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WM. A. TAYLOR, Chief

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March 20, 1922

**PECAN ROSETTE**  
**ITS HISTOLOGY, CYTOLOGY, AND RELATION**  
**TO OTHER CHLOROTIC DISEASES**

By

**FREDERICK V. RAND**

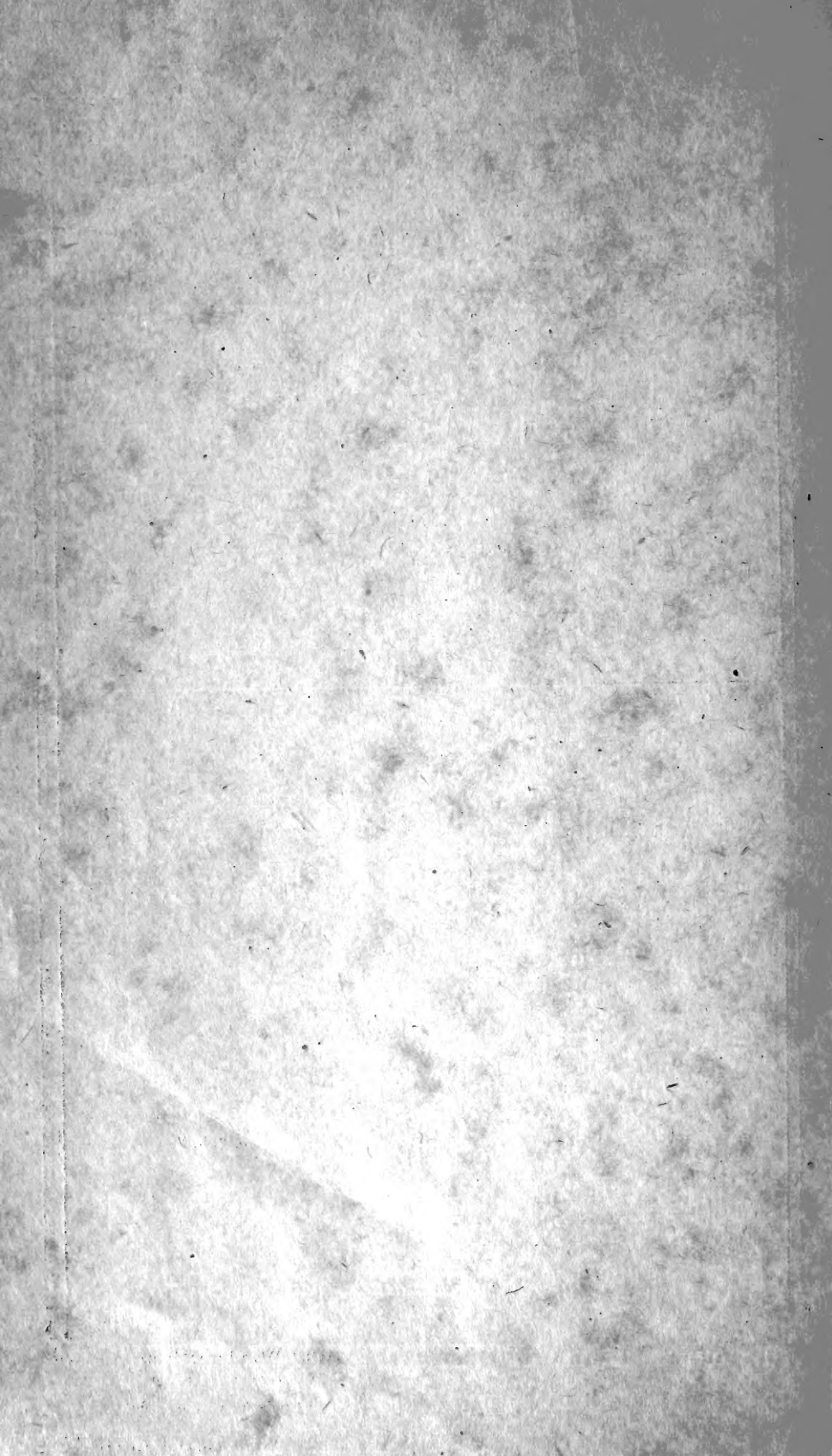
Pathologist, Laboratory of Plant Pathology

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# PECAN ROSETTE

ITS HISTOLOGY, CYTOLOGY, AND RELATION TO  
OTHER CHLOROTIC DISEASES

BY

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EASES.<sup>1</sup>

By FREDERICK V. RAND, *Pathologist, Laboratory of Plant Pathology.*

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TYPES OF CHLOROTIC PLANT DISEASES.

The chlorotic group of plant diseases to which pecan rosette belongs has long been recognized and has presented to the investigator some of the most baffling problems in plant pathology. The potato-mosaic group began to assume alarming proportions in the British Isles and on the Continent toward the end of the eighteenth century and at the beginning of the nineteenth century (27, 45, 59).<sup>2</sup> Peach yellows was known and much written about in the United States near the beginning of the nineteenth century (69). Tobacco mosaic was first described by Mayer in 1886 (52), but more fully treated by Beijerinck in 1898 (17). Other well-known chloroses will readily come to mind in addition to those recently discovered or not so generally recognized.

It is not the purpose of this paper to consider in detail all types of plant variegation. Chloroses have to do with the reduction or

<sup>1</sup> The present study was largely carried out under the direction of Dr. R. A. Harper, of Columbia University, and was completed under the direction of Dr. Erwin F. Smith, of the Laboratory of Plant Pathology, Bureau of Plant Industry.

<sup>2</sup> The serial numbers in parentheses refer to "Literature cited" at the end of this bulletin.

total suppression of chlorophyll, but since a yellowing or bleaching of normally green parts may result from a wide variety of causes chlorosis in itself is a symptom rather than a disease. There are, for example, the chloroses of etiolation and of the normal autumnal ripening of leaves. Moreover, a yellowing of chlorophyll frequently follows upon some types of insect injury, such as that of root aphids on the peach and the grape, and it is a constant accompaniment of certain diseases caused by parasitic bacteria and fungi, such as bacterial black-rot of cabbage and *Fusarium* wilts of cabbage and potato. Again there are the more or less general chloroses due to unfavorable soil or climatic conditions, and finally those infectious chloroses of obscure origin which present a fairly regular sequence of pathological signs, including fundamental derangements in both metabolism and morphogenesis.

Some of these chloroses are true diseases in the restricted sense. Others are not diseases except under a broad application of the term, and certain forms of chlorophyll restriction are clearly not diseases at all.

There are, however, two fairly well marked types of chlorophyll disturbance which are usually included under the chlorotic group of plant diseases. These are (1) the infectious chloroses which are communicable through expressed plant juices or through those juices as directly transmitted within the living plant tissues, and (2) the noninfectious chloroses due to unfavorable soil or atmospheric conditions.

The present study of pecan rosette deals with the histology and cytology together with the sequence of gross symptoms of the disease. In order to place the results of this study in proper relation to other diseases of this type it is necessary to review briefly some of the work of other investigators.

#### CHLOROSSES DUE TO SOIL OR ATMOSPHERIC CONDITIONS.

With respect to those chlorophyll changes due to physical or chemical conditions of soil or atmosphere it is difficult to say just at what point the normal state ends and chlorosis begins.

Certain plants prefer an acid condition of the soil, others tolerate it, others are restricted for their optimum development to neutral or alkaline situations. Nevertheless, neither macroscopic nor microscopic examination of such a plant as field sorrel (*Rumex acetosella* Linn.), for example, would give a clue to its acid-soil toleration. In response to certain environmental changes, however, Transeau (78) has shown that this species does develop anatomical changes. In moist situations, with soil and air temperature approximately identical, the leaves of this species are relatively large, with a loose

arrangement of the tissues, a poorly developed palisade tissue of one-cell layer, three layers of sponge cells, and an epidermis of large thin-walled cells with delicate cuticle. On the other hand, when grown in dry sand or in undrained sphagnum bogs where the soil temperature was several degrees below that of the air the leaves were thickened, reduced in size, and revolute margined. The mesophyll tissue was more compact, with two or three layers of palisade, and two layers of sponge cells. The epidermal cells were smaller, with outer walls and cuticle thickened. In addition to the development of these other xerophilous characters, drops of oil or resin, characteristic of bog plants, were formed on the epidermis and cells adjacent to the bundles; these are absent under moisture conditions more favorable for this species.

Warming (82) states that in acid soils intimately associated with high water content, in a cold or temperate climate, the tendency of plants is toward the development of leaf coatings of hairs, papillæ, or wax; thickened cuticle; mucilage; erect and ericoid, terete, or filiform leaves; with bilateral internal structure. Since these characters develop on wet, moor soils the world over, he considers that there must be a connection between these soils and the xeromorphic structure, and that consequently these soils must be "physiologically dry." These facts also account for the xeromorphic structure of plants in the extreme north or at high altitudes.

The experimental results obtained by Mrs. Clements (23) show that the xerophyte tendency is toward the development of a diplophyll palisade (bilateral) tissue with restricted air spaces and with or without water-storage cells. This prolate closely packed type of cell tends to reduce transpiration. The mesophyte type, on the other hand, approximates an equal development of palisade and sponge cells with moderate looseness of structure. The hydrophyte type consists in the development of simple globose cells and large air spaces. She found that decreased light and increased water absorption caused an increase of leaf surface but a decrease in thickness, while increased light and decreased water absorption brought about a reduction in leaf surface but an increased thickness. Extremes of any factors not at the optimum tended toward dwarfing.

Hanson (39) found differences in total thickness between leaves from the south periphery and the center of the same tree usually greater than the differences hitherto reported between leaves of mesophytic and xerophytic forms of a species. Leaves from the south periphery, as a whole, developed more palisade, greater compactness of structure, and thicker epidermis and cuticle than leaves from within the crown.

Halophytes, or "salt-loving plants," usually develop thick, fleshy leaves which are more or less translucent, owing partly to the abun-

dant cell sap and poverty of chlorophyll and partly to the smaller intercellular spaces. The thickness of the leaves is caused by the enlarged roundish sponge cells and by the massive, often transversely divided palisade. Sodium chlorid thus appears to act morphologically, Warming says, in much the same way as sunlight (82). In the cell sap the solution of salt is more concentrated than in the soil. Side by side with this increased salt content goes a decrease in the development of chlorophyll, due to a reduction both in size and number of chloroplasts. Succulent halophytes usually show at first a dark-green color, later passing over into a yellowish green or red. Wax coatings are characteristic of many salt-loving plants. Some species, such as *Solanum dulcamara* Linn., are dimorphous, exhibiting halophytic forms and also inland forms with thin leaves.

Such general environmental factors as those enumerated may and do have appreciable developmental results. Plants thrive or fail to thrive and may even die, or again various general adaptive morphological changes are brought about, but there are no fundamental derangements in morphogenesis or in metabolism which could properly characterize these conditions as disease.

In this same category of general environmental effects are to be placed the calciphilous or lime-loving plants; and the calcifugous or lime-avoiding plants. Plants grown upon a lime soil, Warming says, tend to a greater pubescence and to a bluish green color and a more divided condition of the leaf (82). Moreover, not only are the chemical but also the physical characters of lime soils to be taken into consideration.

It is but a step, now, from these general lime relations to some of the more specific lime effects usually considered as diseases. In the pineapple chlorosis of Porto Rico sometimes the plant becomes almost ivory white. In other cases the leaves are yellowish white with streaks or patches of green, or the outer leaves may be green while the later developing leaves at the center are white from the start. In still other instances the leaves, normally green for several months, may gradually develop a light-colored mottling until finally the whole leaf becomes blanched. The plants are dwarfed and the reddish or pinkish fruit finally cracks open and decays. Gile (35-37) has definitely shown that this pineapple trouble is primarily caused by a lowering of the availability of iron to plant absorption due to calcium carbonate in the soil. A chlorosis of pineapple in Hawaii (46) appears to be caused by a similar depression of the availability of iron due to the high manganese content of the soil.

Similar lime chloroses of rice and of sugar cane in Porto Rico have been shown by Gile and Carrero (38) to be caused by a lack of sufficient iron absorption. In all these cases spraying with a solution of



iron sulphate evokes a development of chlorophyll and at least a temporary resumption of growth.

Grape chlorosis may also result from this same set of causes and in such cases responds to the ferrous-sulphate spray treatment. According to Mazé (53), grape chlorosis may also result directly from excessive absorption of lime, and in other cases from a deficiency of sulphur in the soil due to lowering of its availability by the action of lime. Furthermore, as a result of water-culture experiments he found chloroses of maize due to lack of both iron and sulphur. Chlorosis in maize was also experimentally induced by the addition to the solutions of various toxic substances, such as lead or methyl alcohol.

The overabsorption of lime was reported by Clausen (22) as causing a chlorosis of oats in Europe.

These plant relations to various salt constituents of the soil bring up the questions of plant absorption, antagonism, and changes in permeability investigated more recently by Loeb, Osterhout, Brooks, Waynick, and others. As the result of a careful series of experiments Waynick (83) concluded that no "optimum calcium-magnesium ratio" appears to exist and that a balance between all ions present in a solution appears to be far more important than any single ratio. He found by chemical analysis that the composition of plants in inorganic constituents may be enormously altered by variations in the surrounding medium. The permeability of the plasma membrane appeared to be changed by the nature and balance of the solution around the roots. The same salt was found to act differently at different concentrations, preserving the normal permeability at certain concentrations but at other strengths allowing a large penetration. With regard to each salt tested, its presence in toxic concentration always resulted in increased permeability of the plant tissues to calcium and magnesium. On the other hand, normal growth was always accompanied by an approximately equal percentage of calcium and magnesium in the plants tested; and in nearly all cases where growth was markedly decreased the amounts of calcium and magnesium were greatly increased in the tissues. The degree of absorption of any salt seemed, over a wide range, to be independent of the concentration present; and growth was the same under widely varying ratios of calcium and magnesium. The findings of Loeb, Osterhout, and Brooks were confirmed in that antagonistic salt action tends toward the preservation of normal permeability.

It will readily be seen that there are many closely intergrading steps or degrees of environmental effects. These are—

- (1) The adaptations without visible changes in structure or metabolism.
- (2) The general adaptive changes in anatomy or physiological processes in response to physical or chemical stimuli from without.

- (a) The dwarfing effects of unfavorable soil or climatic conditions.
- (b) Starvation phenomena due to insufficiency or absence of essential nutrients.
- (c) Chloroses caused by the absorption of toxic amounts of mineral or organic soil constituents.
- (d) The general chloroses due to insufficient or to overabundant water supply.
- (e) Restrictions in chlorophyll development due to reduction of light.
- (f) Finally there are chloroses due to lowering of temperature.

