient to employ the same time unit for this statement as is employed in the rate unit itself, and this is done in the present study. Thus any mean growth rate is characterized by the combination of one spatial value and two time values , we say that the mean rate of enlargement of a given fungus for

an exposure period of *c* days, with the given medium and with a certain maintained temperature is *s* mm. per time period of *n* hours. These three factors enter into the statement of the time rate of every process. In the present case *h* is always taken as 24 hours, or 1 day, and *d* is always stated in terms of *h*, so that *d* becomes *nn*, n being the number of days of the exposure period. It is of course not supposed that the rate remains constant throughout the exposure period; the values are simply mean 24 hour rates for the given period.

The following tabulation of values obtained from table III for Pytheacytis, temperature 30° c, illustrates these propositions in a concrete manner.

Length of exposure period (d)	Length of observation period or time factor of rate (n)	Mean rate of diameter increase (s)
1	1	7.0
2	1	7.5
3	1	7.6
4	1	7.4
5	1	7.5
6	1	7.1

Of course all three factors are considered as only approximately measured in any case, they are taken as true only within the limits of variation due to unknown or neglected conditions, within the limits of what are commonly termed the unavoidable errors of observations, measurement or experimentation. All that was necessary in the present study is that fluctuation in the approximately ~~xxx~~ maintained temperature, and uncorrected errors in the measurement of the length of the exposure period should be of no greater relative magnitude than were the unknown and neglected fluctuations in the approximately controlled material and of the nutrient medium. A decision in such matters, for physiological studies, still depends largely on general judgment rather than upon mathematical calculation.

It has been emphasized, by Lehenbauer (1914) and others, that the optimal temperature for a given physiological process, with a given complex of controlling conditions, can be logically stated without reference to the length of the exposure period only when it is assumed that the process rate is constant throughout that period. If the rate in question changes in any way with the lapse of time the index of optimal temperature can have no very precise meaning unless the length of the period considered is definitely stated. This is a consideration frequently neglected in experimental determinations of temperature optimal for physiological processes. Of course this whole matter of the relation of length of exposure period to the three cardinal points of the temperature relation specifically involves the consideration of progressive

alteration [illegible] complex [illegible] [illegible] climaxes [illegible] [illegible] after [illegible] [illegible] [illegible] be correlated [illegible].

From [illegible] of these mycelial disks, [illegible] inspection [illegible] given in [illegible] tables III - VI [illegible] optimal temperature indices [illegible] Table VII. These indices of course represent temperature ranges rather than temperatures. Each value should be read as if preceded by the word about, to indicate that the given value really represents a temperature somewhere within the optimal range.

TABLE VII.

Temperatures representing the optimal ranges for the enlargement of mycelial disks, for each of the four fungi studied, for the non-temperature conditions uniformly employed and for various lengths of exposure period. The values are given in degrees, C.

LENGTH OF EXPOSURE PERIOD.

FUNGUS	1 da.	2 da.	3 da.	4 da.	[illegible] da.	[illegible]
Pythiacystis	27.5	[illegible]	[illegible]	[illegible]	[illegible]	
Phytophthora	24.5	[illegible]	[illegible]	[illegible]	[illegible]	
Phomopsis	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]
Diplodia	30	30	30			

The data of table VII indicate that the optimal temperature ranges are not the same for all four fungi, and that for two of them, Pythium and Phytophthora, it is not the same for all exposure periods even of the same fungus.

Since the consecutive temperature actually tested were rather far apart in all cases, these observed optimal temperature values do not necessarily represent the medium points in the respective optimal range, and they stand for these ranges only in a very rough and general way. In order to study these differences between the fungi and to bring out the relation that holds between the age of the culture and its growth rate, the rate rates for the consecutive days of the exposure period must be employed.

MEAN RATES FOR SUCCESSIVE OBSERVATION PERIODS IN THE EXPOSURE PERIOD.

Introductory : If the mean 24-hour rate of enlargement for the same fungus, with any given maintained temperature, is different for different lengths of exposure period, this must of course be due to progressive alteration of the instantaneous rate with the lapse of time from the beginning of the culture. This makes it desirable to study the march of the instantaneous rate value throughout the period of exposure, and this is best done by resorting to graphs. For each of the four fungi a growth-temperature graph was prepared to represent each one of the successive 24-hour observation periods (within the exposure period) for which adequate data were available.

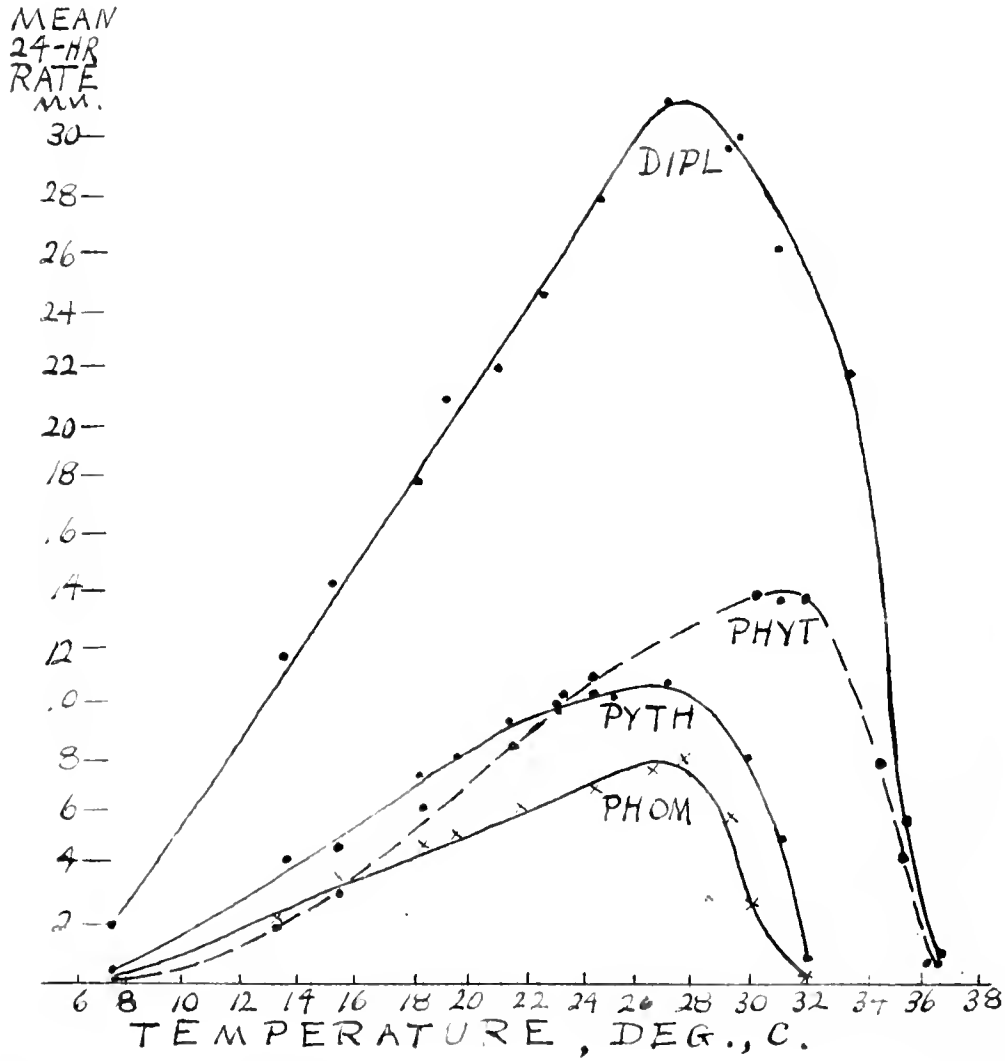
This treatment resulted in several graphs for each fungus, and a comparison of these graphs brings out certain features of the manner of the growth-temperature relation as it changed throughout the exposure period.

These growth-temperature graphs were constructed in the ordinary way. For the given fungus and observation period the mean 24 hour rates (first part of Tables III - VI) were plotted as ordinates and the indices of maintained temperature were plotted as abscissas. After the points were in place a smoothed graph was drawn in the regular manner.

To illustrate this process of smoothing, the four graphs for the second 24-hour period after inoculation are shown together in Figure 1. The points shown on or near each smoothed graph represent the mean rates taken from the table. It is seen at once that they arrange themselves in a very satisfactory manner as regards the smoothed graph, i.e., that the process of smoothing introduces only very slight alterations from any of the values derived directly from observations. These four second-day graphs are representative of the others. All are shown (without the points -- the value for which may be obtained from tables III - VI, however) in figures 2 -5, each figure presenting the several smoothed graphs for a single fungus.

Discussion of graphs of Figures 2 - 5 : The four fungi all agree in showing very different growth-temperature graphs for the successive observation periods. For the same fungus, the mean growth rate for any one of the successive 24-hour periods within the entire exposure period is generally

Fig. 1. Smoothed growth-temperature graphs for second 24-hour periods for each of the four fungi employed. The points represent the actual increments as given in tables III-VI.



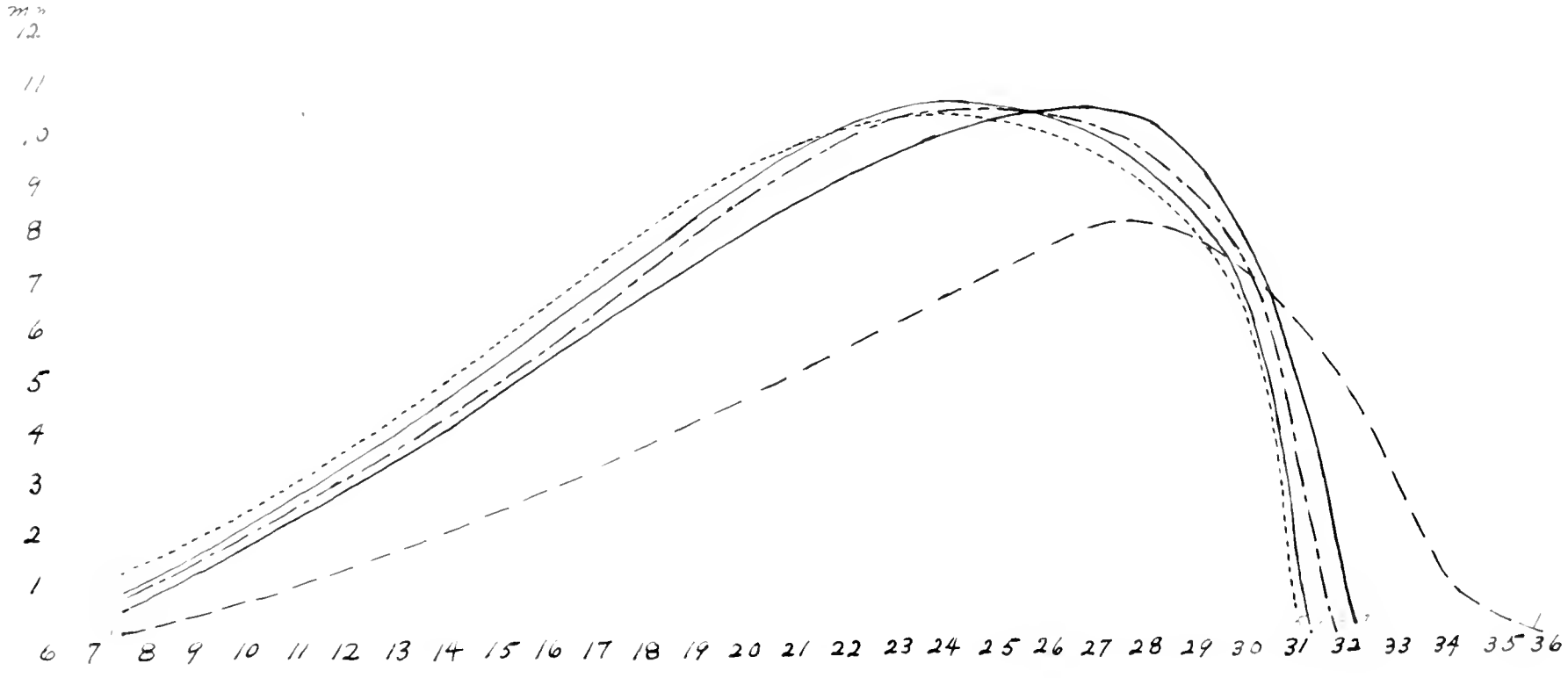


Fig. 2. Smoothed growth temperature graphs for each of the first five 24-hour observation periods, for *Y. chiarvstis*.