All these reactions, however, are rather general effects which are more or less comparable to starvation, overfeeding, or direct poisoning. There are no profound or strictly localized derangements in both metabolic and anatomical development, and it is a question whether in the restricted sense some of these phenomena should be regarded as diseases at all.

### INFECTIOUS CHLOROSSES.

As opposed to the general chloroses caused directly by soil or climatic conditions are those specific chlorotic diseases of infectious nature and obscure origin in which are simultaneously brought about fundamental derangements in both physiological and structural development. Concomitant with the rise of plant pathology as a science there have come to light an increasing number of diseases of this type until now it seems apparent that almost every plant group may have one or more infectious chloroses.

Reports of early scientific investigations upon two of the principal types of infectious chlorosis appeared at nearly the same time—those upon tobacco mosaic by Mayer (52) and Beijerinck (17) and upon peach yellows by Penhallow (58) and Erwin F. Smith (69, 70).

In peach yellows the sign often first to appear is a red blotching of the fruit on one or more branches, with the color extending through the flesh to the pit. A yellowing of the foliage always occurs at some stage of the disease. Another characteristic feature consists in the premature development of the buds of several series into spindling depauperate shoots with dwarfed and linear and often curled or inrolled leaves. A premature ripening of the fruit also usually takes place. The disease ordinarily affects one or more branches at first, but may develop signs at once over the whole tree. Penhallow (58) found an abnormally loose cellular structure in the bark, but a reduction in the size of the cells and an abnormally dense structure of the wood. Assimilation is profoundly affected and translocation of starch is delayed. The leaves become gorged with starch, and excessive storage occurs in the cortex rather than in the inner bark and the wood, as in normal trees. The oxidizing enzymes are increased in the diseased leaves, and a larger tannin content has been found in the diseased fruit. In one instance (18) delayed starch translocation was also found in an apparently healthy branch con-

tiguous to a diseased branch, showing that external signs may be preceded by deep functional disturbances. Conditions unfavorable to growth tend to intensify the signs of yellows but do not cause the disease (71). Yellows is transmitted by budding or grafting from diseased trees to healthy stock, and infections through the roots as well as the stems may take place in this way. However, all attempts to infect with the expressed plant juices have thus far failed, nor have insect relations been discovered.

Peach rosette (70, 72) and little peach (73) differ in the character and sequence of the signs, but are similar in type to peach yellows. All three diseases induce deep changes in assimilation, translocation, structure, and development. All may appear first in one branch, and they are transmitted by budding or grafting. The disease progresses gradually from the point of infection, requiring longer for the development of external signs in the top when infected by root grafting than when grafted into the branches. That rosette may not affect the whole tree at once was shown in one case where buds from branches showing external signs transmitted the disease, whereas those from the apparently healthy side failed to give infection. The normal side of this tree, however, developed rosette the following season. The outer leaves of rosettes fall early.

In spike disease of the parasitic sandalwood we find a close resemblance to the peach-yellows group. The spikelike appearance of the leaves standing out stiffly from the branches suggested the name "spike disease." The entire tree is not attacked at once over the whole top, but symptoms appear first on one, then on several branches, and gradually spread over the tree. The internodes become shortened and the leaves reduced in size and narrowed. The continuous development of buds into new leaves and branches throughout the year produces a growth closely resembling the "witches'-brooms" of the peach, and with the progress of the disease the leaves become smaller and more chlorotic. No blossoms or fruit are borne in the later stages, though sometimes flowers and fully developed fruit are formed on portions of trees still retaining their normal appearance. Death of the haustoria and fine root ends keeps pace with the progress of the disease.

Spiked leaves and branches contain a marked accumulation of starch (24). In the diseased leaves this starch is distributed throughout the parenchyma, especially in the sheaths of the fibrovascular bundles, and no marked difference in quantity is found at different periods of the day. In the diseased twigs the starch occurs as grains of considerable size in the pith, in the medullary rays, in the wood, and in the bast fibers; whereas in the normal twig such grains are rarely found except when the leaves are fully matured. This accumulation in the twigs precedes external signs of the spike disease both

in natural and artificial infections. Diastase activity in the healthy leaves was found to be almost double that in the spiked leaves. The mesophyll tissues are hypertrophied and the leaves increased in thickness and the vascular tissues become reduced in advanced stages of the disease. Chemical analysis (24) shows a higher percentage of nitrogen and of most of the ash constituents in healthy leaves. Since young, healthy leaves pass through a stage comparable in chemical composition to that of spiked leaves, it seems probable that in the latter case development has for some reason been checked in the early stages of growth.

In a large percentage of Coleman's grafting experiments (24) spike was successfully transmitted to originally healthy stocks, and in almost every case the disease first appeared in the stock on branches closest to the point of grafting and spread from these regions to the other parts of the stock. A considerable time always elapsed before external signs of disease appeared. In all cases examined, infection had spread to the roots and resulted in the death of the root ends and haustoria.

The occurrence of spike disease is not dependent on the fertility of the soil, nor does injury to the roots have any relation to the disease. Venkatarama (81) found experimentally that isolation of the trees from all possible hosts by digging trenches did not cause signs of spike even after two years under observation. Cutting the root connections and removing the haustoria, injecting the lateral roots with strong sulphuric acid, and girdling to the heartwood did not cause the disease. Thousands of trees previously growing under a heavy covering of vines were exposed to the light, but the increased loss of water resulted in nothing resembling spike disease. Fischer (34) states that the spike disease spreads from a center, not appearing simultaneously over considerable areas.

In tobacco mosaic the yellow and green mottling of the leaves is a prominent sign. Not only leaves but also the calyx may be mottled, and the corolla becomes flecked with red and white blotches instead of exhibiting the normal even red or white color. The light areas of the leaves are usually slower growing than the green areas, thus often resulting in distortions which may become extremely marked in young leaves. Often, however, such leaves almost recover from these malformations as they mature (1). Sometimes the laminae are almost suppressed, and in other cases a long, sinuous, ribbonlike leaf is produced. In many cases abnormally dark-green blisters develop on the immature leaves.

Koning (47) and Heintzel (40) reported a separation of the cells in diseased foliage which often leaves spaces nearly as large again as the cell itself. The chlorophyll bodies become distributed in irregular groups, chlorophyll disappears, the cell walls disintegrate, and finally

complete disorganization follows. Woods (84, 85) found that in the lighter areas of badly diseased leaves the palisade parenchyma had not developed at all, but the tissue consisted entirely of a respiratory parenchyma with cells packed together rather more closely than normal. In healthy leaves the palisade cells were four to six times as long as broad, whereas in the moderately diseased leaves these cells were almost as broad as long. The leaf surface becomes depressed in the light areas and raised in the green areas, thus giving a roughened appearance to the lamina.

As first shown by Woods (84), the oxidizing enzymes are greatly increased in the diseased areas. He also found more starch in the form of granules in the yellow areas than in the green areas of the same leaf. The cells were often completely gorged with starch. Examination in the early morning showed only a slight decrease, while healthy tissue at the same time was empty or contained only a trace. Starch translocation in the diseased leaf is greatly delayed in spite of the fact that diastase is present often in larger amount than in the normal leaf, and Hunger (41) from experiments *in vitro* concluded that the retarding effect upon diastase action is caused not by the oxidizing enzymes, but by reducing substances including tannin.

Mayer (52) first showed that transmission of tobacco mosaic could take place through the expressed juices of diseased plants. Iwanowsky (42), and Beijerinck (17) independently demonstrated that the infective principle would pass through the pores of a Chamberland filter, though such a filtrate was less infective than the unfiltered juice. Allard (4) proved that infection fails to result after the juice from diseased plants has been passed through a Livingston porous-clay cup filter. Transmission of the disease by an infective principle in the expressed juice was thoroughly demonstrated, and it was shown by Allard and others that oxidizing enzymes do not constitute this infective principle (4). Such plant juices diluted to 1 to 1,000 in water were quite as infective as the undiluted juice; attenuation was indicated at 1 to 10,000, while at greater dilutions infection was found unlikely to take place (2). The virus is infectious to all susceptible plants, but such plants never develop mosaic so long as chances for infection are excluded, and this regardless of soil and climatic conditions. The infective principle may be present in all parts of a diseased plant except within the seed and has been demonstrated even in the trichomes (3, 6). Furthermore, infection may occur through inoculation of the trichomes alone. Cutting the midrib at the base or severing the larger veins on one or both sides does not prevent the final dissemination of the infective principle to all parts of the leaf and to other leaves of the plant. Environmental conditions may partly or even wholly mask the external signs for a time, but can neither cause nor cure the disease.

Nishimura (55) found that the bladder cherry (*Physalis alkekengi* Linn.), after inoculation with tobacco-mosaic virus is capable of acting as a carrier without itself showing external signs of the disease. Cross-inoculation experiments (5) have shown that mosaics in tobacco, tomato, pepper, and petunia are caused by the same infective principle. On the other hand, the mosaic of pokeweed (*Phytolacca decandra* Linn.), though readily transmitted by expressed plant juices within the species, was not found to be cross inoculable on tobacco or vice versa (8). Tobacco mosaic is transmissible by insects.

Another type of infectious mosaic is that carefully worked out by Baur (11-16) in the Malvaceæ. He showed that the Abutilon mosaic is transmitted only by grafting and not by inoculation with the expressed juices, as occurs in tobacco mosaic. As in the latter disease, however, seed transmission does not occur. He found that when scions of the immune *Abutilon arboreum* Sweet are grafted on the variegated *A. thompsoni* Hort. they grow vigorously and remain apparently normal. However, if scions of the green but susceptible *A. indicum* Sweet are now grafted upon the immune *A. arboreum* they become infected and develop the typical mottling. On the other hand, the contagium passing through the immune *A. arboreum* is not capable of remaining there and giving infection if this portion of the shoot is subsequently grafted into a susceptible stock. In the case of the immune *Lavatera arborea* Linn., however, there is no transmission at all when double grafted between the mottled *Abutilon thompsoni* and the green susceptible *A. indicum*. Baur succeeded in transmitting this mosaic by grafting to about 50 species and varieties of Abutilon and related plants.

It was found that if the leaves of variegated plants were removed, or if the shoots were cut back so that no leaves remained and the new shoots developed in darkness, only the first two or three leaves were mottled. If these mottled leaves were then removed the plants remained permanently green in the light unless they were grafted with scions from other variegated plants. However, if axillary buds on old parts were forced into growth these produced shoots with mottled leaves which in turn infected all the newly formed leaves on the plant. Furthermore, when scions of a green but susceptible variety were grafted upon defoliated, mottled plants the scions remained green; but here again, if a mottled shoot was allowed to develop from the stock it rapidly infected the whole plant. The contagium is,<sup>3</sup> therefore, capable of infecting only the embryonic leaves, and in the buds it may be stored up for months in inactive form.

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<sup>3</sup>The term "contagium" has been suggested by Dr. H. M. Quanjer as synonymous with any infective principle, whether of known or unknown origin. Its use in the place of "virus" with reference to the so-called "filterable-virus" diseases does away with objectionable connotations and leaves nothing to be taken back.

In varieties where the size and distribution of the yellow spots made it possible, Baur found that by carefully cutting out all yellow spots and continuing this process on all newly developing leaves for one or two weeks, finally green leaves only were formed. From this result he considered it certain that the contagium is present in the yellow spots but only in sufficient quantity to infect about three or four newly developing leaves at the growing point. After this it is apparently used up, and leaves subsequently formed remain green. Darkening the assimilating leaves of a mottled plant led to a similar result. Here the first leaves to develop thereafter were yellow spotted, but if these also were darkened before they began to assimilate, the subsequently developed leaves were all green.

Girdling experiments demonstrated that the contagium is carried only through the bark. Different species and varieties of Malvaceæ were found to vary widely in susceptibility and also in the incubation period, but several days at least are required. Baur also demonstrated infectious chloroses in *Fraxinus*, *Laburnum*, *Sorbus*, *Ptelea*, *Euonymus*, and *Ligustrum*.

In potato mosaic the mottling is irregularly distributed irrespective of the venation; and, moreover, profound dwarfing, with curling, crinkling, and further distortions of the foliage occur in the more severe attacks of the disease. The parenchyma tissues are less perfectly developed in the light-colored areas, the palisade cells tend to shorten up and chlorophyll development is restricted. Potato mosaic (65, 67) is transmitted by the tubers, by grafting, and by inoculation with the expressed juices of diseased plants; and it is also disseminated by aphids.

In potato leaf-roll the leaves become inrolled from the margin, reduced in size, and of a paler green to yellowish cast. A necrosis of the phloem (60-62) seems to be characteristic of the disease, but whether this condition is specific for leaf-roll is still a moot point. The disease is transmitted by means of the tubers, by grafting, and by insects (66).

In both potato mosaic and leaf-roll portions of the healthy plants growing near diseased plants contract the disease, but not all tubers from such secondarily infected plants necessarily develop diseased progeny. Affected leaves in both these diseases exhibit deep-seated assimilatory derangements, including increase in activity of the oxidizing enzymes and gorging of the leaves with starch, with greatly delayed starch translocation. In both these diseases tuber formation is greatly restricted. In neither case are these diseases induced or cured by soil or climatic conditions. However, certain environmental factors may temporarily mask the external signs though such diseased plants still retain their power to infect.

The infectious chlorosis of the sugar beet known as curly-top is not transmitted by expressed plant juices nor by way of the seed, but is disseminated by the sugar-beet leafhopper (*Eutettix tenella* Baker). This insect is capable of producing infection only after a definite incubation period subsequent to feeding upon diseased plants. Apparently there is no other agent of transmission for this disease (10, 68).

Some extremely interesting insect relations of spinach blight have recently been worked out by McClintock and Loren B. Smith (49). Not only were healthy plants successfully inoculated by needle-pricks with the contagium from diseased plants and with the crushed juice of aphids fed upon diseased plants but the potato aphid (*Macrosiphum solanifolii* Ashmead) and the spinach aphid (*Rhopalosiphum persicae* Sulzer), free from infection at first, were demonstrated to transfer the blight to healthy spinach after feeding upon diseased plants. Control plants invariably remained healthy. Later, these two species were obtained from four different States where spinach blight did not occur, and they failed to induce the disease on healthy plants until after they had fed on blighted spinach. The same two species collected locally and tested at the same time produced the disease. These investigators demonstrated that the contagium may be carried from spring to fall by a direct line of aphids. Transmission tests with several other species of insects gave negative results, thus also tending to show that the insect relation to spinach blight is not that of a purely mechanical disseminator.

Sugar-cane mosaic has been shown by Brandes (19-21) to be transmitted by cuttings, by expressed juices from diseased plants, and by certain insects (*Aphis maidis* Fitch) fed upon infected plants. No evidence of seed transmission was found. Insect transmission of corn mosaic has also been demonstrated by Brandes.

That cucurbit mosaic is transmitted by the expressed plant juices and by insects has been definitely proved by Doolittle (28), by Jagger (43, 44), and by Doolittle and Gilbert (31); and the latter investigators have apparently shown that at least in some cases the disease is carried over by the seed (30). In this disease both foliage and fruit become yellow mottled and distorted, and growth of the entire plant is seriously checked. The dark-green portions of diseased leaves are slightly thicker than normal, thus accounting for their blistered and distorted appearance. The yellow areas, though thinner than contiguous dark-green parts, are of about the same diameter as in the normal leaf. The palisade cells of the green areas are crowded closely together and are somewhat longer and narrower than in the normal leaf. In the yellow parts these cells are more nearly isodiametric and less in number than normal per unit of area.



The spongy parenchyma of these parts is also more compact, and the intercellular spaces are smaller than in the green areas. The chloroplastids are decidedly smaller and often are pressed so closely against the cell wall as to be almost invisible. In the fruit the structural derangements are similar in general to those occurring in the diseased leaves.

Dilutions of the virus up to 1 to 1,000 were found to be just as potent as the undiluted juice expressed from diseased plants, but at dilutions greater than 1 to 10,000 no infections took place. Where infections took place with the higher dilutions, the incubation period was no longer than when undiluted juice was used, thus showing a rapid reproduction of the virus within the plants. This virus was found to be entirely removed by passage of the expressed juices through porcelain filters of the finer grades (29).

Taubenhaus (76) reported experiments in which mosaic of sweet peas was transmitted by insects and by needle inoculations with plant juices.

Reddick and Stewart (63, 64, 75) found mosaic of beans transmissible by rubbing the young leaves of normal seedlings with crushed leaves from mosaic plants and obtained a high percentage of mosaic by sowing seeds from diseased plants. In cases of inoculation external signs usually appeared after about four weeks.

Many other chlorotic diseases such as aster yellows, cassava leaf-curl, mulberry dwarf, cotton leaf-curl, little-leaf of the vine, citrus mottle-leaf, raspberry leaf-curl, apple rosette, and mosaics of peony and sweet potato present characters suggesting a possible relation to the group of infectious diseases. However, they have scarcely received sufficient study for any final statements regarding infectivity or causes. With this brief general review the particular disease under investigation may now be considered.

## STUDIES OF PECAN ROSETTE.

### RESULTS OF PREVIOUS WORK.

Pecan rosette was recognized by orchardists as far back as 1900, but no early published references to the disease have been found. Field investigations by W. A. Orton were undertaken in 1902 and continued about four years. During the years 1910 to 1913 field studies were carried out independently by the writer. The results of these two sets of field studies were brought together and published as a joint paper (56). With the exception of a few brief references to the disease, this was the first published account of pecan rosette.

The disease is fairly well distributed over the pecan-growing regions of the Southern States, but has not been reported from the northern limits of pecan culture.

## TRANSMISSION.

In the investigation by Orton and Rand (56) negative results were obtained with inoculations using bits of diseased buds or tissue taken from beneath the bark of rosetted shoots, inserted into slits in the terminal branches of healthy nursery trees. Cultural methods and microscopical examination likewise gave negative results, showing the apparent absence of fungi or bacteria in still living rosetted twigs and branches.

Normal buds or scions worked on rosetted stocks all developed rosetted shoots except in one case where the stock itself recovered. On the other hand, rosetted buds and scions worked on apparently healthy stocks usually developed into normal shoots; in the cases where rosette did develop in such buds or grafts the percentage was no greater than in adjacent stocks worked with supposedly normal buds or grafts in the commercial propagation.

## ENVIRONMENTAL RELATIONS.

The observations and orchard records by Orton and Rand (56) showed that pecan rosette is not absolutely limited to any soil type, topography, or season. The disease was found at least to some extent in practically all kinds of soils where pecans were observed, with the single exception of parts of some orchards where the land tended to be swampy. In the latter case very little growth was made, and the trees finally developed a diffuse general chlorosis, but no signs of any phase of rosette. The disease, however, was observed to be particularly prevalent under poor soil or cultural conditions for the species, such for example as in the dry uplands of Texas, or the washed-out hillsides of the Southern States. The disease was found to be comparatively rare in the alluvial river bottoms of Texas, Louisiana, and Mississippi, where the tree is under native environmental conditions.

In most cases where rosetted trees were transplanted into apparently better local soil conditions, the larger percentage of such trees and often all of them resumed normal development; and all rosetted nursery trees recovered when shipped from the South and transplanted in the open or in potted garden soil at Washington.

Nearly all healthy trees used to replace rosetted orchard trees subsequently developed the disease; whereas only about half of those replacing healthy trees later contracted the disease.

In a three-year fertilizer test on level uniform soil cases of rosette developed in 9 out of 11 plats where lime was used; and the largest number of cases and severest attacks occurred on two plats each receiving lime<sup>4</sup> and acid phosphate, in one case combined with

<sup>4</sup> Fertilizers were applied at the following rates: Lime (CaO acted on jointly by air and water), 1 bushel per tree; nitrate of soda, 8 pounds; cottonseed meal, 32 pounds; muriate and sulphate of potash, 8 pounds; acid phosphate, Thomas phosphate, and ground bone, 24 pounds; stable manure, a liberal application.

muriate of potash, in the other with nitrate of soda. The two limed plats free from rosette received in addition cottonseed meal and Thomas phosphate, respectively. In the five plats without lime no rosette at all developed with the exception of doubtful signs in two trees immediately contiguous to a limed plat. The four lime-free plats showing no traces of rosette were the control, untreated, and three plats treated respectively with muriate of potash and acid phosphate, stable manure alone, and stable manure with ground bone. During this period no other cases of rosette developed in the vicinity of the experimental block, though cases appeared in other parts of this orchard of 700 acres. In two other fertilizer tests where the disease was already present at the start, it increased somewhat in severity of attack or in the number of new cases in the plats receiving lime.

Analyses of the subsoil around normal pecan trees in parts of an orchard free from rosette gave 0.5 to 9.5 per cent of calcium. It appears then that the disease is not caused by the presence of lime alone, since more lime occurred here than in parts of the orchard where rosette was present. Ash analyses of normal and diseased leaves and twigs showed only slight or highly variable differences. Apparently, however, the percentage of potassium is greater in the diseased leaves and twigs.

In one spray test with Bordeaux mixture on rosetted trees negative results were obtained.

It is evident from numerous orchard records covering periods of 2 to 12 years that pecan rosette fluctuates from year to year without any variation in fertilization or cultural methods. The diseased trees may apparently make a complete recovery and remain normal for an indefinite period, or after one or more years may again contract the disease. However, in the majority of cases of recovery observed, the trees had not reached the stage where the branches were dying back. It seemed thus (56, p. 165, 169) highly probable that seasonal climatic changes, such as variations in precipitation or moisture content of the soil, might have at least an indirect relation to the prevalence of rosette. In large orchards the more or less simultaneous appearance of rosette in patches, and its usual limitation to these areas, suggested some connection with soil phenomena.

From the apparent nontransmissibility through the seed, negative results in attempts at isolation of organisms, the apparently negative results of budding and grafting between normal and diseased trees, and the results of transplanting tests it was concluded that the disease was probably nonparasitic.

From pruning and "dehorning" tests, transplanting and fertilizer experiments, dynamiting of soil around rosetted trees, and from orchard records of disease fluctuation it was at that time considered

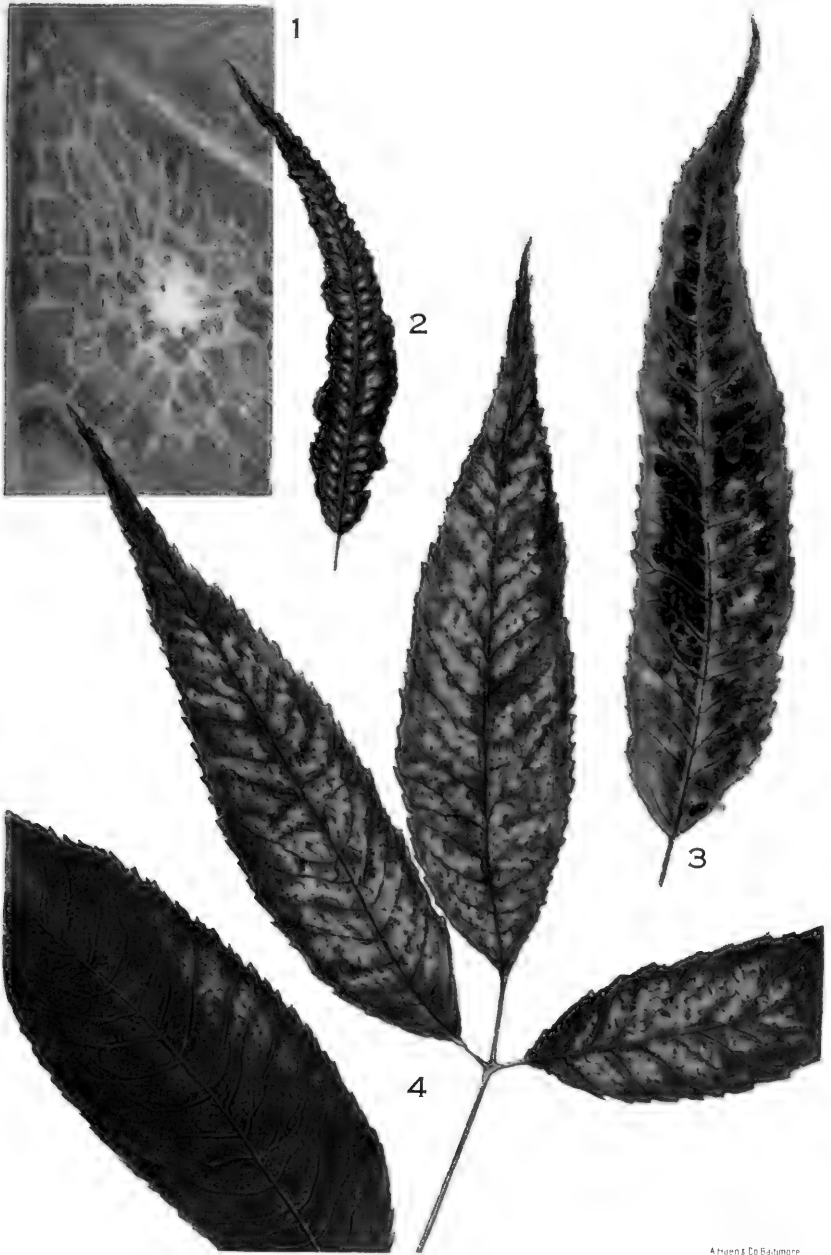
highly probable that pecan rosette belonged to the group of non-transmissible chlorotic diseases caused by improper nutritive supply or injurious physical conditions. The possibility of the presence of some parasitic organism was not entirely precluded, but it was thought highly probable that the ultimate cause would be found in some lack of balance in nutritive supply, or possibly in some toxic substance or substances in the soil. These conclusions were based entirely upon the results of field experimentation.

Miller (54) observed that buds from rosetted trees worked upon healthy stock in most instances grew into normal trees, but that when a tree was decidedly rosetted its buds would sometimes develop the disease when worked upon healthy stock. He is of the opinion that rosette is due to soil relations and observed that in dry seasons the disease is more prevalent. Rosette and a proper amount of moisture, he says, do not go together. Impoverished soil, lack of humus, overstimulation of growth, and use of improper fertilizers all favor rosette. He states that the severest cases are incurable or at least not curable by practical means.

Fawcett (33) refers to pecan rosette in a short paragraph, and Crittenden (26, pp. 44-45) briefly summarizes the work of Orton and Rand.

Matz (51, pp. 139-141) in a bulletin relating to various pecan diseases and insects devotes a short section to rosette. He states that the disease apparently occurs on all types of soil and at all seasons, but that wherever it occurs it is most abundant during late summer. He also says that rosette is more abundant on higher and more exposed soils than in low and more protected situations. After briefly describing the signs of the disease he adds that many of the dead leaves adhere to the branches throughout the winter unless blown off by strong winds. The disease is favored by planting in open, sandy soil or where a hardpan exists near the surface.