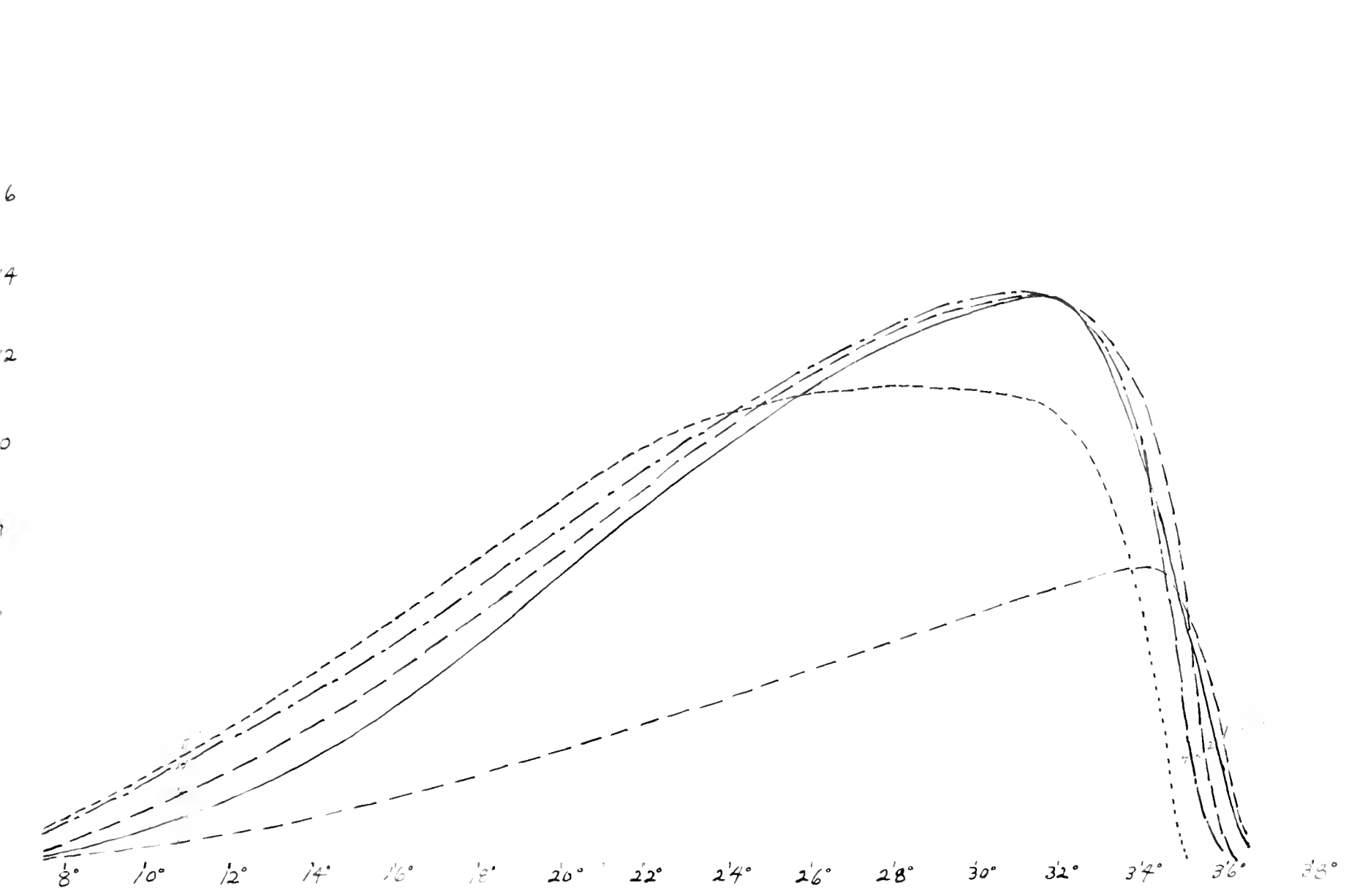
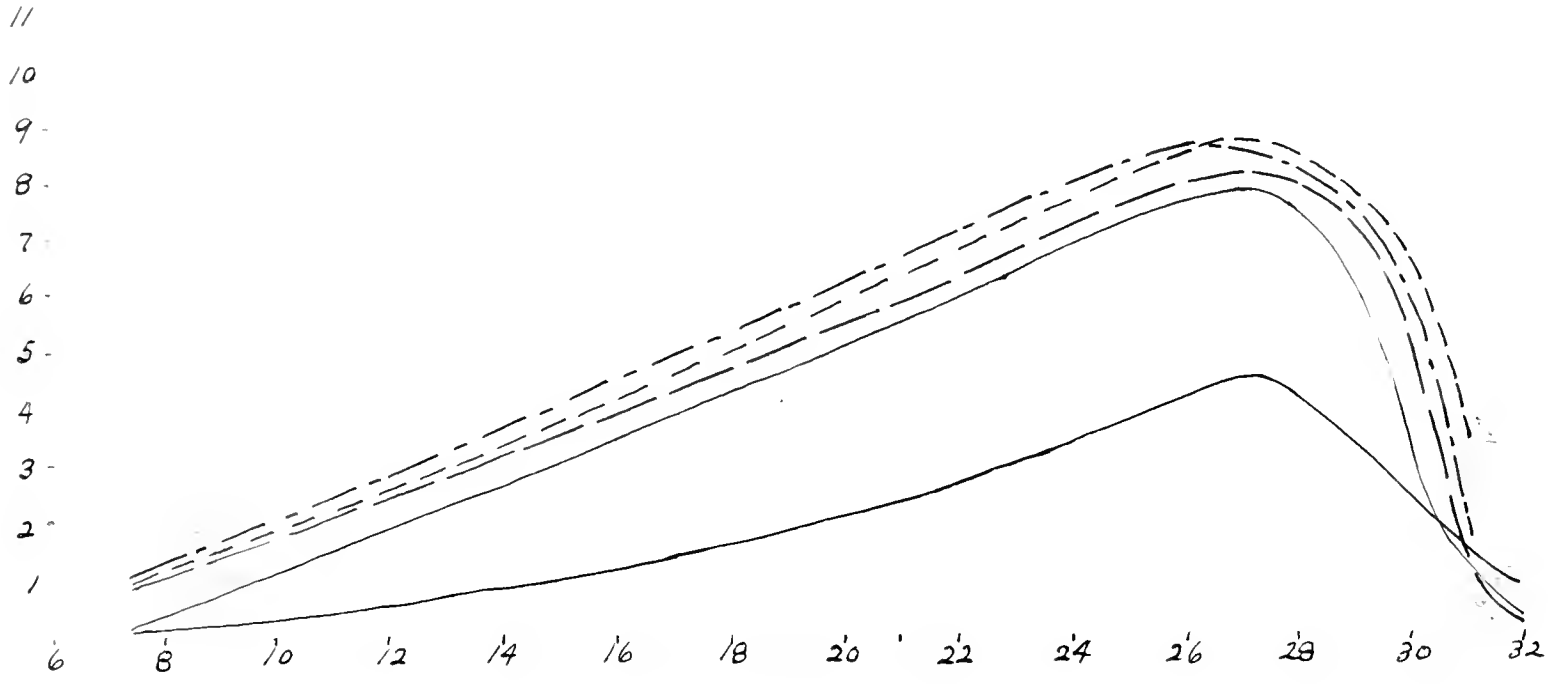


Fig. 3. Smoothed growth-temperature graphs for each of the first five 24-hour observation periods, for Phytophthora.



ig. 4. Smoothed growth-temperature graphs for each of the first five 24-hour observation periods, for Phonopsis.