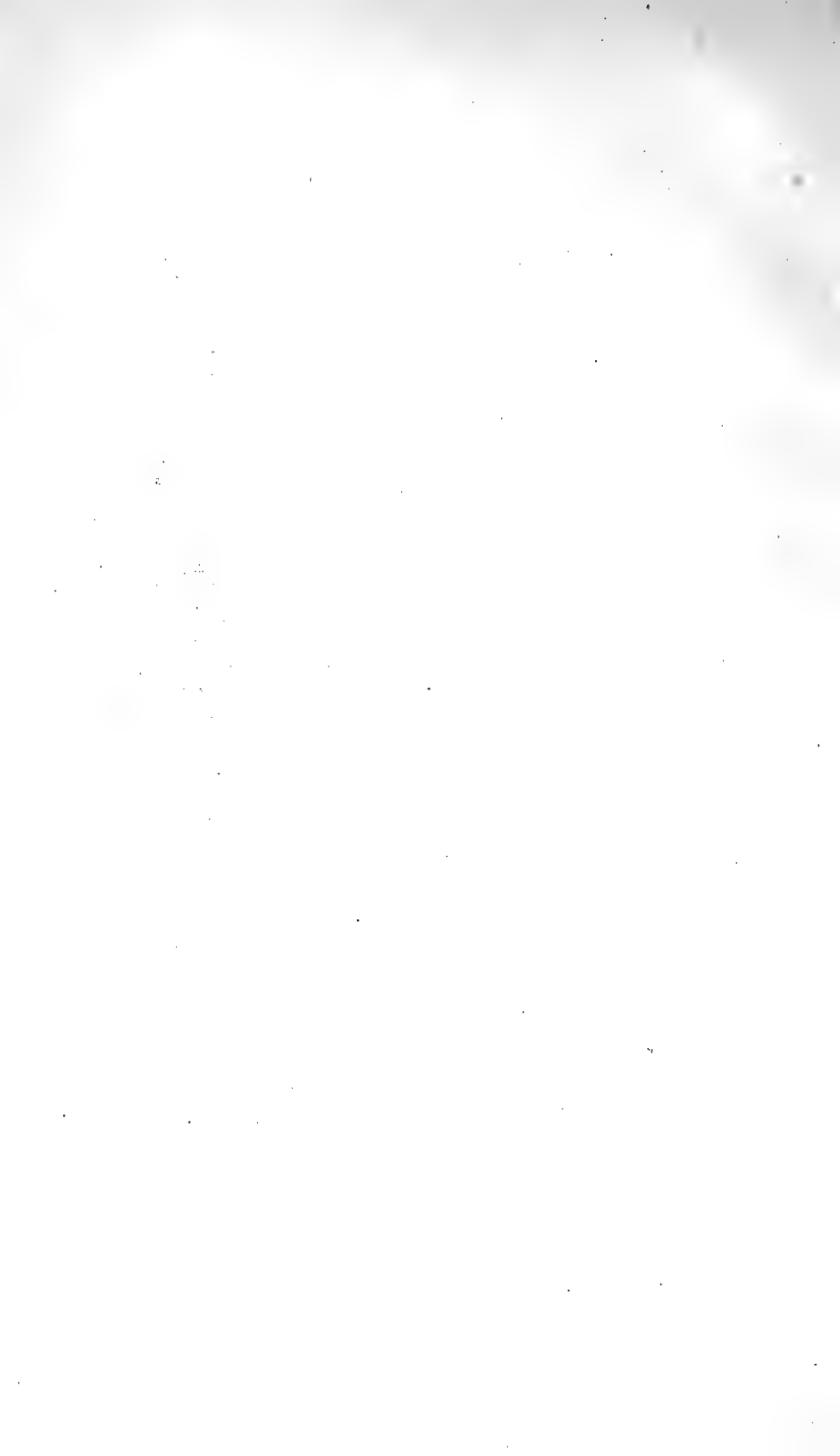
McMurrin (50) in a paper dealing with field experiments and observations states that rosette is generally considered to be the most serious pecan disease. It is found upon a wide range of soil types and under various conditions of culture and fertilization; but one factor, he says, appears to run through it all. On the river flood plains of Southern Louisiana the disease is practically unknown. Here the soil is deep and black and of high fertility and presumably of high water-holding capacity as compared with the typical sand, sand-clay, and clay soils of the Atlantic and Gulf Coastal Plains where the disease is so prevalent. In Georgia and Florida probably 90 per cent of the affected trees are on hilltops and slopes. All cases on the bottoms that have been examined were found to be in deep sand or in a clay or sand-clay soil underlain at 2 or 3 feet by sand. It was noted further that large healthy trees 5 to 10 years old



DIFFERENT STAGES OF PECAN ROSETTE.

[ILLUSTRATION BY J. F. BREWER.]

FIG. 1.—Enlargement of one of the yellow spots on a pecan leaf in the secondary stage of rosette showing the distortion of vein islets and their radial expansion around a focal center. X about 30. FIG. 2.—Pecan leaflet in the secondary stage of rosette, showing the distribution of yellow spots between the side veins and the crinkling and roughening of the leaf surface. Natural size. FIG. 3.—Pecan leaflet showing the red-brown stage of rosette which often follows either the primary or the secondary stage. Natural size. FIG. 4.—Pecan leaves in the primary stage of rosette, showing no external signs of the disease except the yellow mottling between the side veins. Natural size.





**NORMAL PECAN LEAF, FROTSCHER VARIETY.**

Collected on August 25, 1919, at Thomasville, Ga. Photographed by transmitted light to show the texture and opacity of the leaf.  $\times \frac{1}{2}$ .



ROSETTED AND MOTTLED LEAVES OF PECAN, FROTSCHER VARIETY.

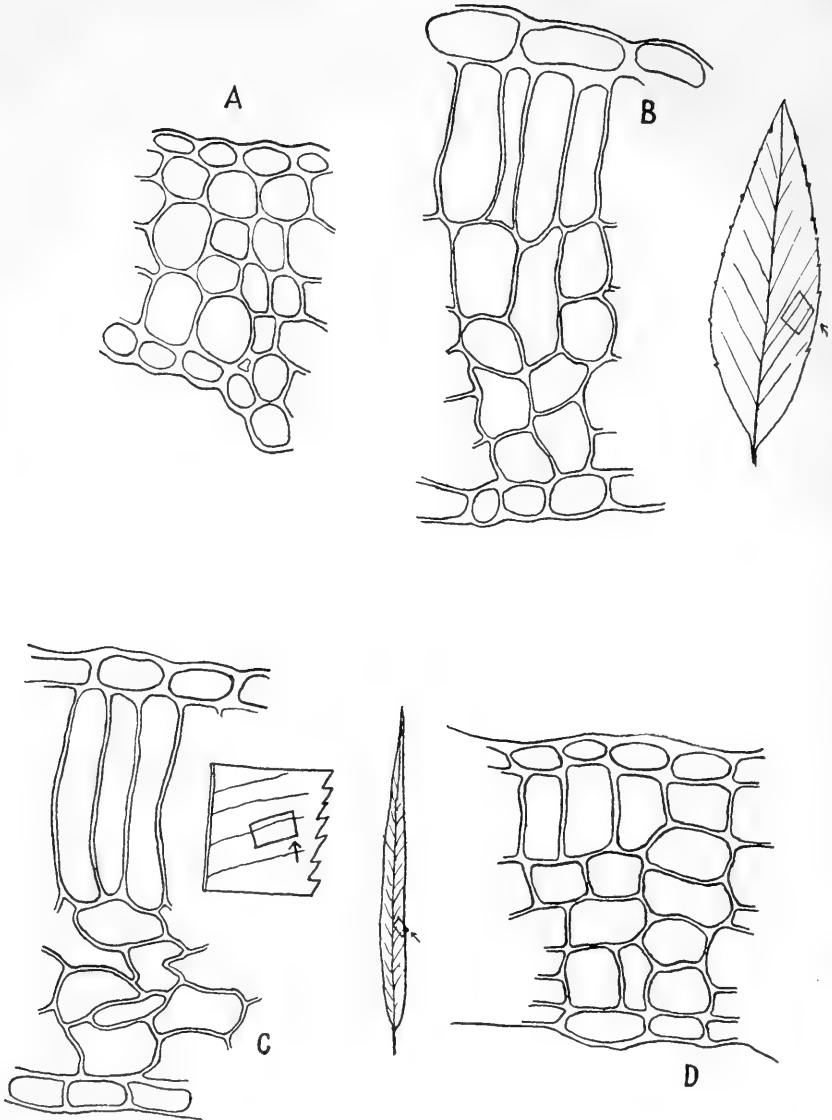
Collected on August 25, 1919, at Thomasville, Ga. Photographed by transmitted light to show reduction in size, yellow mottling, and abnormalities in form and texture of the leaves.  $\times \frac{1}{2}$ .





**MOTTLED LINEAR LEAVES OF PECAN, FROTSCHER VARIETY.**

Collected on August 25, 1919, at Thomasville, Ga. Photographed by transmitted light to show abnormal shape and texture.  $\times 4$ .



FREEHAND VERTICAL SECTIONS OF PECAN LEAVES, FROTTSCHER VARIETY.

Semidiagrammatic camera-lucida drawings of diseased and healthy leaves, showing tissue changes due to rosette. Collected on August 25, 1919, at Thomasville, Ga. All of same magnification. The small side diagrams show the relative size and shape of the leaves and the locations of the sections. A.—Section through the center of a thin, yellow area, showing close packing of the cells and lack of differentiation. B.—Section at the margin of a leaflet, showing the close packing of the cells and the partial decrease in the long axis and increase in the short axis of palisade cells. C.—Section through a healthy leaflet, showing palisade and spongy tissue well developed and the looser arrangement of the spongy cells, the narrower openings being intercellular spaces. D.—Section of a mottled, linear leaflet, showing the close packing of the cells and only partial differentiation of the palisade cells. Drawings by the writer.

often showed marked signs of rosette the first year after transplanting. Trees in low situations where humus and fertility accumulate from year to year were almost always found to be uniformly vigorous and free from disease. Briefly stated, 90 per cent of the cases of rosette were found under conditions indicating lack of humus, plant food materials, and moisture.

Fertilizer tests (50) showed in two years a marked improvement in rosette cases and also many cases of [apparent] recovery. Stable manure, particularly, gave excellent results. Rosetted trees in the plat that received ground limestone at the rate of 3 tons to the acre not only failed to improve but were more severely attacked at the end of the third season following its application than at the beginning.

Examination of large numbers of trees (50) showed that the feeding roots are distributed through the surface soil, and in proportion as this is deep and fertile do pecan trees usually attain their normal development and vigor. Long hot, dry periods often kill many of the feeding roots in the shallow surface soils; and deep sand, clays underlain by sand, and eroded hillsides were found particularly to favor rosette. An acid soil, according to McMurrin, is probably not the cause, since river flood plains nearly all exhibit an acid soil, and pecan rosette under these conditions is a rarity.

#### EXTERNAL SIGNS OF ROSETTE.

Every phase of the disease is observed on trees of all ages, from young seedling or budded and grafted stock in the nursery row to trees of long-established maturity.

In every distinct case the constant sign of rosette consists in the final development of undersized, more or less crinkled and yellow-mottled leaves (Plate I, fig. 2; Pls. II to IV), particularly at the ends of one or more branches. This phase may be properly designated as the secondary stage of the disease. The chlorotic areas are situated between the principal veins, while portions adjoining these veins and along the margins of the leaflets are green. In severe cases these intervascular chlorotic areas are thinner than in healthy leaves, while along the midrib and principal veins the blade is often somewhat thicker than normal. This condition gives the leaf a peculiarly rough and furrowed appearance and causes the veins to stand out characteristically. Such leaves do not attain their normal size, are often linear (Pl. IV) and otherwise malformed, and present a crinkled or undulated appearance of the laminae. Parts of the laminae are often suppressed; sometimes the leaflet consisting merely of the midrib bordered by an edging of ragged tissue. In laminae otherwise fairly normal in general form, portions of the mesophyll

tissue occasionally fail to develop; as a result of subsequent growth stretching in contiguous tissues the blade becomes dotted with smooth-margined holes suggestive of healed insect punctures.

During the early course of the disease, or in cases of very slight attack, yellow mottling may be the only external sign (Pl. I, fig. 4), the size, shape, and texture of the leaves appearing normal. This primary stage is less characteristic than the secondary stage, but here also the chlorotic mottling is confined to areas between the principal side veins. The regions along the veins and leaf margins remain green. With advance of season the chlorotic areas of both primary and secondary stages often turn a dark reddish brown. (Pl. I, fig. 3.) Signs may appear over the whole tree at once, but frequently only one or more branches on a tree are affected at first. Early indications of rosette often consist in the appearance late in the season of a few mottled leaves near the tip of one or more branches, the remainder of the tree appearing normal. Leaves developing signs earlier in the season often present a general bronzed appearance during late summer and fall, and particularly is this true under conditions of drought.

Later, where the branches also become affected, there is considerable reduction in growth, so that the aborted leaves become clustered together on a shortened axis, giving the characteristic bunched appearance of the foliage at this stage (Pl. III, figs. A and C). It was this close bunching of the leaves that led Orton originally to apply the name "rosette" to the disease (56, p. 151). A few nuts are often borne on branches not too severely attacked, but they are usually malformed and reduced in size.

Affected trees may continue thus for several years, or they may appear to recover completely after showing moderate signs for one or more seasons. However, in severe cases where the signs have spread over the whole tree and in some instances where only one or more branches are severely attacked, the affected branches begin to die back from the tip during the latter half of the growing season. At first brownish spots and streaks develop in the chlorophyllous inner bark, and these dead areas increase in size until the bark and cambium are disorganized and the end of the twig or branch dies. This staghorn phase is followed during the current and subsequent seasons by development of abnormal numbers of shoots from dormant and adventitious buds. Usually in young rosetted trees the first shoots of the season are abnormally large and succulent, and the leaves are dark green and larger than normal. This is probably in part due to the severe pruning induced by the staghorn phase, since similar results are obtained by severe artificial pruning during earlier phases of the disease. Toward midseason, however, the mottling begins to appear and the later developed leaves present the

dwarfed, mottled, and roughened appearance typical of the secondary phase. Dormant and axial buds of one to several series may and usually do prematurely develop into depauperate shoots, and toward the end of the season clusters of dwarfed branches are usually put out from dormant and adventitious buds farther back on the branches or main trunk. With each repeated sequence of premature abnormal growth and subsequent dying back of the branches, the new twigs and leaves tend to become more and more depauperate, so that a well-marked case of several years standing presents a characteristically gnarled appearance.

#### HISTOLOGICAL AND CYTOLOGICAL STUDIES.

No less striking than the external changes brought about by rosette are the internal abnormalities of structure and metabolism in the leaf. The abnormal histological characters develop with the development of the leaf, and the most far-reaching internal derangements are found in cases of the greatest external malformation.

Within the yellow thin areas between the larger side veins (secondary stage) the tissue is usually less developed than normal, and the cells are more closely packed together (Pl. V, figs. A and B; Pl. VI, figs. E and F). In less severe cases the palisade tissue is well developed, but the intercellular spaces become almost obliterated (Pl. VI, figs. A, B, and D). In other instances the palisade cells are differentiated, but the individual cells are greatly shortened vertically (Pl. V, figs. B, C, and D) and may be only two or three times as long as broad, instead of seven to ten times, as in the normal leaf. In the most severely affected leaves with extreme variations in leaf diameter there is no differentiation into palisade and sponge tissue at all in the center of these yellow spots (Pl. VI, figs. E and F), but the tissue within these parts of the leaf consists entirely of closely packed, more or less isodiametric cells without conspicuous air spaces. Under these conditions the number of cell divisions may be somewhat increased, the cells remaining smaller than normal (Pl. VI, figs. E and F). Usually, however, the number of divisions is reduced, and in some instances a parenchyma tissue only three cells deep has been found. (Pl. V, fig. A.) Occasionally the entire tissue between the margin and the midrib consists of undifferentiated cells. That this lack of differentiation is not due to immaturity was shown by examination of young healthy leaves just after their emergence from the bud. Even at this young and only partially expanded stage the palisade tissue of healthy leaves was found to be well differentiated (Pl. VII, fig. C).