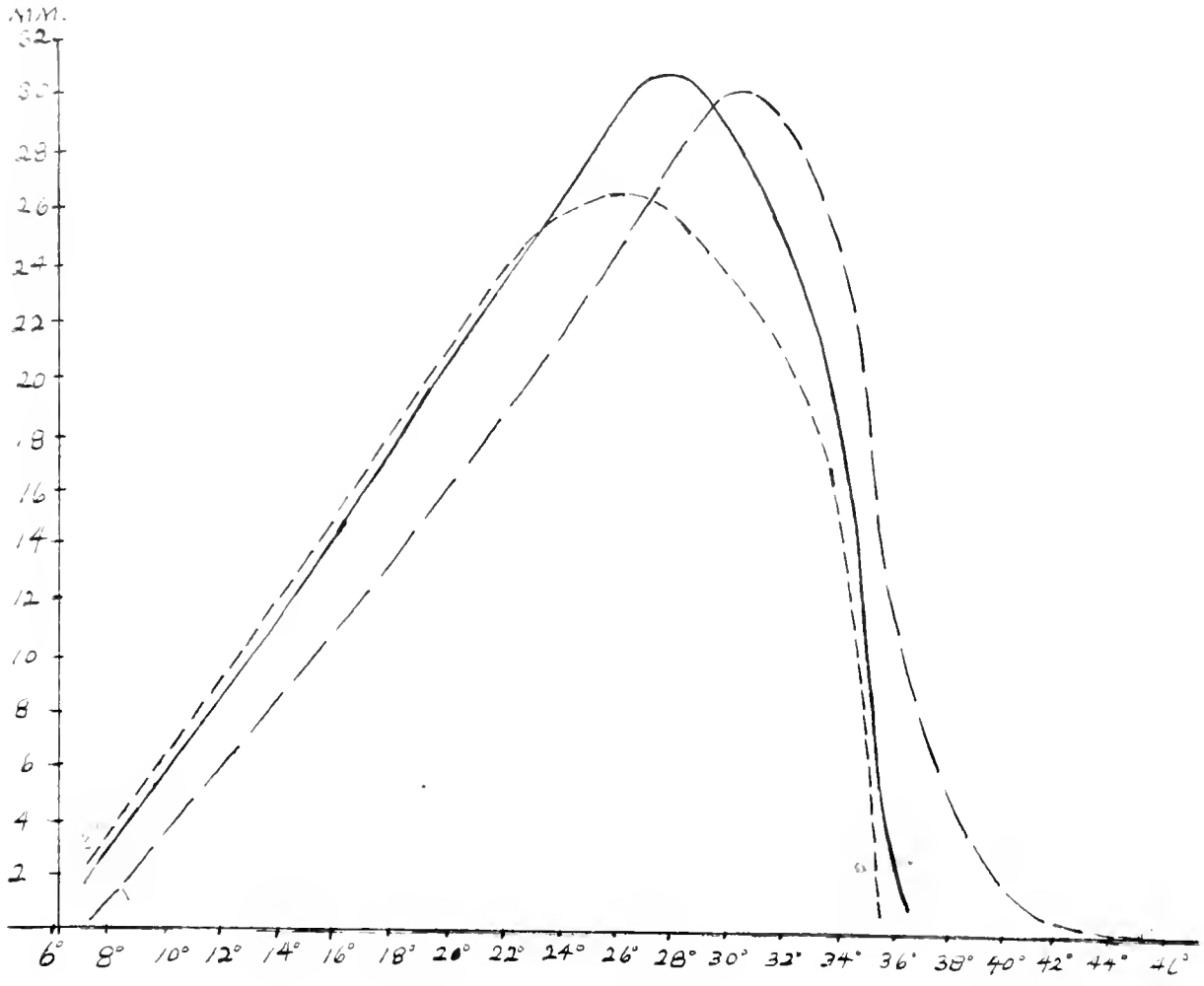


Fig. 5. Smoothed growth-temperature graphs for each of the first three 24-hour observation periods for Diplodia.

nc[illegible] same as that for any [illegible] graph. It follows from this that the growth-temperature graph for each organism alters its shape as it proceeds from one observation period to another in the continuous series, as is clear from superficial inspection of the figures. This progressive change in the form of the growth-temperature graph of course represents a corresponding progressive change in the growth-temperature relation of the fungus as the stages of investigation. Since the external conditions of these experiments are considered as not altering with time, this apparently gradual change in the growth-temperature relation must be evidence of internal changes occurring in the organism. Just what these changes may be is not a matter that can be considered as yet, but it is possible to study them as they affect the growth-temperature relation; the progressive alteration in the form of the graph representing this relation is itself a clear indication of certain features of the effectiveness of the internal alterations within the organism.

All the graphs agree in showing a very small value to the growth rate with the lowest maintained temperatures studied. In all cases the minimum temperature (below which no growth occurred under the conditions of these experiments) was apparently below 7° C.

With maintained temperature progressively higher the the minimum the rate values are progressively larger, till the maximum rate represented by the graph is reached. With still higher maintained temperature the rate is progressively lower, so that the optimal temperature region is the maximum in the graphs, considered as a curve. This maximum growth rate is

fers, among the several graphs, in the exact place it occurs with different maintained temperatures and it represents rates of different magnitudes. For example, the first period graph for Pythiacystis (fig. 2) shows an optimal temperature range about 27.5°C., while the fourth period graph for the same fungus shows this critical point as about 24°C. Also in the first case the maximum ordinate value is about 8 mm; while in the second case it is about 10.6 mm.

In general form and shape the growth-temperature graphs of the four fungi for any given period are much alike. Beginning with the lowest temperature tested the graphs all rise gradually being slightly concave upwards at first but becoming decidedly convex upwards as the graph maximum is approached. Beyond this maximum region the graphs descend rapidly to the graph minimum (maximum temperature for growth). Just where the reversal of direction of curvature direction occurs in this downward slope is difficult to determine, on account of the short temperature interval between the optimal point and the temperature maximum. It appears that the curvature increases very rapidly as the temperature maximum is approached. But this matter cannot be settled definitely for all the graphs without much more detailed study. It is clear that the growth optimum always lies far above (to the right of) the middle of the total temperature range, and that the upward slope of every graph is much less steep than the downward slope.

In these general characteristics these graphs resemble those of Edgerton (1915) for the growth of Glomerella

those of Lehenbauer (1914) for the growth of maize seedlings and those of most other students of life-processes -- temperature relations based on short time and temperature intervals. The graphspublished by Brooks & Cooley[13] showing the relations of growth of a number of apple rot fungi to temperature for 5-degree intervals, also suggest the same general type of curve.

If the curves for the successive 24-hour periods for each fungus (figs. 2-5) are compared, certain general features may be noted. For every fungus there is a shift of the apparent maximum temperature downward (to the left in the graphs) with each successive observation period. This shifting is much more pronpunced between the first and second 24-hour periods than between any other two consecutive periods, except in case of Phytophthora. For Pythiacytis the maximum shifts from about 36° for the first 24-hour period, to about 32° for the fifth period ; for Phytophthora the corresponding shift is from about 33 to about 35° ; for Diplodia the maximum temperature shifts from about 46 for the first 24hour period to about 35° for the third period. The maximum temperatures for Phomopsis are also uncertain.

A similar shifting of the apparent temperature optimum is shown for all the fungi excepting Phomopsis. The apparent optimum temperature for Pythiacystis shifts from about 27.5 for the first day to about 24 for the fifth day, the corresponding shift for Phytophthora is from about 34° to about 29° for the same time and for Diplodia the optimum shifts from about 31° for the first day to about 27° for the third day. But

[13] Brooks, C and Cooley, J.S., Temperature relations of apple rot fungi. Jour. Agric. Res. 9 , 139 - 164, 1917.

the optimum temperature for Phomopsis appears to remain nearly constant throughout the first five 24-hour periods.

Aside from the shifting of the apparent maximum and optimum temperature values just considered, it should be noted that a similar shifting is evident for growth rates lying within a large part of the sub-optimal region of the growth-temperature graphs for each fungus. Throughout a large portion of this sub-optimal region the ordinate value for any given maintained temperature is greater for every observation period after the first than it is for the next preceding period. This statement is true for Pythiacystis for the first five 24-hour periods after inoculation and for maintained temperature up to 21°C. It is true for Phytophthora and Phomopsis for the first five observation periods, for maintained temperatures up to 23.5° and 26°C., respectively. For Diplodia it is true for the first three 24 hour periods and for maintained temperatures up to about 21°C.

In much of the supra optimal region on the other hand, the value of any given ordinate value is usually less than that for the next preceding period. These shiftings in the specific relations of growth rate to maintained temperature bring it about that the growth temperature graph for each successive observation period crosses the next preceding one. The only apparent exception to this statement is for two of the graphs for Phomopsis, for the fourth and fifth 24-hour periods..

Discussion of graphs of figures 6 and 7: To compare the curvatures of different graphs it is convenient to express all the ordinate values of each in terms of the maximum and to

Fig. 1. Growth-temperature graphs for Pythiacystis and Phytophthora for the second 24-hour period after inoculation, the ordinate values being expressed in terms of the corresponding maximum growth rate taken as unity in each case.

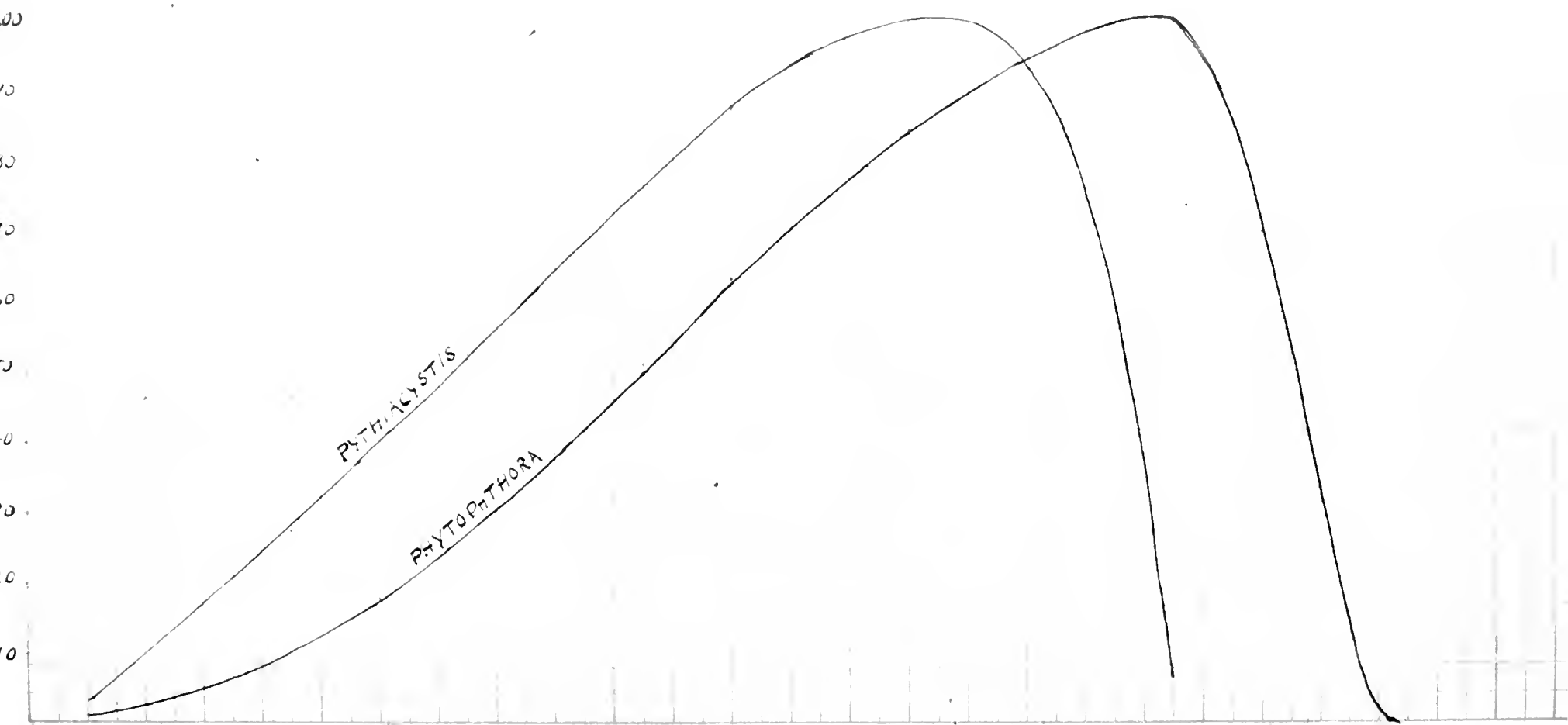
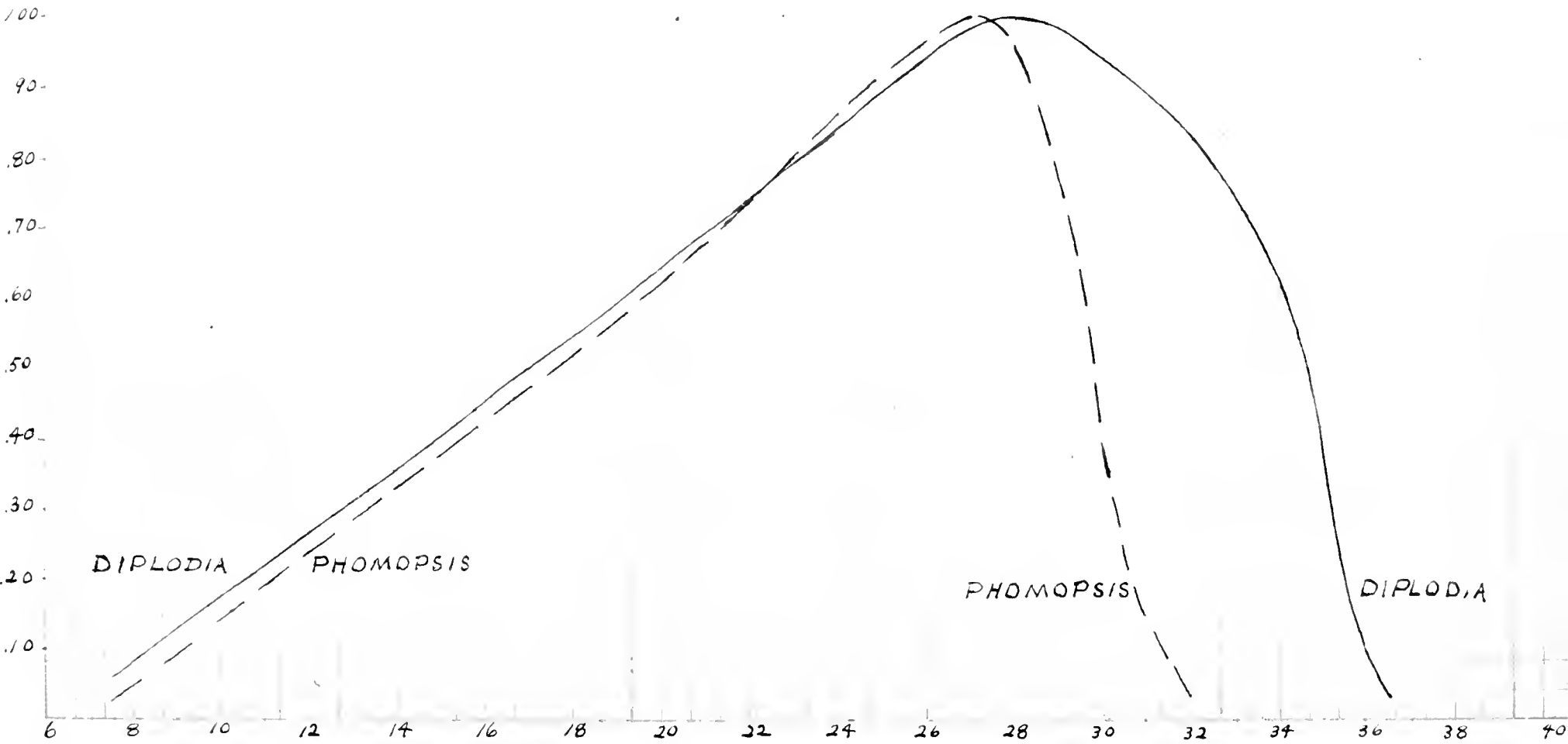