In the thickened portions along the veins, or sometimes throughout the laminae in the aborted nonmottled leaves of depauperate rosettes, the size of cells may become increased in all three dimensions without

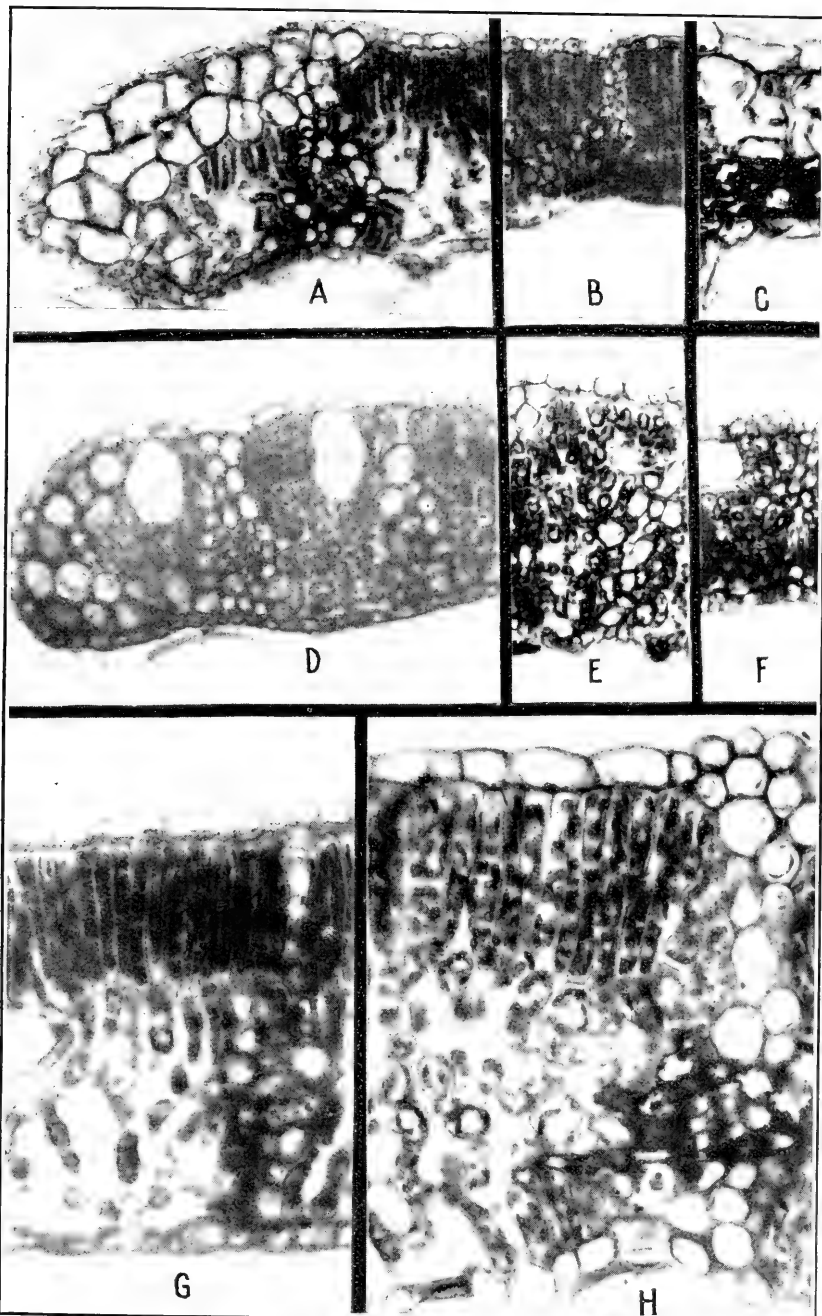
essential change in ratio between spongy and palisade tissue. (Pl. VI, figs. G and H; Pls. VIII and IX.) In some cases a slight increase in number of cell divisions also takes place (Pl. VI, fig. E). The individual cells appear abnormally healthy, and increase in leaf thickness here is due largely to increase in size of the individual cells (Table I). Along with this increase in cell size there is a reduction in size of intercellular spaces within the spongy parenchyma, and in the severely attacked leaf an almost complete obliteration of air spaces results (Pl. VI, figs. B to F).

TABLE I.—*Tissue and cell measurements of normal pecan leaves compared with those of leaves hypertrophied with rosette.*

Variety and leaf tissue.	Tissue and cell measurements (microns).			
	Normal.	Rosette, about halfsize.	Rosette, aborted.	Rosette, linear.
Frotscher variety, Thomasville, Ga.:				
Palisade cells...dimensions..	50 to 52 by 5.5 to 6.	57 to 60 by 6.5 to 9.5	58 to 67 by 8.5 to 10.	.....
Spongy tissue...thickness..	87 to 100.....	76 to 116.....	114 to 116.....	.....
Spongy tissue where palisade tissue is lacking:				
Upperepidermis.....	9.4 to 12.6.....	10 to 12.2.....	11.2 to 14.....	.....
Lower epidermis...do....	10.3 to 11.....	10 to 11.....	9.4 to 11.....	.....
Van Deman variety, Cairo, Ga.:				
Palisade tissue...thickness..	53.....	69.....	.....	57
Spongy tissue...do.....	84.....	125.....	.....	94

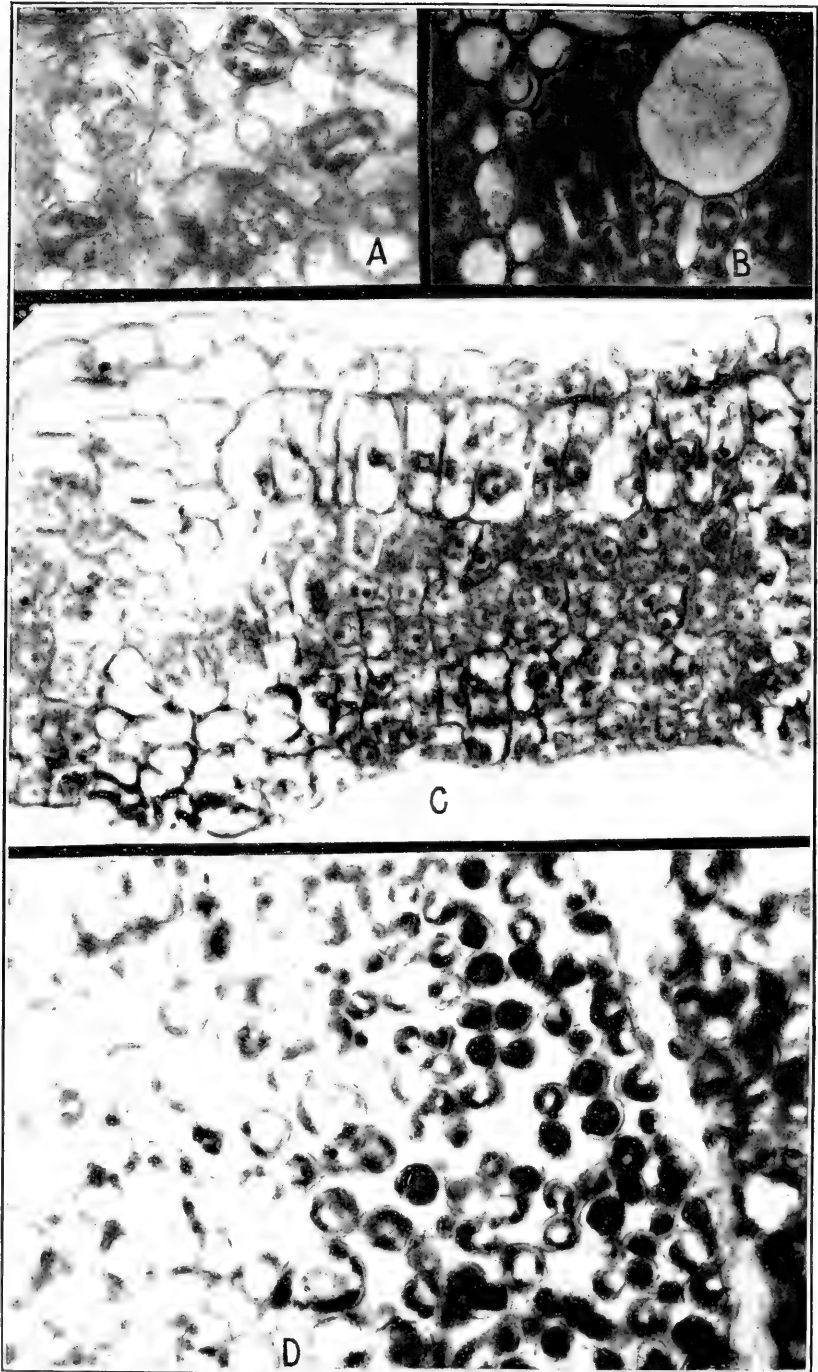
Linear leaves may or may not show a differentiation into palisade and spongy tissue, but where the palisade cells are developed they are usually shortened vertically and the spongy cells of the parenchyma are more closely packed together than the cells in healthy leaves (Pl. V, fig. D). The average thickness of these leaves tends to be less than normal and this reduction is due partly to decrease in the number of cell divisions and partly to the shortened vertical axis of the palisade cells. The elongated shape of the leaves, however, is not due to variations in cell shape but rather to a decrease in the number of cell divisions in which the central spindles are perpendicular to the midrib. This would tend to keep the cells closer to the main water supply of the leaf.

Amelung (9) working with plants and Conklin (25) working with animals have shown that normal tissue cells of corresponding organs or parts of organs within a species or variety are in general of the same size and that the size of organs is primarily due to differences in the number rather than in the size of cells. Mrs. Tenopyr (77), as a result of investigations in several species of plants, found that difference in the shape of leaves of the same plant or related species is not correlated with difference in the shape of their cells. Linear leaves are not composed of longer, narrower



· VERTICAL SECTIONS OF PECAN LEAVES KILLED WITH CARNOY'S FLUID.

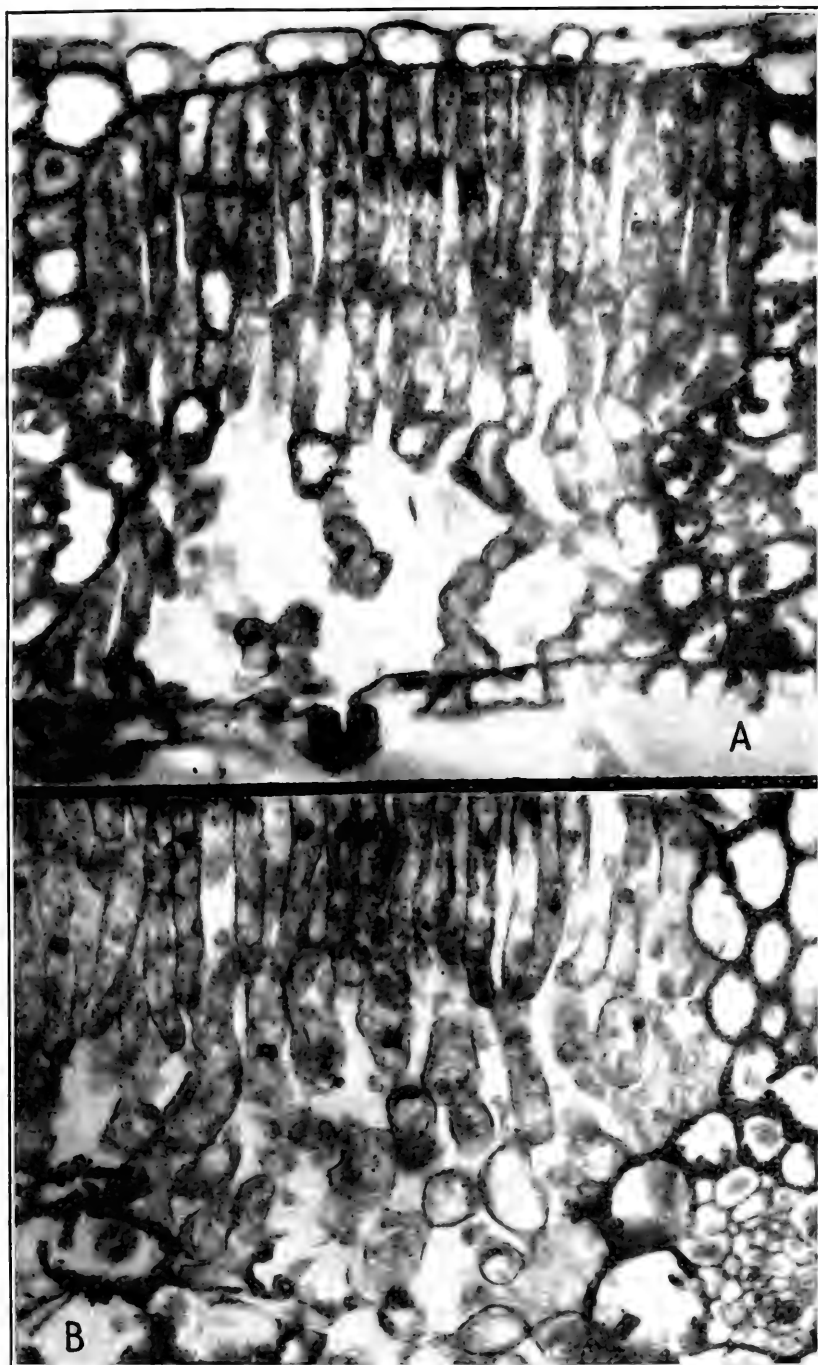
Fleming's triple stain used. A, C, and E to H, Van Deman variety. Collected in July, 1913, at Belleview, Fla. A.—Section of healthy leaflet at the margin, showing well-defined palisade and spongy parenchyma and large intercellular spaces. B.—Section of a yellow, thin area in a large rosetted leaflet, showing a differentiated palisade, but close packing of the cells. C.—Section through the yellow area of a much-aborted leaflet where the tissues have partially collapsed. There is no well-defined palisade tissue. D.—Section of a much-aborted leaflet at the margin, showing the close packing of the cells. E and F.—Sections through the yellow, thin portions of a badly mottled but nearly full-sized leaflet, showing the lack of differentiation into palisade and spongy parenchyma, almost complete obliteration of intercellular spaces, and difference in the leaf diameters in the center (F) and toward the margin (E) of a yellow spot. G.—Normal leaflet, showing palisade cells well differentiated and typical loose arrangement of the spongy parenchyma. H.—Section through the green tissue around a yellow spot of a mottled leaflet, showing slight hypertrophy of the palisade and spongy parenchyma cells and the reduction in size of the intercellular spaces in the spongy tissue. A to F,  $\times 220$ ; G and H,  $\times 420$ . Photomicrographs by the writer.



EPIDERMIS AND SECTIONS OF PECAN LEAVES.

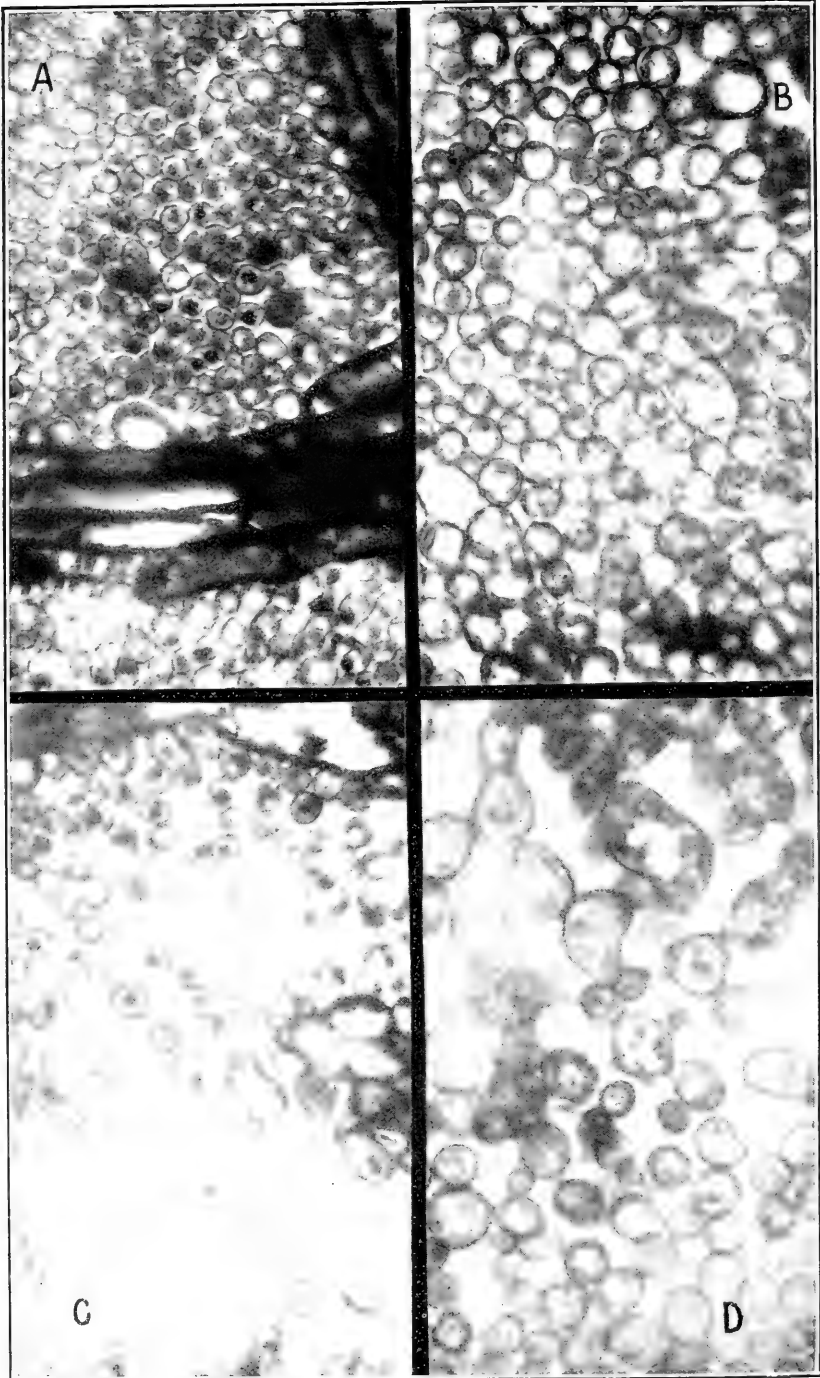
A.—Epidermis of a healthy pecan leaflet collected before sunrise and stained with iodine to show the retention of starch in the guard cells.  $\times 540$ . B.—Vertical section of a pecan leaflet, showing a calcium oxalate crystal aggregate.  $\times 420$ . C.—Vertical section of a young pecan leaflet collected just after emergence from the bud, showing the differentiation of the palisade tissue at this early stage of development. Killed in Carnoy's fluid; Fleming's triple stain used.  $\times 540$ . D.—Horizontal section of spongy parenchyma at the margin of a yellow spot, secondary stage, stained with iodine to show the presence of starch in the green periphery but a smaller quantity or total absence of starch toward the center of the yellow area.  $\times 540$ . Photomicrographs by the writer.





VERTICAL SECTIONS OF NORMAL AND MOTTLED PECAN LEAFLETS, FROTSCHER VARIETY.

Collected in August, 1919, at Thomasville, Ga. Killed with Carnoy's fluid; Fleming's triple stain used.  $\times 540$ . A.—Section of normal leaflet. B.—Green portion of mottled leaflet, showing the enlargement of the cells, the reduction in size of the intercellular spaces in the spongy parenchyma, and the unevenness in the arrangement of the palisade cells. Photomicrographs by the writer.



**HORIZONTAL SECTIONS OF NORMAL AND MOTTLED PECAN LEAFLETS.**

A and B.—Horizontal sections through the palisade tissue illustrated in figures A and B of Plate VIII, showing the enlargement of the short diameter of the palisade cells in the mottled leaflet (B). C and D.—Horizontal sections through the spongy parenchyma illustrated in figures A and B of Plate VIII, showing the enlargement of the cells and the reduction in size of intercellular spaces in the green area surrounding a yellow spot in the mottled leaflet (D). All figures  $\times 540$ . Photographs by the writer.

cells than rounded leaves of the same plant, but the narrower form of such leaves is due to a larger number of cell divisions with spindles parallel to the long axis.

In pecan rosette the general shape of the leaf seems to follow this rule, depending upon the orientation of the cell divisions rather than upon differences in the size or the shape of the cells. That is, the linear shape is not due to the development of cells elongated parallel to the midrib, but to a difference in the number of cell divisions in the two axes; nor are reduction in both length and breadth of leaf caused by a decrease in the size of the cells, but rather to a decrease in the number of cell divisions in both axes. On the other hand, there is often a large and localized change in both the size and the shape of the diseased cells, but not as related to leaf shape nor necessarily to leaf diameter. In the linear type of leaf the cells are as likely to be enlarged parallel to the short as to the long axis of the blade, and in portions of leaves profoundly reduced in both length and breadth the palisade and the spongy cells are at the same time often considerably enlarged in all three dimensions.

As a result of a considerable number of measurements of the thickness of leaves from healthy and rosetted trees of like age and variety, striking differences were found associated with the disease. (Table II.) Comparisons were made between leaves collected from the north and from the south sides of trees, but no constant differences were found which could be referred to situation, since all leaves were taken from the lower, outstanding branches, and under these conditions those on the north received nearly or quite as much light as those on the south periphery.

In each case the figures are based on 10 to 15 measurements of the thickness of each of several sections from comparable parts of each of 10 or more leaves. From such measurements it was found that the average variation from the greatest thickness in normal, individual leaves was 18 per cent, with extremes varying between 10 and 22 per cent. In the various types of rosetted leaves the extreme differences in thickness varied between 11 and 62 per cent of the greatest thickness. The least variation was found in the nonmottled linear or aborted leaves, while the greatest differences occurred in mottled leaves. Extreme variations in thickness of normal leaves of the Frottscher variety were 131 to 187 microns, while in diseased leaves of the same variety the range was from 70 to 234 microns. The smallest measurements were taken at the thin places in the leaves where tissue differentiation was lacking. The Van Deman specimens examined had slightly thicker leaves, but the same relations in thickness were found to hold between the healthy and the rosetted leaves of this and several other varieties.

TABLE II.—*Variation in the thickness of normal and rosetted pecan leaves, using the thickest part of each leaf as the standard of comparison.*

Description of material.	Measurements of thickness.		
	Average.	Extremes.	Variation average.
NORMAL LEAVES.			
Frotscher variety, Thomasville, Ga.:	<i>Microns.</i>	<i>Microns.</i>	<i>Per cent.</i>
North side of tree.....	162	131-187	18
South side of tree.....	147	122-173	18
Van Deman variety, Cairo, Ga.....	168	154-187	18
ROSETTED LEAVES.			
Frotscher variety, Thomasville, Ga.:			
Mottled leaves, about half size—			
North side.....	158	108-206	38
South side.....	167	80-210	37
Much-aborted leaves, south side—			
Nonmottled.....	152	70-215	26
Mottled.....	158	72-229	42
Linear leaves, south side—			
Nonmottled.....	118	103-141	26
Mottled.....	113	70-173	50
Mottled leaves, about half size, northeast side.....	139	75-234	55
Variety not stated, Cairo, Ga.:			
Mottled, red-brown stage, about half size.....	151	94-187	50
Van Deman variety:			
Mottled, about full size.....	198	140-281	45
Mottled, linear.....	140	78-195	47

The average thickness of linear leaves was always less than that of healthy or of other types of rosetted leaves. That this difference is not normally related to leaf size was shown by measurements comparing the thickness of the large juvenile leaves of normal young seedlings with that of the first type leaves above and that of large tip leaflets with small basal leaflets on single juvenile leaves. (Table III.) In the case of large juvenile leaves as compared with type leaves above there was a 67 per cent variation in the area of the laminae, but only a 7 per cent variation in thickness. In the vascular portion of the large side veins, however, a 72 per cent variation in the area of the cross section was correlated with this increase in leaf size. (Table IV.)

TABLE III.—*Leaf thickness and vein diameter as related to size in normal young pecan seedlings.*

Description of material.	Thickness (microns).			Leaf variation (per cent).	
	Leaf average.	Leaf extremes.	Large veins.	Thickness.	Area.
Large juvenile leaves.....	103	91 to 117	515	7	67
First type leaves above.....	96	89 to 103	388		
Large juvenile tip leaflets.....	112	98 to 126	538	10	94
Small juvenile basal leaflets on same leaves.....	101	98 to 112	243		

In the large and small leaflets of single leaves a 94 per cent variation in leaf area gave only 10 per cent variation in leaf thickness; but here again there was a 92 per cent variation in the area of the cross section of the vascular tissue, corresponding to the 94 per cent increase in the area of the leaf blade. (Table IV.) It will be readily seen that the differences in leaf thickness are at the lower range of variation found in normal leaves. In these juvenile leaves the variation in area of the total cross section of veins was high, but not quite as great as the difference in leaf area; the area of the vascular part, however, increased in direct proportion with the size of the leaf, as would be necessary to carry an adequate supply of water and nutrients to and from the larger leaf blade. In these leaves very little variation was to be found in the vertical diameter of either palisade or spongy tissue.

TABLE IV.—*Tissue measurements as related to size in normal young pecan seedlings.*

Description of material.	Thickness of tissue.		Diameter of large side veins.		Area of cross section, large side veins.		Variation in areas.		Area of cross section, relation of vascular part to total veins.
	Palisade.	Spongy.	Total.	Vascular part.	Total.	Vascular part.	Cross section, vascular parts.	Leaf.	
	Microns.	Microns.	Microns.	Microns.	Square microns.	Square microns.	Per cent.	Per cent.	Per cent.
Large juvenile leaves.....	39	41	515	351	207,460	96,211	72	67	46
First type leaf above on same plants.....	40	30	388	187	118,215	27,172			
Large juvenile tip leaflets....	35	43	538	351	227,285	96,211	92	94	42
Small juvenile basal leaflets on same leaves.....	35	39	243	103	45,987	8,171			

The total area of the cross section of comparable veins in healthy leaves and in aborted leaves of approximately half size (Pl. III, fig. D) was nearly the same for a given variety and set of external conditions. However, the area of the vascular portion of these veins was reduced from 43 per cent of the total area of the normal to about 33 per cent of the total area of the diseased veins. In the greatly aborted leaves (Pl. III, figs. A and C) the area of the total vein cross section was about half that in the normal leaves, while the cross section of the vascular portion of these veins was only 10 per cent of the total vein cross section as opposed to 43 per cent in the veins of normal leaves. (Table V.) While the area of total vein cross section, except in the most aborted leaves, tended to remain the same as in normal leaves, the development of vascular tissue within the vein became greatly reduced with the reduction in the

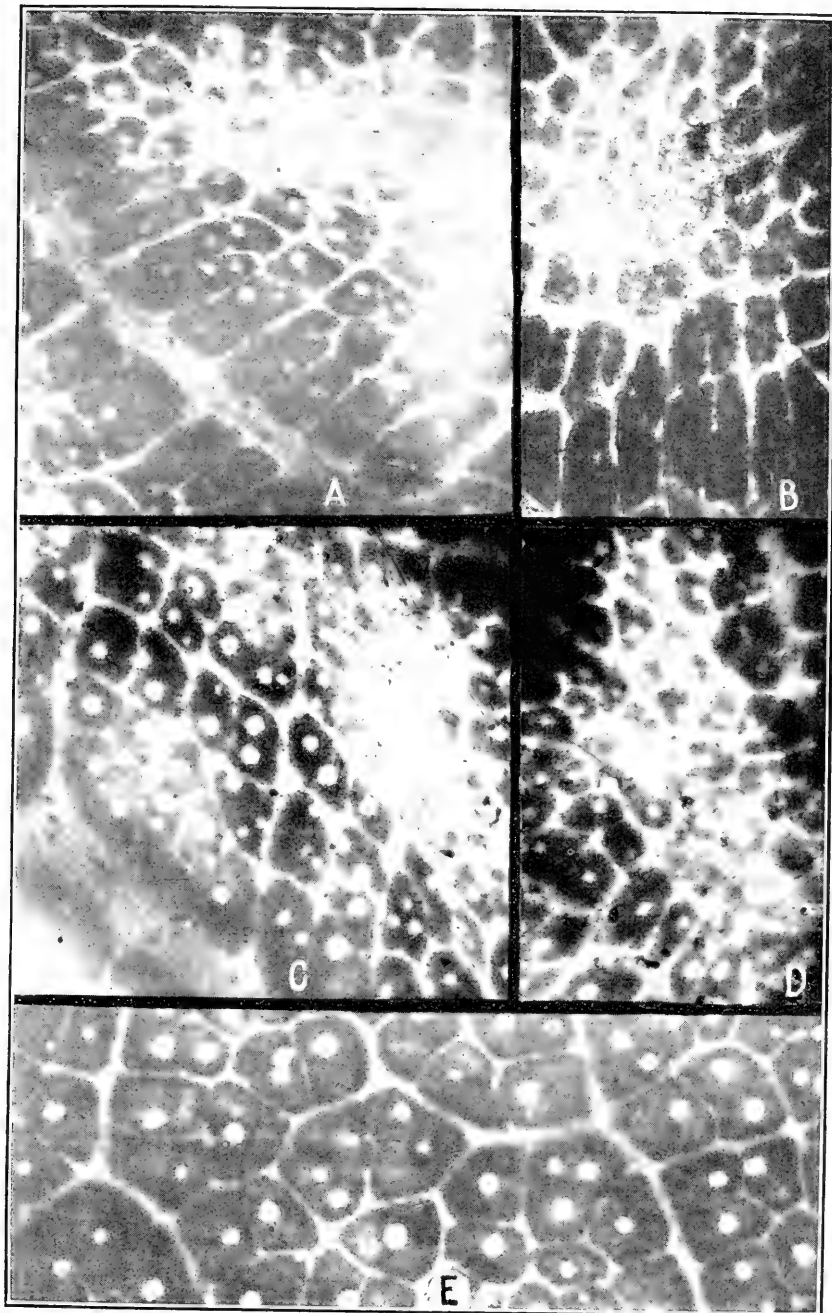
size of the leaf. In other words, the total size of vein in rosetted leaves of reduced size tends to be as great as that normally developed to support the full-sized leaf, but the development of vascular tissue within the vein becomes reduced with the severity of attack and consequent reduction in the size of the leaf blade.