Fig. 7. Growth-temperature graphs for Phomopsis and Diplodia for the second 24-hour period after inoculation, the ordinate values being expressed in terms of the corresponding maximum growth rate taken as unity in each case



replot the graphs using the values thus derived. This treatment removes apparent differences in curvature due to differences in the magnitudes of the maximum ordinates. Such relative graphs for the second 24-hour observation period for each fungus are presented in figures 6 and 7. Constructed in this way, the unit of growth rate is different for the different graphs, being always the maximum value for that fungus, but the value is always considered as unity, hence all the graph maxima appear to be equal for these graphs. The upward and downward slopes of these four graphs are strictly comparable as to direction or angle, always with reference, not to actual growth rates in millimeters, but to relative growth rates, in terms of the corresponding maximum rate.

Referring to figures 6 and 7, the relative degrees of steepness of the graphs are nearly the same for the sub-optimal region, and the same is true for the supra-optimal region, excepting that the graph for Diplodia is here somewhat less steep than the other three. The four graphs differ considerably in other details, however, mainly in regard to total temperature range and in regard to minimum, optimum and maximum temperature values.

It is to be noted that if the entire graph for Phytophthora were moved to the left through 4 degrees of temperature, e. g., if all the rates for this fungus were plotted at temperatures 4 degrees lower, this graph would follow closely the actual curve for Pythiacystis except that the first part of the downward slope is a little steeper. The difference in the two curves is therefore mainly one of location of the temperature range and the actual values of the increments. The

TABLE VIII

Characters of the graphs of figures 6 and 7, for the second 24-hour period after inoculation.

Name of fungus	Approximate Maximum temperature	Temperature range for rates equal to or greater than 0.1 of maximal rate.			Optimal temperature		Percent of partial range (rate = 0.1 or more of maximal rate).	
		Extent of range	Lower limit of range	Upper limit of range	Actual	Percent of partial range	Above maximal temperature limit of range.	Below maximal limit of range
	Deg. c.	Number of degrees	Deg. c.	Deg. c.	Deg. c.			
Pythiacystis	32.5	27.2	4.7	31.9	16.5	59.7		23.4
Phytophthora	37.0	24.1	12.0	36.1	31.5	80.5		19.5
Phomopsis	33.0	22.3	9.1	31.4	27.0	80.2		19.7
Diplodia	37.0	27.6	9.4	36.0	28.0	72.0		28.0

The data of table VIII show, for these second-day growth temperature graphs, that the approximate maximum temperature is about 37°C. for the two fungi Phytophthora and Diplodia, while it is about 32.5° for Pythiacystis and about 33° for Phomopsis. The actual optimal temperatures (as read from the graphs) are quite different for all four fungi; this value is lowest (26.5) for Pythiacystis and highest (31.5°) for Phytophthora. As to the extent of the temperature range for rates equal to or greater than 0.1 of the maximum rate, the four fungi are all considerably different; Diplodia has the greatest range (27.6°) and Phomopsis has the smallest (22.3°). This partial temperature range has its lower limit lowest (8.5°) for Diplodia, a little higher for Pythiacystis (9.7°) and Phomopsis (9.1°) and highest for Phytophthora (12.0°). But the four fungi do not stand in this relation in regard to the upper limit of this range, for Phytophthora and Diplodia show about the same limit (36.1° and 36.0°) while Pythiacystis and Phomopsis also nearly agree in this respect (31.0° and 31.4°), the value for the last two being markedly lower than for the first two.

It is especially interesting to observe the relative positions of the optimal temperature values in the respective partial temperature ranges (for relative rates of 0.1 or more), as shown in the last two columns of table VIII. Roughly speaking, it may be said that from about 70 to about 80 per cent. of the temperature range here considered lies below the optimal temperature, with from about 30 to about 20 per cent. lying above. But the agreement among the four fungi is not more striking in this respect than in the other respects mentioned above. The optimal temperature occurs lowest, in this

partial temperature range, for Diplodia (72.2 per cent.), it occurs higher in the range for Pythiacystis - (77.7 per cent) and it lies still higher in the range for Phytophthora (80.5 per cent.) and Phomopsis (80.2 per cent).

By these various criteria it appears that the four relative graphs under discussion (figs. 6 and 7), although they are alike in a very general way, are yet unlike as to details of form. While two or three of them may resemble one another more than they resemble the remainder of the four, yet the four graphs do not fall into the same grouping by any two of the criteria here employed for their comparison. Of course comparisons like the ones just made might be instituted between different fungi with reference to any other time period than the one here employed; only the mean rates of enlargement for the second 24-hour period after inoculations are here considered.

RELATION OF GROWTH RATE TO AGE OF CULTURE.

General considerations

It has been emphasized that the growth rates as measured in the work here reported differ not only for different fungi with the same maintained temperature and for different maintained temperatures with the same fungus, but also for different consecutive observation periods with the same fungus and same maintained temperature, and it has also been pointed out that these last differences in growth rate must be regarded as due to progressive alterations in the internal con-

ditions of the fungus as the culture becomes older. This relation of growth rate to age of culture may be studied by reference either to the original mean 24-hour rates for the different consecutive observation periods and for the four fungi (tables III- VI), or to the derived rates given in tables IX - XII or by reference to graphs of figures 2 - 5 and figures 11 - 13.

Inspection of the tables IX - XII and graphs (figures 11 - 13) shows that the mean rate of enlargement alters with the age of the culture in three general ways. (1) For lower temperatures the rate increases throughout the culture period, the rate of increase being generally greatest for the first two days and much more gradual afterwards. (2) For a few higher temperatures the rate first increases and then decreases, sometimes remaining constant for two 24-hour periods or longer. (3) For the highest temperatures the rate decreases throughout the culture period, this decrease soon bringing the value to zero for the very highest temperatures studied.

Changes in Maximum and Optimum Temperature with Lapse of Time. It is a common observation that at certain high temperatures near the maximum temperature, the rate of certain processes diminish rapidly with time. As has been pointed out, an examination of the data of tables III - VI, figures 2 - 5 and figures 11 - 13 show that at certain temperatures that were below the maximum for the first observation period, the rates fell off rapidly to zero with time in all the fungi so that the maximum temperature was definitely moved back at each subsequent 24-hour period.

In two of the fungi, Pythiacystis and Diplodia, the

ig. 11. Graphs showin. relation of enlargement to age of culture, for Pythiacystis grown with various maintained temperatures. Ordinates are 24-hour increments and abscissas are number of d s from moment of inoculation.

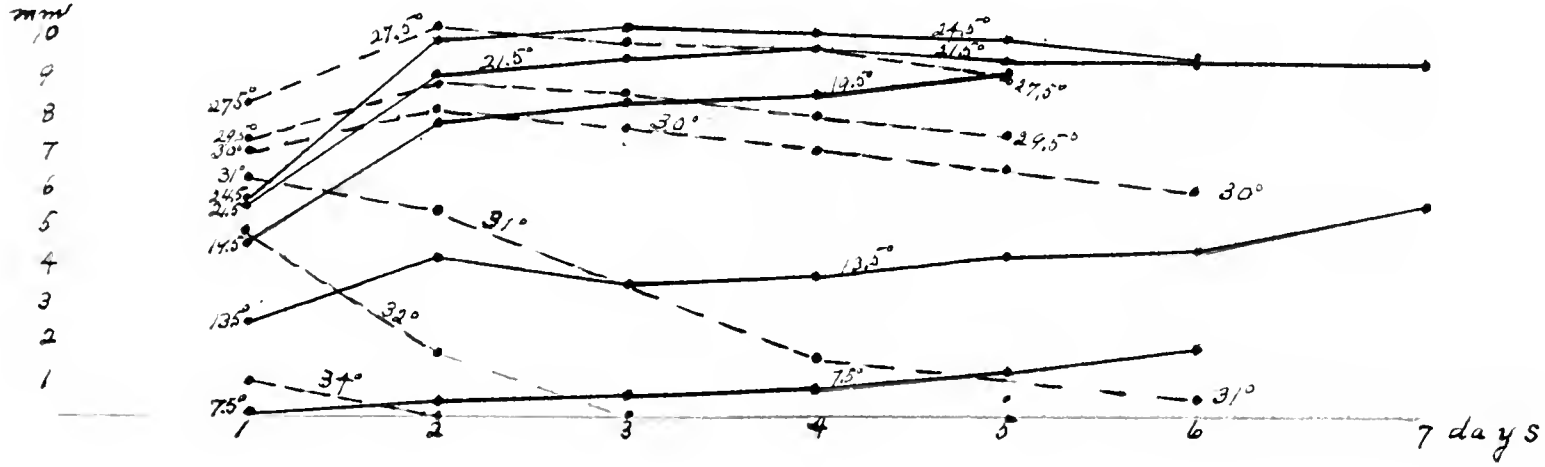


Fig. 12. Graphs showing relation of enlargement to age of culture, for Phytophthora grown with various maintained temperatures.

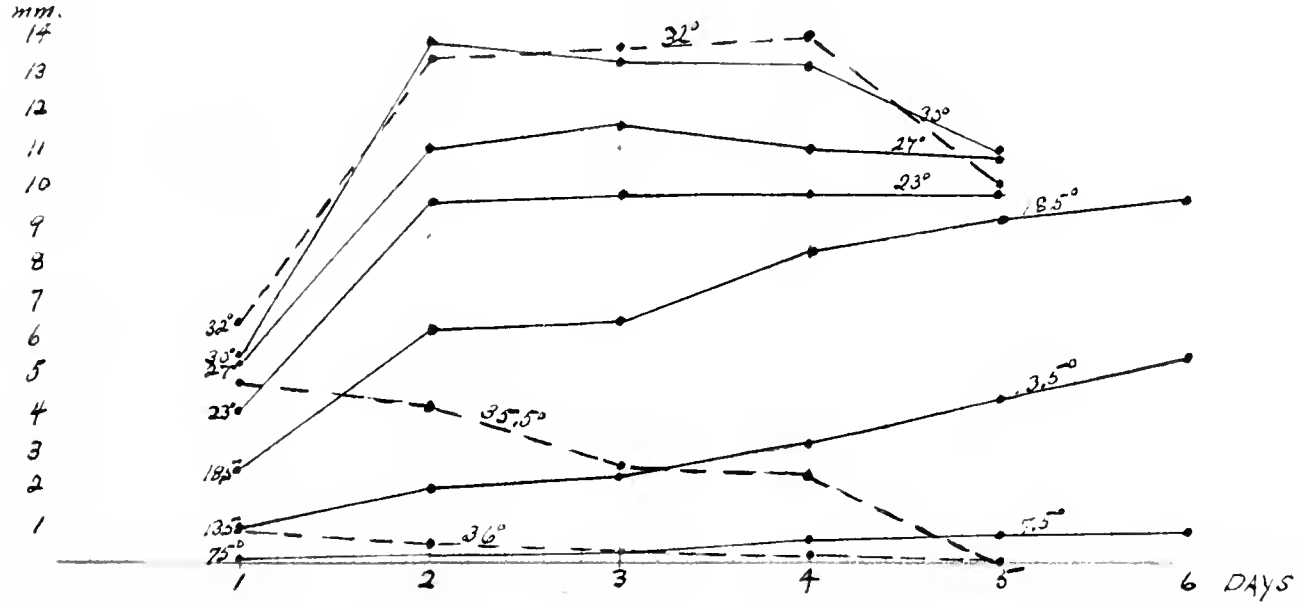
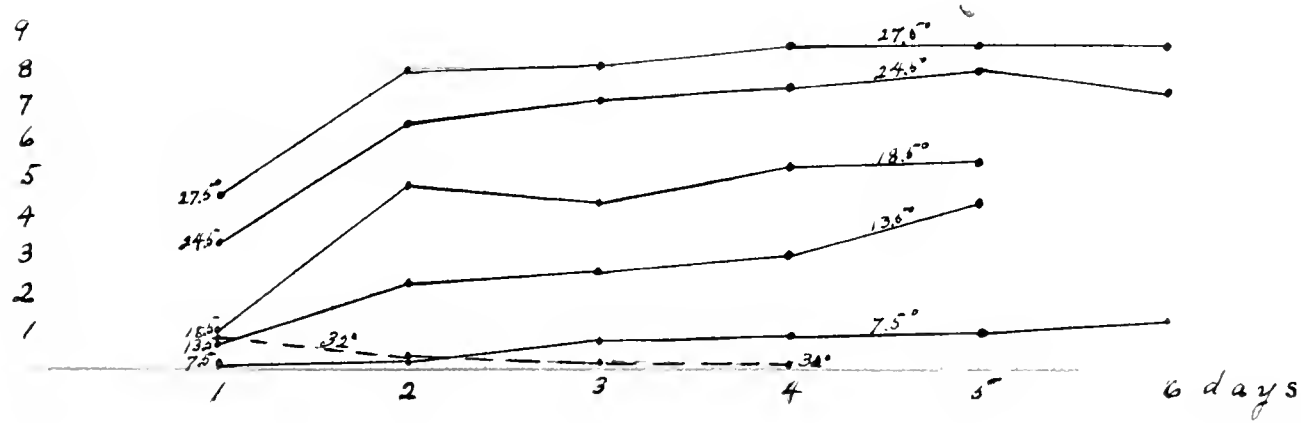


Fig. 13. Graphs showing relation of enlargement to age of culture for Phomopsis grown with various maintained temperatures.



optimum temperature of the growth-temperature graph also appeared to move back from day to day upon the temperature scale but not so rapidly as did the maximum for the same corresponding periods. This tended in general to make the supra-optimal slope of the curve for each 24-hour period somewhat steeper than for the previous 24-hour period. In Phytophthora there was also a shifting of the optimum, but this shifting was definite from the data only between the first and second and between the fourth and fifth 24-hour period. In Phomopsis there appeared to be a shifting of the optimum backward only between the fourth and fifth 24-hour period.

TEMPERATURE COEFFICIENTS.

Introductory: A temperature coefficient derived from the rates of a given process for two different temperatures, is usually defined as the factor by which the rate for the lower temperature is to be multiplied in order to give the rate for the upper temperature. While the temperature interval may be any value desired it has been usual to consider an interval of ten degrees centegrade and to derive temperature coefficient on the basis of a rise of ten degrees. In some investigations where the increase in the rate of the process with rise of temperature is very great, however, temperature coefficient for smaller intervals have been used, as temperature coefficients for one degree, two degrees or 5 degrees.

The temperature coefficient for the rate of any process for a rise of 10 degrees is conventionally represented by the symbol Q_{10}. When derived directly from experimental data showing rates at ten degree intervals it is of course the quotient of the rate at the higher temperature by the rate at the lower.

Temperature coefficients for physiological processes have been much discussed in the literature. The statement occurs in numerous papers that the rate of a certain process under consideration does or does not obey the "Vant Hoff-Arrhenius rule" for chemical reaction velocities with change in temperature, this rule being commonly understood to mean that the reaction velocity is doubled or trebbled for each rise of 10 degrees centegrade. It has been usual for biologists to use this rule as thus understood, to decide whether a given

process was chemical or physical in its nature. If the rate of process in question was found to have a temperature coefficient for 10 degrees between 2 and three, it was considered an indication that the process was a chemical one or was controlled by chemical reactions. If on the other hand the 10 degree temperature coefficient proved to be much below 2 it was taken as an indication of a physical reaction. In many discussions of temperature influence on process rate it has been assumed that if this coefficient appears to be more or less nearly constant and has a magnitude between 2.0 and 3.0, for the particular range studied, then the process follows the Van't Hoff-Arrhenius rule. If, on the other hand, the coefficient is not constant but varies greatly above or below this range of magnitudes it is considered that the rule does not hold. This common, narrow interpretation of the Van't Hoff-Arrhenius rule (or R. G. T.[14] rule as it is also called) appears to have been based on a general misconception of the Van't Hoff formula. As has been clearly pointed out by Stuart[15] this formula itself makes clear that a constant coefficient is not a part of the rule, even for chemical reactions, and that Van't Hoff[16] himself

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14 Reaktionsgeschwindigkeit — Temperatur - Regel

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15 Stuart, C. P. Cohen, A study of temperature-coefficients and Van't Hoff's rule. Koninklijke Akademie van Wetenschappen te Amsterdam Proceedings of the section of sciences of the Royal Acad. Amsterdam. (Translated from Verslagen van de Gewone Vergaderingen der Wis-en Natuurkundige afdeeling) 11 - 2nd part. 1173 - 1173. 1912.

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16 Van't Hoff, J. H., studies in Chemical Dynamics (Revised and enlarged by D. Ernst Cohen and translated by Thomas Ewan) 1896.

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recognized that the coefficient value is by no means to be taken as constant but generally decreases with rising temperature. The curve representing the relation between chemical reaction-velocities and temperature is therefore not an exponential one as seems to have been supposed by many writers. If it is not usual for the temperature coefficient to be constant for simple chemical reactions, it is not to be expected that it would be constant for physiological processes where much more complex reactions take place. An examination of the experimental data on the relation of a large number of live processes to temperature[17] shows that the temperature coefficients for such processes (with rare exceptions) tend to diminish in value from lower to higher ranges of temperature. Kanitz[18] appears to have been one of the first to regard this feature as essential in the relationship between rates of life processes and temperature.

Trantz and Volkmann[19] gave considerable attention to this lowering of temperature-coefficients in certain chemical processes.

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17 The data for a large number of life process rates, with citations of 363 papers has been collected and compiled in a monograph by Kanitz. Kanitz, A., Temperatur und Lebensvorgänge Die Biochemie in Einzeldarstellungen. Berlin 1 : 1-175 - 1915.

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18 Kanitz, A., Über der Einfluss der Temperatur auf die Kohendroxydassimilation. Zs. Elektrochem. 11 : 689 - 1915.

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19 Trantz, Max and Volkmann, Karl, Der Temperaturkoeffizient Chemischer Reaktionsgeschwindigkeiten Uschr. für anyeik. chem. 64 , 55-58, 1908.

Snyder[20] also pointed out clearly that, since it is the rule for the temperature coefficient of chemical reaction to vary with temperature, variation should be expected in physiological processes.