TABLE V.—*Relation of vascular tissue in leaves to rosette.*

Description of material.	Diameter of large side veins.		Area of cross section, large side veins.		Area of cross section, relation of vascular part to total veins.
	Total.	Vascular part.	Total.	Vascular part.	
<i>Frotscher</i> variety, Thomasville, Ga.:	<i>Microns.</i>	<i>Microns.</i>	<i>Square microns.</i>	<i>Square microns.</i>	<i>Per cent.</i>
Normal leaf, south side.....	347	227	94,006	40,107	43
Rosetted leaf, about halfsize—					
North side.....	359	202	100,641	32,041	32
South side.....	346	202	94,006	32,041	34
Aborted leaf, south side.....	259	83	52,269	5,280	10
Linear leaf, south side (midvein).....	487	180	85,472	25,442	30

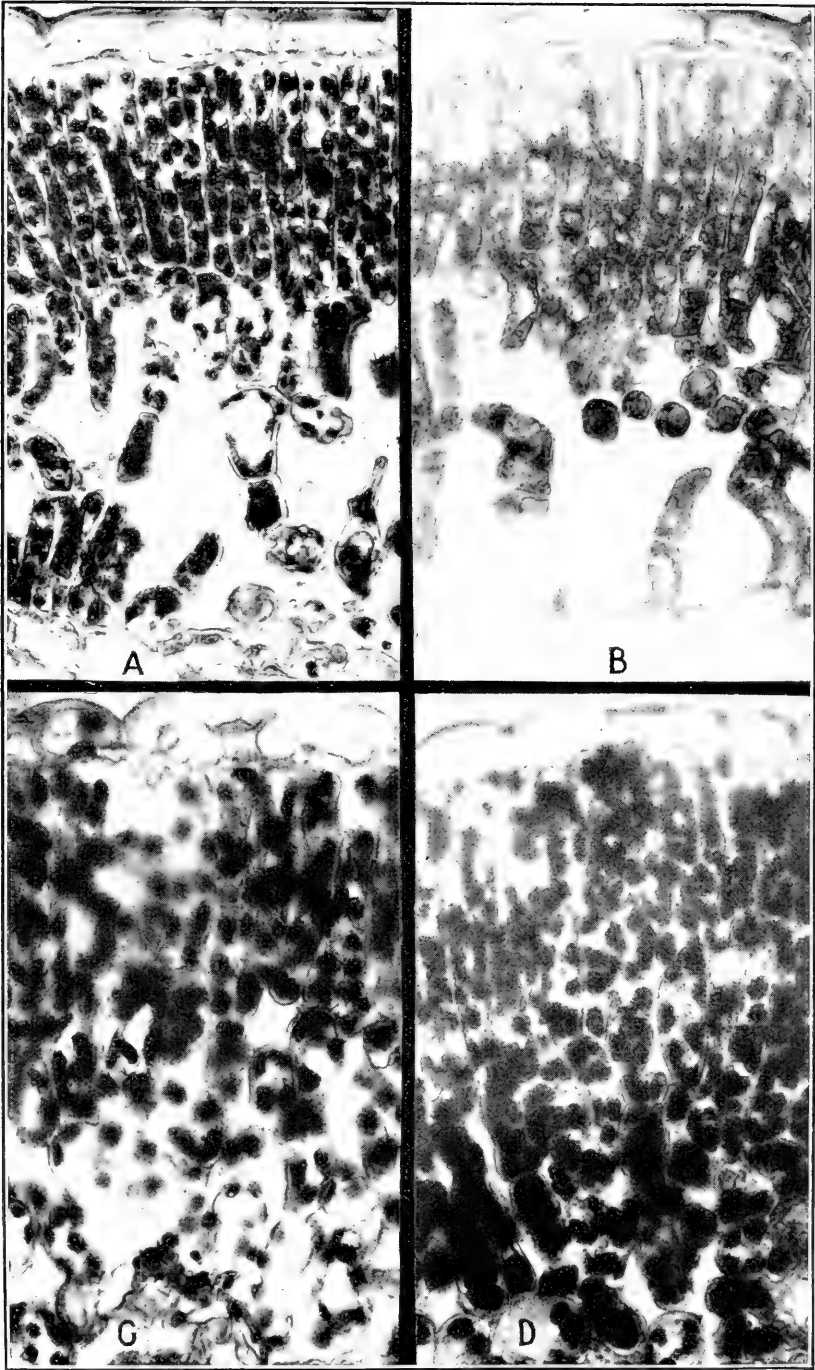
An examination of the vein islets of healthy and diseased leaves (secondary stage) has revealed striking differences in size, shape, and arrangement. Over the entire normal leaf blade these tiny areas bounded by the small, anastomosing veinlets tend to be isodiametric and of uniform size (Pl. X, fig. E). In the yellow areas, on the contrary, great differences in size and shape are the rule (Pl. X, figs. A to D). At the center of these spots the vein islets are smallest and become larger and larger with increasing distance from the center until often in the neighboring green parts they are considerably larger than in the healthy leaf. Their appearance suggests an inhibitory influence generated from the center, which largely prevents normal growth and differentiation there, but acts as a poison more and more feebly with receding distance from the focal center until in the neighboring green parts it has become sufficiently attenuated to function as a stimulant rather than as an inhibitory factor. This theory is also borne out by the writer's histological studies.

Not only are the vein islets highly variable in size, but they are often greatly distorted in shape. In many cases they are linear in outline, and with reference to the spot they approximate the arrangement of spokes in a wheel (Pl. I, fig. 1; Pl. X, fig. B). Thus it will be seen that the direction of their greatest expansion may or may not parallel the direction of greatest expansion in the leaf blade as a whole. That is, the size, shape, and arrangement of the vein islets in these chlorotic spots of the secondary stage are controlled from the focal center of the spot rather than by the normal morphogenic forces of the leaf.



**VEIN ISLETS OF UNSECTIONED AND UNSTAINED PECAN LEAVES.**

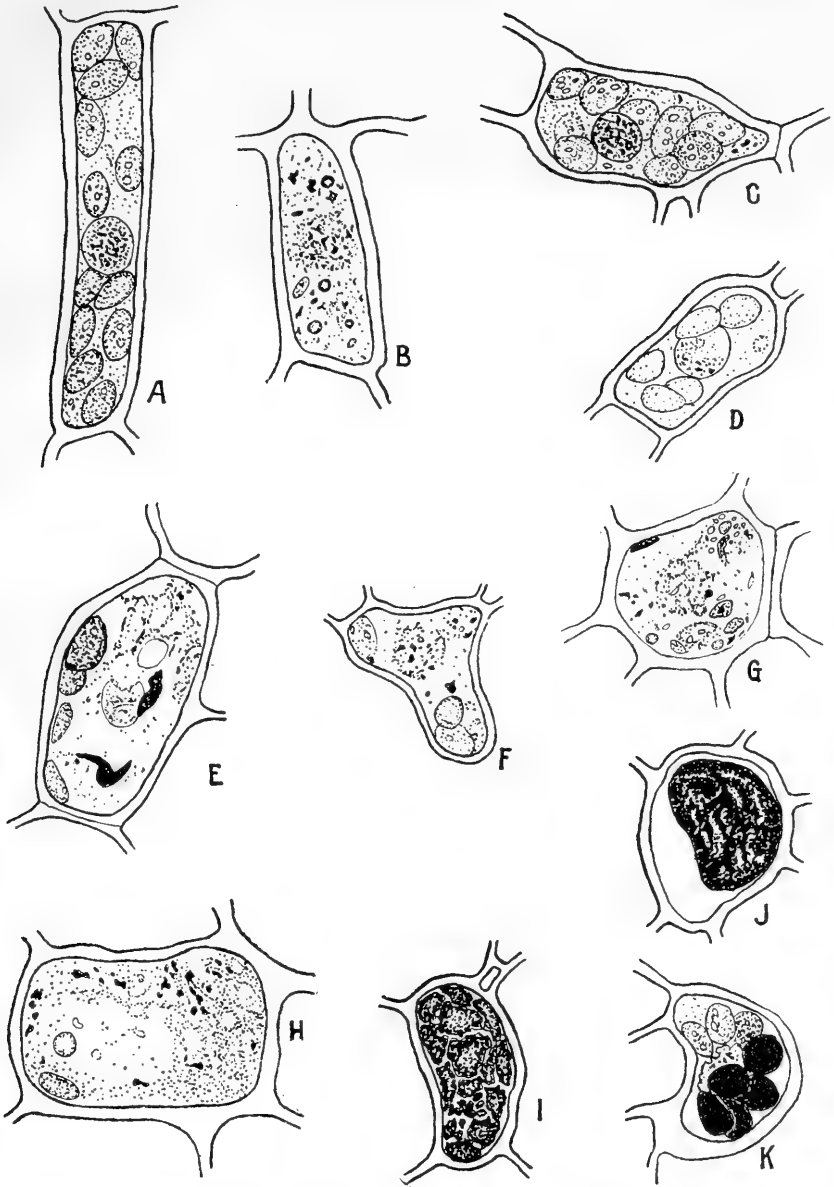
A, and C to E are from photomicrographs of leaves preserved in alcohol. B is from a photomicrograph of a dry herbarium specimen. All at the same magnification.  $\times$  about 60. The white spots are calcium-oxalate crystal aggregates. A to D.—Yellow spots of rosetted leaflets in the secondary stage, showing the distortion in shape and the variation in size of the vein islets. E.—Similar view of vein islets in a normal leaflet, showing approximately uniform size and regular arrangement. Photomicrographs by the writer.



VERTICAL SECTIONS OF HEALTHY AND DISEASED PECAN LEAFLETS,  
FROTSCHER VARIETY.

Collected on August 25 and 26, 1919, at Thomasville, Ga. All figures at the same magnification.  $\times 510$ . A.—Healthy pecan leaflet collected at sundown, killed in Carnoy's fluid, and stained with iodine to show the presence of starch in the palisade and spongy cells. B.—Healthy pecan leaflet collected in the morning, killed in Carnoy's fluid, and stained with iodine to show the absence of starch. C and D.—Pecan leaflets aborted with rosette, collected and treated in the same way, respectively, as in the preceding two normal leaflets. Chloroplasts in both night (C) and morning (D) specimens were gorged with starch, and apparently no translocation had taken place during the night. Note also the enlargement of the cells in the diseased material. Photomicrographs by the writer.





FRESH LIVING CELLS OF PECAN LEAVES.

Camera-lucida drawings of freehand vertical sections, mounted in water. All at the same magnification ( $\times$  about 1,200) and oriented the same as in the leaf. A.—Healthy palisade cell, showing well-defined nucleus and plump livid-green chloroplasts. B.—A disorganization stage of a palisade cell in a leaf affected with rosette; chloroplasts disintegrated and nuclear outline vague. C.—Healthy cell of the spongy parenchyma. D.—Slightly diseased cell of the spongy parenchyma in which the chloroplasts have lost a part of their green color. E.—Spongy parenchyma cell at the margin of a yellow area. The protoplasmic structures on the side toward the spot (right) are disorganized and the nucleus is fragmenting. Chloroplasts next to the green periphery of the spot (left) are still green and unfragmented, though a part of them are smaller than normal. F and G.—Spongy cells at further stages in disorganization. H.—Spongy cell showing entire disorganization of contents. I.—Tannin degeneration products gathering into flocules at a later stage of the disease in a spongy cell. J.—Spongy cell at the red-brown stage with more or less homogeneous reddish brown contents plasmolized. K.—Spongy cell at the margin of a yellow area (right) showing chloroplasts red-brown, but unfragmented on the side toward the spot. This form of injury is rather uncommon. Drawings by the writer.



A comparison of the size and shape of the vein islets in large and small mature leaflets on the pecan leaves and in large (mature) juvenile (undivided) and type leaves on the same normal seedlings showed very little variation. Ensign (32) found in healthy citrus leaves that the shape varied in different parts of the leaf, but the size was independent of the shape or location on the leaf. The size and shape of the vein islets in citrus were approximately the same in normal leaves and in chlorotic leaves of plants dwarfed from starvation. A comparison of the vein islets in large and small leaves on the same plant showed that the size was constant irrespective of the size of the leaf. A comparison of the leaves of all ages on the same plant showed that the youngest leaf had the smallest vein islets and with increasing maturity a corresponding increase in the size of the islets took place. Ensign concluded that the main skeleton of the vascular system is laid down very early in the history of the leaf and that but little differentiation later takes place. It is clear that in pecan rosette the size, shape, and arrangement of the vein islets are considerably altered from the normal. In fact, there is in the mottling of the leaves in pecan rosette much that appears comparable in origin to Liesegang's diffusion patterns, for example, those obtained with drops of silver-nitrate solution on a layer of solidified gelatin in which potassium bichromate had been dissolved. These "Liesegang phenomena" have also been compared by Küster (48) to the formation of growth rings by *Armillaria*, *Penicillium*, and other fungi, to the alternative lighter and darker areas of some leaf-spot diseases of fungous or bacterial origin, and to a great variety of pattern phenomena in both plant and animal kingdoms. The end results in all these cases of organic origin are similiar in appearance to those obtained by diffusion experiments carried out *in vitro*. Furthermore, while great caution should be exercised in the interpretation of such experiments the results are extremely suggestive that diffusion in colloidal systems plays a prominent rôle in the development of the pattern phenomena in both health and disease.