Livingston[21] pointed out that the temperature coefficient of the growth rates of maize seedlings, as determined by Lehenbauer (1914), could be regarded as following the van't Hoff rule, as commonly understood, only for a very limited range of temperatures.

The data of Miss Leitch's[22] paper giving the results of carefully controlled experiments on the effect of temperature on the rate of growth of pea seedlings show that the temperature coefficient varies quite regularly from a high to a low value passing from 3 to 2 between 10 and 29°c.

Rahn[23] taking his data from experiments of Marshall Ward[24] on the rate of development of Bacillus ramosus and

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20 Snyder, Chas. D., On the meaning of variationin the magnitude of temperature coefficients of Physiological processes. Amer. Jour. Physiol. 28 : 167 - 175. 1911.

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21 Livingston, B. E., Physiological temperature indices for the study of plant growth in relation to climatic conditions - Physiological Researches . 1 : 399 - 420. 1917.

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22 Leitch, Miss I., Some experiments on the influence of temperature on the rate of growth in Pisum sativum. Am. Bot. 30 : 25 - 46. 1916.

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23 Rahn, Otto, Der Einfluss der Temperatur und der Gift auf Enzymwirkung, Garung and Wachstum. Der Bioch. Zeitsch. 72 : 351 - 372, 1916.

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24 Ward, Marshall, On the biology of Bacillus ramosus. Proc. Roy Sol. London. 58: 265 - 283. 1895.

B. coli, construct some curves of the temperature coefficients showing how they decrease rapidly from high values at low temperatures to low values at higher temperatures.

Temperature coefficient in the present study : The growth temperature relations of the four fungi used in the work here reported were studied in certain respects by means of such temperature coefficients as have previously been described. Since unexplained fluctuations in growth rate as related to temperature are to be neglected, it being desired to obtain information of a general nature only, the mean 24 hour rates for the various 24 hour observation periods (given in tables III - VII) were not employed in calculating the temperature coefficients. Instead of these, the length of the ordinates for each degree on the smoothed graphs of figures 2 - 5 were used. These ordinate values are presented in tables IX - XII. The arrangement and notation of the first parts of tables III - VI are here followed.

TABLE IX.

Mean 24-hour rates of enlargement for consecutive 1-day observation periods, for Pythacystis, as determined by measuring the ordinates of the smoothed graphs of figure 2.

Temperature	First day	Second day	Third day	Fourth day	Fifth day
Deg. C.	mm.	mm.	mm.	mm.	mm.
8	0.3	0.8	0.9	1.1	1.4
9	0.5	1.2	1.4	1.6	2.0
10	0.7	1.7	1.9	2.2	2.4
11	1.0	2.3	2.5	2.8	3.1
12	1.3	2.9	3.0	3.4	3.7
13	1.6	3.5	3.6	4.0	4.2
14	2.0	4.1	4.3	4.7	5.0
15	2.4	4.8	5.0	5.4	5.8
16	2.8	5.5	5.7	6.2	6.5
17	3.3	6.2	6.5	6.8	7.2
18	3.7	6.8	7.1	7.5	8.0
19	4.2	7.4	8.0	8.3	8.7
20	4.7	8.0	8.7	8.9	9.2
21	5.1	8.6	9.3	9.5	9.7
22	5.7	9.1	9.8	9.9	10.0
23	6.1	9.6	10.2	10.3	10.2
24	6.6	9.9	10.4	10.5	10.3
25	7.1	10.2	10.4	10.4	10.1
26	7.6	10.4	10.3	10.2	9.9
27	8.0	10.4	10.1	9.8	9.5
28	8.1	10.2	9.6	9.3	8.8
29	7.8	9.4	8.7	8.3	7.9
30	7.1	7.8	7.4	6.9	6.4
31	6.1	5.3	3.5	1.5	0.5
32	4.8	0.7	0.0	0.0	0.0
33	2.6	0.0	-	-	-
34	0.9	-	-	-	-
35	0.4	-	-	-	-
36	0.0	-	-	-	-

TABLE X.

Mean 24-hour rates of enlargement for consecutive 1-day observation periods, for Phytophthora, as determined by measurin the ordinates of the smoothed graphs of figure 3.

Temperature	First day	Second day	Third day	Fourth day	Fifth day
Deg. c.	mm.	mm.	mm.	mm.	mm.
8.0	.07	0.25	0.40	1.0	1.1
9.0	0.13	0.40	0.90	1.5	1.6
10.	0.22	0.70	1.2	2.0	2.1
11	0.30	1.0	1.7	2.5	2.7
12	0.50	1.4	2.2	3.0	3.3
13	0.70	1.9	2.7	3.5	3.9
14	0.90	2.4	3.3	4.1	4.5
15	1.1	3.0	3.9	4.7	5.2
16	1.4	3.7	4.5	5.3	5.8
17	1.7	4.4	5.3	5.9	6.5
18	2.0	5.2	6.0	6.6	7.2
19	2.4	6.0	6.7	7.2	7.9
20	2.7	6.9	7.4	7.9	8.6
21	3.0	7.8	8.2	8.7	9.3
22	3.3	8.6	9.0	9.3	9.9
23	3.6	9.3	9.8	10.1	10.3
24	4.0	10.0	10.5	10.7	10.7
25	4.3	10.8	11.1	11.3	10.9
26	4.6	11.4	11.8	11.9	11.1
27	5.0	11.9	12.3	12.4	11.3
28	5.3	12.5	12.8	12.9	11.3
29	5.7	12.9	13.2	13.3	11.3
30	6.0	13.3	13.4	13.6	11.2
31	6.4	13.5	13.6	13.7	11.0
32	6.7	13.5	13.6	13.6	10.7
33	6.9	12.2	12.8	12.6	9.2
34	7.1	9.5	11.2	10.2	6.0
35	6.1	5.7	6.8	4.2	1.0
36	2.2	1.7	0.5	0.2	-

TABLE XI.

Mean 24-hour rates of enlargement for consecutive 1-day observation periods, for Phomopsis, as determined by measuring the ordinates of the smoothed graphs of figure 4.

Temperature	First day	Second day	Third day	Fourth day	Fifth day
Deg. C.	mm.	mm	mm	mm	mm
8.0	0.10	0.40	1.1	1.2	1.3
9.0	0.20	0.70	1.4	1.5	1.6
10.0	0.30	1.1	1.7	1.8	2.0
11.0	0.40	1.4	2.1	2.1	2.4
12.	0.50	1.9	2.4	2.6	2.9
13.0	0.60	2.2	2.8	2.9	3.2
14	0.80	2.6	3.1	3.2	3.5
15	1.0	3.0	3.5	3.6	4.0
16	1.2	3.4	3.9	4.0	4.4
17	1.4	3.7	4.2	4.4	4.8
18	1.6	4.1	4.6	4.8	5.2
19	1.9	4.5	5.0	5.2	5.6
20	2.1	4.8	5.4	5.7	6.1
21	2.3	5.3	5.8	6.1	6.5
22	2.6	5.8	6.2	6.5	7.0
23	3.0	6.2	6.6	7.0	7.4
24	3.4	6.7	7.1	7.4	7.9
25	3.8	7.1	7.5	7.8	8.2
26	4.1	7.5	7.8	8.2	8.4
27	4.4	7.7	7.9	8.5	8.3
28	4.2	7.4	7.8	8.2	8.0
29	3.1	6.0	7.0	7.0	7.3
30	2.4	3.0	5.0	6.6	5.9
31	1.5	1.3	1.2	3.3	1.8
32	.9	0.3	0.2	1.0	1.0

TABLE XII.

Mean 24 hour rates of enlargement for consecutive 1-day observation periods, for Diplodia, as determined by measuring the ordinates of the smoothed graphs of figure 5.

Temperature	First day	Second day	Third day
Deg. C.	mm.	mm	mm
8.0	.7	2.7	3.0
9.0	2.0	4.1	4.5
10.	3.2	5.6	5.9
11	4.4	7.0	7.2
12	5.7	8.4	8.9
13	6.8	9.9	10.1
14	8.0	11.3	11.7
15	9.3	12.7	13.1
16	10.5	14.1	14.6
17	11.8	15.7	16.1
18	13.1	17.0	17.6
19	14.3	18.5	19.0
20	15.6	20.	20.5
21	17	21.6	22.2
22	18.4	23	23.6
23	19.8	24.5	24.7
24	21.2	26.1	25.4
25	22.7	27.6	26
26	24.2	29.1	26.3
27	25.6	30.5	26.3
28	27.2	30.6	25.9
29	28.5	30.3	24.9
30	29.7	29.2	23.8
31	29.7	27.4	22.6
32	29	25.6	21
33	27.4	23	19
34	24.9	19	15.9
35	19.9	11	9.3
36	11.4	2.7	-
37	7.4	--	--
38	5.0	--	--
39	3.0	--	--
40	1.5	0.0	--
41	.8	--	--
42	.5	--	--
43	.4	--	--
44	.3	--	--
45	.2	--	--

From these ordinate values of the smoothed growth-temperature graphs, were calculated temperature coefficients for every ten-degree interval by whole degrees, between the lowest and highest maintained temperature tested for each of the consecutive 24 hour observation periods represented in tables IX - XII. To illustrate the method followed, the mean 24-hour growth rate for Pythiacystis, for the first day after inoculation is seen (table IX) to be 0.3 m m for a maintained temperature of 8° c, and 3.7 m m for a maintained temperature of 18° c. The ratio 3.7 to 0.3 is 12.3 which is the 10-degree coefficient (Q_{10}) for the 10-degree interval from 8° to 18° c. Now since the value of Q_{10} varies with the maintained temperature, if its fluctuations are to be studied, it is necessary to calculate the different values, not for successive 10-degree intervals (as 8°-18°, 18°-28°, 28°-38°), but for 10-degree ranges beginning with each successive whole degree for which data are available (as 8°-18°, 9°-19°, 10°-20°, etc) If the value just obtained for 8°-18°c be written $Q_{10}\ (8°-18°)=12.3$ then referring to table IX we may write $Q_{10}\ (9°-19°) = 8.4$; $Q_{10}\ (10°-20°) = 6.7$; $Q_{10}\ (11°-21°) = 5.1$, etc.

For convenience of reference and for convenience in plotting these temperature coefficients for various 10° degree temperature ranges as they are made to shift by one degree intervals, the middle point of each 10-degree range is taken to represent the range itself. Thus Q_{10} for 13° stands for the 10-degree temperature coefficient for the range (8° - 18°) whose middle point is 13°.

The 10-degree coefficient values for all available intervals of 10 degrees for which data are at hand, for all four fungi and for each of the successive 24-hour observation periods employed in the experimentations, are set forth in table [illegible]. The first column of this table presents the different temperature ranges, the second column shows the middle points of these ranges, which will be taken to represent the various 10-degree ranges. The rest of the table falls into four parts, each part giving the data for a single fungus. Each single column gives the coefficients for a single one of the consecutive 24-hour observation periods.

FOLD OUT

Inspection of the coefficient values given in table 1 brings out the fact that, for every one of the four fungi and for each of the consecutive 24-hour observation periods, the 10-degree temperature coefficient for mycelial enlargement is greatest for the lowest temperature shown and regularly decreases toward higher temperatures, becoming smallest for the highest temperatures. The highest coefficient value here encountered (30.0) is that for 13° C. (range from 8° to 18°), for the first 24-hours after inoculation for Phytophthora. This value is progressively smaller for progressively higher temperature, becoming 6.47 for the temperature 31° (range from 26° to 36°). For the temperature 13° (range from 8° to 18°) the lowest coefficient value shown (4.0) is for the fourth 24-hour period for Rhonopsis and this value is progressively smaller for progressively higher temperatures, becoming 0.5 for the temperature 26° (range from 21° to 31°). The lowest coefficient value of the whole table is 0.01, for the temperature 31° (range from 26° to 36°) for the fourth 24-hour period for Phytophthora this value being progressively larger with progressively lower temperatures and becoming 0.6 for the temperature 13° (range from 8° to 18°.

Aside from the regular falling off in the coefficient value for each period and fungus, as we pass from lower to higher temperatures, as just pointed out, the value for any temperature and fungus is always largest for the first 24 hour period after inoculation and generally tends to become smaller with each successive period after the first, although this last statement is not always strictly true for all temperatures. The general truth appears to be that the temperature coeffi-

cient for any temperature decreases in magnitude, from time period to time period for a certain time after inoculation, after which the magnitude regains approximately the same, oscilating slightly, perhaps within the limits of what may be called observational error.

The relation of the value of this temperature coefficient to the maintained temperature representing the middle point of the 10-degree temperature range from which each coefficient value is derived, is shown graphically, for the second 24-hour period after inoculation, for each fungus, in figure 4. Abscissas are the temperatures of these middle points, while ordinates are coefficient values. These graphs have not been smoothed.

These four graphs of 10-degree temperature coefficients are seen to be alike in their general form. Every one begins with a relatively very high value at the left (lowest temperature range tested) and descends, rapidly at first and then less rapidly, with higher temperatures.

From the nature of the temperature coefficient it is clear that the value of any range of maintained temperatures having its lower limit just below the minimum temperature for enlargement must be infinite; as the maintained temperature is supposed to vary from any value below the minimum temperature to any value above the minimum, the rate of enlargement must (by definition) suddenly increase from zero to some positive value, and the ratio of any positive quantity to zero is of course infinity. All of the graphs should therefore become vertical straight lines for some low temperatures, but these

[illegible] limit is approximately the [illegible] for enlargement.

On the [illegible], as the temperature [illegible] the coefficient also if calculated has a [illegible] that approached the maximum temperature for enlargement, the coefficient approaches zero. No matter what may be the temperature [illegible] a plateau, a range of maintained temperature for [illegible] value below the temperature maximum to some value above this critical point must be accomplished by a corresponding change in the rate of enlargement from a positive value to zero, and the ratio of zero any positive quantity is of course zero.

From these considerations it is clear that all graphs of temperature coefficients are vertical and traverse a point whose ordinate is infinite and whose abscissa is near the value of the minimum temperature for the process considered; and they are all horizontal and traverse a point whose ordinate is zero and whose abscissa is near the value of the maximum temperature for the process considered. These two points are the fixed ends of graphs, between them the graph slopes downward to the right, having a curvature varying with the maintained temperature itself. The graphs of figure 4 appear to be all concave upward throughout their upper portions, but they each show a reversal for an ordinate value between 1.5 and 2.0, beyond which point each graph is concave downward for some distance, as the temperature maximum is approached, i.e., as the coefficient value approaches zero.

For graphs such as those here considered it follows (from the points brought out above) that the slope of the

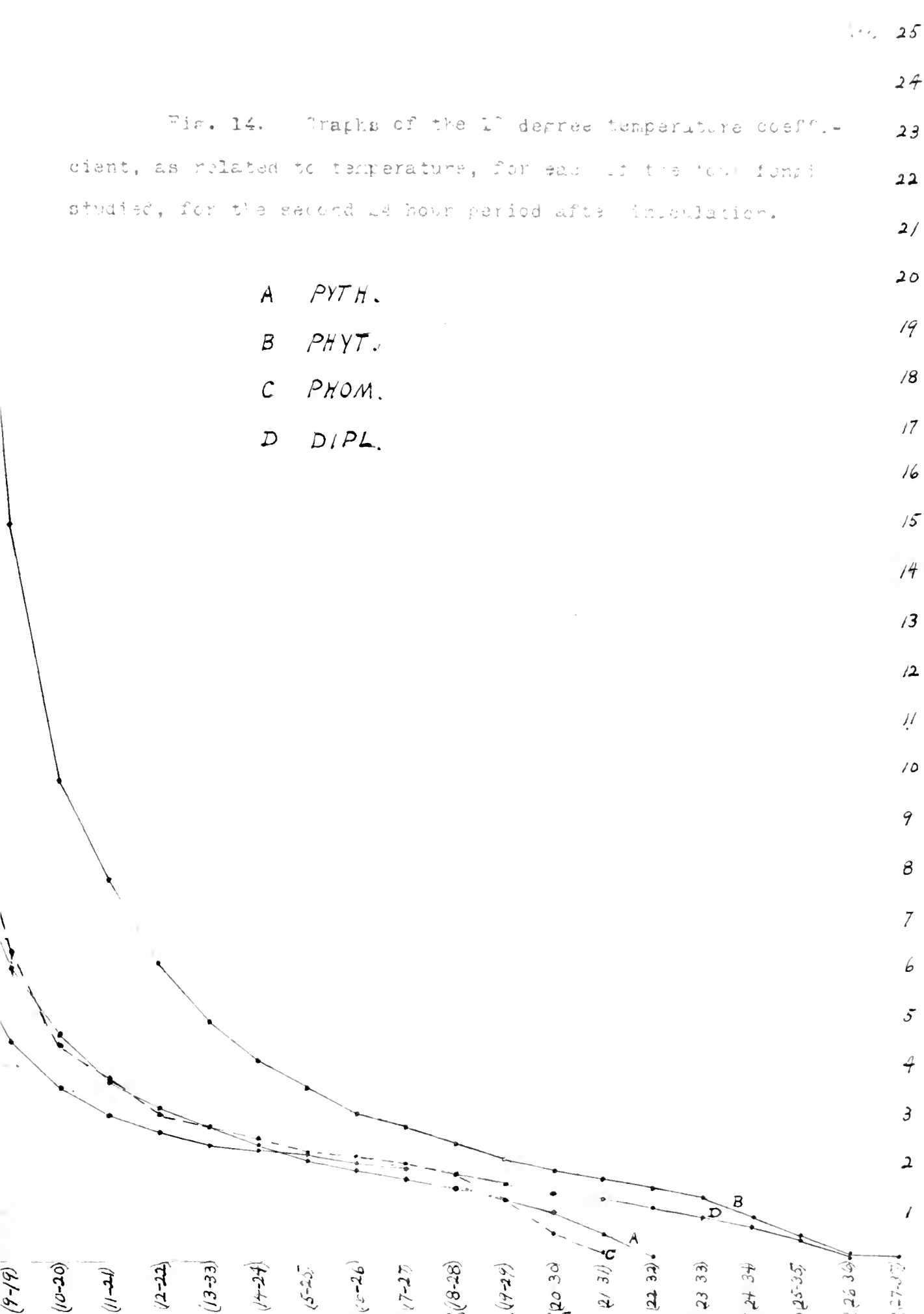
Fig. 14. Graphs of the 1° degree temperature coeff.-cient, as related to temperature, for each of the four fungi studied, for the second 24 hour period after inoculation.

A PYTH.

B PHYT.

C PHOM.

D DIPL.



graph at the point of maximum ordinate. The 'left end' appears to furnish a criterion by which it may be judged, at least in a general way, how nearly the abscissa of this point approaches the minimum temperature value. Since the downward slope must be infinite for a range whose lower limit is just below the minimum temperature, the nearer the actual slope approaches infinity, the more nearly does the corresponding abscissa value approach that of the minimum temperature. It follows clearly that the lowest temperature value here considered (as a middle point of a 10-degree range), which is 13° c., is much more nearly the minimum temperature for enlargement for Phytophthora than it is for any of the remaining three fungi. It is nearer the temperature minimum for Phomopsis than for Pythiacystis and nearer the minimum for Pythiacystis than for Diplodia.

Since the graph of temperature coefficient as related to temperature shows ordinates that decrease in magnitude from infinity to zero, it of course follows that there must be some point on every such graph, at which the ordinate value is unity. For this point the coefficient value is 1.0 ; i.e., a temperature change from a very little below to a very little above the abscissa value for this point is accompanied by no change in the process rate at all. The temperature value, or range, corresponding to this abscissa, or range of abscissas therefore, is near to the temperature optimum for the process considered ; for lower temperatures the coefficient values are all greater than unity , for higher ones they are all smaller than unity.

These considerations immediately suggest that we have here the basis for a new definition of temperature optima or

optimum range. Such a definition may be stated as follows : The temperature optimum for any process is that temperature which is the middle point of a range of temperature for which the temperature coefficient is unity.

It may not be out of place here to outline a procedure by which this derived optimum temperature may be obtained. A smoothed process - temperature graph, similar to the smoothed temperature graphs of figures 2-5, is first constructed, employing as short temperature intervals about the apparent graph maximum as is possible. From this graph are read ordinate values for adequately short intervals, for some distance on either side of the apparent graph maximum. From these ordinate (rate) values are calculated the temperature coefficients for equal temperature ranges whose lower limits are as near together as is possible. Finally, the coefficient values are plotted as ordinates on a graph whose abscissas are the middle points of the temperature ranges employed for deriving the coefficient, and this graph is smoothed in the usual manner. The derived optimum temperature for the process in question is that temperature which is represented by the abscissa of that point on the graph whose ordinate is 1.0. This procedure however has not been followed in the present paper.

Still another point that needs emphasis in a study of the general nature of the temperature coefficients of all processes that have temperature minima and maxima, is this, that every such process must show a certain temperature range for which the temperature coefficient has the value 1.0, or 2.0, etc. Indeed, it must always be possible to find a temperature range that corresponds to any given finite value of the coefficient.

It is therefore quite without any definite meaning to state that the temperature coefficient for any process has a certain value unless the corresponding temperature range is simultaneously stated. The coefficient value may be everything between zero and infinity, depending on the temperature range considered and upon the position of that range within the total temperature range (between minimum and maximum) of the process. The so-called van't Hoff rule, stating that the temperature coefficients of many chemical reaction velocities have values between 2.0 and 3.0 is obviously true, but only, if the proper temperature ranges are considered, and this is true of all other processes (whether of chemical reactions or not) only providing that the processes have minimum and maximum temperature limits. It appears to be true that many simple chemical and physical processes, and many physiological processes also, show temperature coefficient values between 2.0 and 3.0 for certain temperature ranges within the ordinary range of weather temperatures on the earth, and it is perhaps this fact that has led to so much inadequate discussion about these coefficients, especially in physiological literature. Since the mathematical interpretation of the concept of temperature coefficients has not been generally insisted on in these discussions, and since the coefficient may have a constant or nearly constant value for a considerable range of temperatures within the limits of what are commonly known as ordinary temperatures, the coefficient has frequently been regarded as a constant for the process considered, without any specific reference to the temperature range referred to. But van't Hoff and the other writers on this subject have all been perfectly well aware that the value of

the temperature coefficient for any given process varies with temperature, so that any misconceptions which may have arisen have been due more to lack of completeness in presenting the logical analysis of the matters in hand than the actual failure to appreciate the various points of this analysis.

It should therefore be emphasized that there is nothing particularly new about the points brought out in the preceding paragraphs; they are probably clear to everyone who has seriously studied rate-temperature relations. But writers appear to have been prone to look only at certain aspects of this rather complicated problem, and to neglect other aspects just as important. Great emphasis should be placed upon the fact that the temperature coefficient for any process having temperature limits is a continuously varying value, the variation proceeding from infinity to zero.

From this point of view the temperature relations of different processes under-stated non-temperature conditions and with stated exposure periods are clearly comparable, not by means of single temperature coefficient values, but by means of the coefficient-temperature relations as a whole. Practically, the simplest way to present this relation for a given process is to construct such a coefficient-temperature graph as those shown in figures 11. The form of this graph and its position within the angle between the rectangular axes includes a complete description of the rate-temperature relation. If two processes are to be compared in respect to this temperature relation the comparison should be instituted between the two coefficient-temperature graphs, constructed on the same scale. If the two graphs coincide throughout, then the temperature

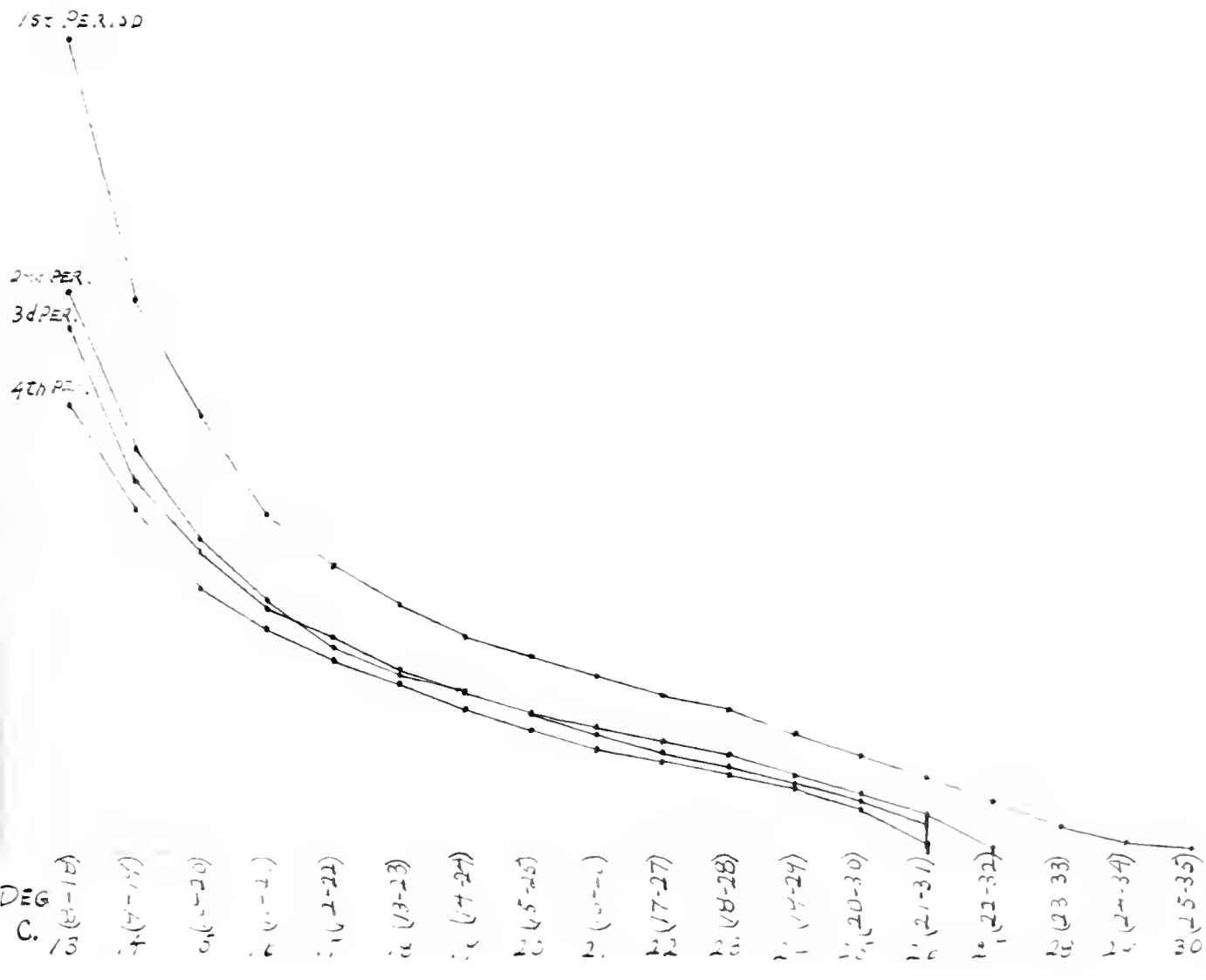
relations of the two processes are alike ; they have the same temperature minima, optima and maxima and the two rates change from one temperature to another in just the same way. If the two graphs fail to coincide throughout, the two rate-temperature relations differ, and just how they differ is apparent from an inspection of the graphs. From any such graph, constructed from adequate experimental data, may be derived the temperature minimum and maximum and also the temperature optimum or optimal temperature range. Furthermore, the different values of the temperature coefficient for the same process, etc., may readily be compared for different temperatures, and the coefficient values for different processes may be compared for the same temperatures. Some of the points brought out by inspection of the group of four coefficient-temperature graphs shown in figure 11. have been mentioned, but many others not here considered may be noted.

The four graphs thus far considered show the relation of temperature coefficients to temperature for the four fungi dealt with in this paper and for the second 24-hour period after inoculation. The four coefficient graphs for Pythiacystis for the first, second, third, and fourth 24-hour periods after inoculation are also shown (figure 15). These graphs are constructed from the data given in tables XIII in the manner employed for figure 14 , they have not been smoothed. The graph for each successive period after the first lies below the one for the preceding period. The progressive lowering (already mentioned) of minimum, maximum and optimum temperature with the successive periods is clearly shown and it is well brought out

that the difference between the growth-temperature relation for the first period and that for the second is by far the most pronounced of all the differences between these relations for successive periods.

Fig. 1. Graphs of [illegible] decline [illegible] incident, as related to [illegible] correspond to [illegible] successive 4-day exposure periods.

PYTHIACYSTIS



From the results of the investigation of the temperature relations of growth in pure cultures of four fungi, (Pythiacystis citrophthora, Phytophthora terrestria, Phomopsis citri and Diplodia natalensis), discussed in detail in the preceding pages, the following generalizations may be now brought together.

It was indicated that there is the usual optimum temperature above and below which the rate of enlargement was smaller with higher or lower maintained temperatures. With maintained temperature progressively higher than the optimum the growth rate is progressively smaller, and the same is true also with temperatures progressively lower, but this decrease in rate is much more rapid in the first case. Growth-temperature graphs (with temperatures as abclassas and growth rates as ordinates) rise from left to right (from lower to higher temperatures) at first slightly concave upward, then becoming convex till the optimu is passed and then fall rapidly toward the temperature axis.

It is to be emphasized that the optimum temperature for the average rate of growth of a given fungus with a given medium is not always the same for different lengths of observation periods.

With culture periods of from three to six days and an observation period 24-hours in length, it was found that in general both the optimum and maximum temperatures for growth shifted to lower temperatures for each successive observation period. There was also corresponding displacement of the apparent maximum temperature downward (from higher to lower temperatures) with each successive observation period. This

displacement was much more pronounced between the first and second 24-hour periods than between any other two consecutive periods except for Phytophthora.

A comparative study of the growth-temperature graphs of the four fungi for the second 24-hour period shows that the total range of temperature within which growth rate values are one tenth or more of the maximum rate, includes from 32.5 to 37 centigrade degrees of the temperature scale. Of this range from 70 to 80 percent is below and therefore only from 20 to 30 percent is above the optimum temperature for growth.

With comparatively low temperatures the growth rate increases with the age of the culture throughout the culture period, and with the highest temperatures it decreases throughout the culture period, this decrease soon bringing the value to zero. With a small range of intermediate temperatures the rate first increases with time and then remains constant, oscillates or decreases.

The 10-degree temperature coefficient (Q_{10}) for each of the four fungi has a high value at the lowest range studied and decreases progressively through lower values to zero. The form of the graph representing the value of the temprrature coefficient as related to different ranges of maintained temperature shows that the value of the temperature coefficient must begin with infinity for some low range, must pass through all finite valtes and then must reach zero for some higher range. For growth-temperature relations of this type the range for which the coefficient is unity will include the optimum temperature, the range for which the coefficient is infinity will include the minimum temperature and the range for which the coefficient is zero will include the maximum temperature.

The use of the coefficient temperature graphs fur-

nishes direct method of comparing the growth-temperature relations of different organisms, no matter in what units the rates have been expressed. If the graphs of two different processes coincide throughout, the growth-temperature relations must be considered to be the same. On the other hand, if the two graphs fail to coincide throughout, their lack of coincidence furnish evidence of the particular manner in which the temperature relations of the two processes differ.

V I T A

The writer was born April 12, 1877, on a farm near Salem, Ohio. His early education was received in the public schools of that vacinity and in the Friend's preparatory school at Westtown, Pennsylvania. After teaching at Stavanger School, Legrand, Iowa, for two years he entered Iowa State College, Ames, Iowa, and was graduated from that institution in 1905, receiving the degree of Bachelor of Science. In the autumn of the same year he entered the University of Florida, as Assistant in Botany and Horticulture.

During the years 1906 - 1908 he was Assistant Plant Pathologist in the Florida Agricultural Experiment Station.

In 1908 he received the degree of Mater of Science from the University of Florida having done his major work under Prof. P. H. Rolfs in an investigation of the fungus parasites of certain insects parasitic on citrus trees. Entomology and Geology were his minor subjects. He was Plant Pathologist of the Florida Agricultural Experiment Station during the years 1908 - 1912.

In 1912 he was appointed Plant Pathologist of the California State Commission of Horticulture, Sacramento, California, and the following year received the appointment of Associate Professor of Plant Pathology in the Graduate School of Tropical Agriculture and of the University of California, located at Riverside.

From Oct. 1916, to June, 1918, he was on leave of absence from the University of California, and devoted this time

to work in the Johns Hopkins University, his principal subject being Plant Physiology, and his subordinate subjects Physical Chemistry and Climatology.