Profound derangements take place in starch assimilation and translocation. In healthy leaves collected at sundown the starch content of the chloroplasts is uniform for each tissue with usually the greatest accumulation in the palisade cells (Pl. XI, fig. A). Stained with iodine the starch is seen in a more or less irregular mass at the center of the chloroplasts but in no case completely filling the plastid. Starch also occurs in the guard cells and in occasional plastids in the bundle sheaths. Healthy leaves collected before sunrise the following morning from similar situations on the same trees in general showed no starch at all (Pl. XI, fig. B). In these healthy leaves the iodine gave merely a yellow stain to the cell contents. An extremely rare

palisade or spongy cell and many of the guard cells were, however, filled with starch as in leaves collected at sundown (Pl. VII, fig. A).

Mottled leaves of all sizes collected at night showed the green portions gorged with starch (Pl. XI, fig. C). The plastids of the palisade and spongy cells were not only filled to the periphery but swollen as if almost bursting with their accumulation of starch. Toward the center of the yellow spots (secondary stage) the chlorophyll bodies are of less and less frequent occurrence until at the center only an occasional starch-filled plastid is to be found (Pl. VII, fig. D). In these areas where the effects of the disease have been in operation from the early stages of bud growth the inhibitory influence has affected not only tissue differentiation but has also prevented normal development of the plastids.

Mottled leaves collected before sunrise the following morning appeared the same as those collected at night (Pl. XI, fig. D). As far as could be determined from the iodine stain, no translocation at all had taken place during the night. Palisade cells, spongy cells, and guard cells of the green areas were full of starch, and the occasional plastids in the bundle sheaths of the veins were also gorged.

The nonmottled, but greatly aborted leaves, showed this gorging of the plastids over the whole lamina. The starch sheaths around the midveins were also black with starch. As in the other diseased leaves the plastids appeared as full of starch in the early morning as at the end of a sunny day.

Wherever plastids are present in either stage of leaf mottling the first sign of disease in these bodies consists in a gradual loss of the green chlorophyll. In the healthy living cell of both palisade and spongy parenchyma the plastids are plump and of a livid green color, while the nucleus is plainly visible as a grayish, more or less centrally located body with definite outline (Pl. XII, figs. A and C). As the disease progresses these chlorophyll bodies first lose their green color (Pl. XII, fig. D), then both nucleus and plastids begin to break down (Pl. XII, figs. B and E to H), first losing their definiteness of outline, or becoming fragmented or appearing as if eaten away at the periphery. Later all the visible remains of the cell structures consist of globules, probably fatty, and darker-colored granules of various shapes and sizes irregularly distributed throughout the cell, with all appearance of disorganization. With ferrous salts many of the brownish granules gave the reaction for tannin. In cases of severe attack the entire tissue within these yellow areas later becomes reddish brown and collapses (Pl. I, fig. 3). In reaching this end stage the tannin degeneration products here described gather into larger and larger floccules (Pl. XII, fig. I) until finally the cell may be filled with a more or less homogeneous reddish brown matrix,

which later recedes from the cell wall (Pl. XII, fig. J), and at last the whole cell collapses and shrivels.

The deposition of crystal aggregates of calcium oxalate is characteristic of pecan leaves (Pl. VII, fig. B). These crystals begin to form in giant binucleate cells of the palisade just after the young leaf emerges from the bud. Finally the protoplasmic contents disappear, and the crystal aggregate nearly fills the cell. These crystal aggregates are distributed with considerable regularity in the healthy leaf and in the majority of cases one to each vein islet (Pl. X, fig. E). In the yellow spots of the secondary stage of the disease, on the other hand, their formation and distribution vary widely. At the focal centers in severe cases few or no crystals at all are formed, whereas in the surrounding green parts they are often far more numerous and sometimes larger than in the comparable healthy leaf.

Averaging a large number of counts in cross sections of green parts of diseased and normal leaves of the Frottscher variety collected at Thomasville, Ga., it was found that in the normal leaves 20 crystal aggregates occurred to every 100 vein islets observed in section, while in the diseased leaves 60 crystal aggregates were found to every 100 vein islets. In Van Deman leaves collected at Baconton, Ga., there were 16 crystal aggregates to every 100 vein islets in the healthy leaves as compared with 54 in the diseased leaves. Furthermore, after all due allowance for differences in cortical area of the diseased and healthy leaves compared, a much larger number of these crystals was found in the cortex of petioles and midveins in rosetted leaves. In view of the fact that waste organic acids usually accompany carbohydrate formation (57, p. 173) these results are significant. Since growth at the periphery of the yellow spots in the secondary stage is often abnormally active, as is shown by the size of the cells, an unusually large accumulation of such organic acids would naturally be expected to take place in that region. Conversely, with the reduction of chlorophyll formation and assimilation in the centers of the spots a smaller production of such acids would occur.

Development of the shield-shaped resin glands occurring mostly on the lower surface of the leaves is also profoundly affected by the disease. On the normal leaf these glands are rather regularly and sparsely distributed over the surface of the blade and contiguous to the veins and veinlets. In diseased leaves of the secondary stage, on the contrary, the focal centers of the yellow spots are often thickly covered with these resin glands both contiguous to the veinlets and well within the vein islets themselves. The more severe the general effects of the disease the more numerous were these glands found to be, until in places where the tissues were practically de-

funct or bordering wounds where such tissues had fallen out, they presented a continuous, rough layer of glandular shields. Examination of ordinary leaf wounds such as those made by insects showed no such abnormal development of resin glands. These conditions have been frequently observed in the general examination of leaves collected during several seasons and in different varieties and localities. Furthermore, using a simple binocular microscope, observations have been checked up by exact counts. For example, counts were made of the numbers occurring in a single field in different parts of 10 normal leaves of the Frotscher variety collected at Thomasville, Ga. (1916), and in the yellow areas of 10 comparable rosetted leaves. The average for normal leaves was 8 to a field, while in the diseased leaves there were 92 to a field. In a lot of the Schley variety collected at Orangeburg, S. C. (1920), the average for healthy leaves was 43 and that for comparable yellow areas of diseased leaves 212 to a field. Similar results were found in material collected at Belleview, Fla., and at Baconton and Cairo, Ga. In some older spots of the secondary stage these resin glands were so closely packed together that counting was impossible.

This increased development of resin glands is characteristic of many halophytes and xerophytes and in these cases apparently bears some relation to the condition of physiological dryness. Furthermore, Tschirsch (80) has demonstrated that the secretion of resin is produced within the cell wall itself just below the cuticle. This region he calls the "resinogenous layer." Nutrient substances and water pass out of the protoplast into this resinogenous layer, to be there further molded into the final product, resin. This process, then, is participated in by both protoplast and cell wall and necessitates loss of material from both and a final breaking down of the cell wall itself. In pecan rosette this abnormal development of resin glands is then probably to be connected in some way with the gradual and general degeneration of the cells involved.

In order to determine whether new spots may be formed after the full expansion of the leaf and whether yellow spots already formed may increase in size, resort was made to careful field observation and microscopical study of fresh living material. On July 20, 1920, at Orangeburg, S. C., the outlines of several hundred spots on 56 different leaves of both primary and secondary stages were carefully traced with India ink on the upper surface of the blade. At the same time the outline of one leaflet on each leaf was traced on paper for comparison with its size at the end of the observational period. Furthermore, nonmottled areas of diseased leaves were marked in a similar way; and finally also certain areas of normal leaves were so marked as a check on possible injury by the India ink. These leaves were located on one normal and five rosetted trees in a 45-acre

pecan orchard of the Schley variety at Orangeburg, S. C. Final notes were taken on a portion of these leaves at the end of 10 days, while the remainder were left until September 9, 51 days later.

In leaves of the primary stage, after 10 days, 17 yellow spots had developed within 170 previously marked green areas. Of 130 yellow spots already present, 11 in all had increased in size but only 2 conspicuously so. In slightly affected leaves of the secondary stage, 36 green areas had developed 13 yellow spots; and out of 26 spots already present 19 had increased in size, 15 of them decidedly. In the considerably aborted leaves, 109 green areas had developed 137 new spots; and out of 270 spots present at the start 193 had increased in size, 70 of them by at least 50 per cent of their original diameter. These leaves were all fully expanded at the beginning, since no appreciable increase in size of blade could be discerned on comparing the leaflets with their original tracings. The check leaves remained normal and evidenced no ill effects from the presence of the India ink.

Four uniformly pale leaves recently out of the bud and not fully expanded at the first observation, at the end of 10 days, had developed a conspicuous green color around the margins and along the veins, but had failed to develop further chlorophyll in the centers of the areas between the lateral veins. These leaves were now typical of the secondary mottled stage.

In those leaves left for 51 days, 33 green areas in the primary stage leaves had developed only 2 yellow spots. Of 180 spots already present, 86 had increased in size, but only 15 decidedly so. In aborted leaves of the secondary stage, 50 green areas had developed 17 spots and of 66 spots present at the beginning 50 had increased in size, and 37 of them decidedly so.

Most of the leaves of the primary stage were at the beginning older and fully matured. The leaves of the secondary stage were fully expanded, but for the most part soft and immature. It is thus apparent that these yellow spots are not necessarily laid down in the bud stage of the leaf or in a definite and unchangeable pattern, as may be the case in certain heritable variegations. It is clear also that spots may develop *de novo* even after expansion of the leaf blade and that those already formed may increase in size, though less rapidly and frequently after the leaf has fully matured.

That yellow spots may increase in size was also shown by a study of free-hand sections of fresh, living material. Here on the margins of the spots an occasional cell was found which showed disorganization of its internal structure on the side bordering the yellow area, while that part of the cell contents toward the green periphery approximated the normal. (Pl. XII, figs. E and K). Here again appearances suggest the outward diffusion of some toxin from a focal center of its production.

These changes in mature cells affect the protoplasmic structures rather than the cell size, shape, or other so-called histological characters. They consist in such changes as loss of chlorophyll, breaking up of the nucleus, and degeneration of the cytoplasm. The situation seems to be that the toxic substances which in the embryonic stages may produce the profound alterations in morphogenetic processes of leaf formation described above, in the mature leaf cells are simply destructive of the still plastic protoplasm. What the ultimate expression of the disease will be depends, then, upon the ontogenetic stage of the plant organ at which the cause becomes effective.

#### SUBSIDIARY EXPERIMENTS.

In order again to test the effect of subjecting pecan trees to varying environmental conditions, further transplanting and fertilizer experiments were conducted on a small scale at the New York Botanical Garden.

In one of these experiments 32 large nursery trees showing severe attack of rosette during the summer of 1913 at Monticello, Fla., were transplanted late in the fall to large pots of garden soil in one of the botanical garden greenhouses. On account of the length of the tap roots these trees were so severely root pruned that only a part of them survived. However, the 18 remaining trees were under observation for two seasons, and at no time during this period did any sign of rosette develop although before transplanting all were badly mottled and dying back from the tip.

In another test several pecan nuts were germinated in fine quartz sand in each of 32 glazed crocks and in 4 similar crocks of garden soil. All were uniformly watered by the porous clay cup autoirrigation method. The crocks containing sand were divided into 8 lots receiving the following different fertilizer treatments:

(1) Liberal application of a commercial fertilizer containing muriate of potash, ammonium sulphate, and acid phosphate.

(2) Liberal application of a fertilizer made up of sulphate of potash, slaughter-house tankage, and Thomas slag.

(3) An excess of stable manure.

(4), (5), and (6) An excess, respectively, of slacked lime, magnesium sulphate, and ferrous sulphate.

(7) Equal quantities of slaked lime and magnesium sulphate.

(8) Control, untreated.

Lot 9 consisted of the 4 crocks with garden soil alone. All seedlings germinated normally and were under observation during one season. All those in lots 1, 2, 3, and 9 made good growth and appeared dark green and healthy throughout the experiment. Seedlings in the other lots started out well but after using up the nutrients in the seeds they became stunted and finally developed a general



yellowing of the foliage. However, none of the mottling or other signs of rosette appeared at any time during the season.

Two large, healthy seedling pecans were transplanted to a plat of garden soil containing a great excess of lime where mortar had previously been mixed for building purposes. These trees have been under observation for three seasons, and no signs of any type of chlorosis have at any time become evident.

In another small experiment run for two months, 10 seedlings potted in garden soil were given liberal and fairly uniform applications of water throughout the period, while 10 similar potted seedlings were given only sufficient water to prevent wilting. In the latter case but little growth was made, and a part of the leaves developed a general chlorosis. At no time, however, were any signs of rosette to be seen.

#### PROBABLE NATURE OF PECAN ROSETTE.

In the writer's opinion the pathogenic picture of pecan rosette as shown in the preceding pages, including histological and cytological features, is much more in agreement with the infectious type of chloroses, including the yellows and mosaic groups, than with those chloroses known to be caused directly by soil or climatic conditions. It is not considered, however, that adequate proof has yet been given on either side, and the present study is offered merely as one more step in the study of this baffling group of diseases and as a suggestion for further research.

In the environmental type of chlorosis structural changes may occur. The xerophytic tendency is toward a bilateral palisade with restricted air spaces reducing transpiration. Hydrophytic conditions produce large, globose cells and loose arrangement of tissues. Excess of sodium chlorid develops enlarged, rounded, spongy, and massive palisade cells and causes reduction in the number of chloroplastids. Wax coatings are also characteristic of many salt-loving plants. Lack of sufficient water or soil nutrients results in the dwarfing and hardening of the tissues, often followed by general chlorosis but without morphogenic changes. The dimorphism of certain plants when grown under differing environmental conditions is well known, but is in no sense comparable to a diseased or other deranged condition. Changes in metabolism also may be caused directly by soil or climatic conditions. All these changes, however, in the main, are general changes affecting the plant tissues as a whole or tending to affect all similar tissues of the plant alike.

In pecan rosette profound alterations and derangements in metabolism, anatomy, and morphogenesis occur together in great complexity. Different types of tissue derangement are found in the same leaf. Reduction and increase in the number of cell divisions, tissue

differentiation and lack of tissue differentiation, hypotrophy and hypertrophy of cells may all occur together within an area only a few millimeters in diameter. There seem to be focal centers out from which these alterations spread, and the resulting abnormal development appears to be controlled from these centers rather than by the normal morphogenic forces. The factors controlling morphogenesis as related to cell differentiation, to development of tissues in diseased areas, and to general pattern of leaf are profoundly altered. No less far-reaching are the derangements in metabolism evidenced by the altered assimilation and translocation of starch.

Pecan rosette frequently appears first on the tip leaves of a single branch and seems as likely to affect a part of the tree first as to occur at once over the whole top.

Careful field observations have given no evidence of varietal differences in susceptibility or resistance to pecan rosette. This same statement would apply equally well to most infectious chloroses.

Fertilizer and transplanting experiments and field observations indicate that rosette is affected, at least indirectly, by soil and climatic conditions, but similar relations are exhibited by chloroses of known infectious nature as well as by many diseases of bacterial or fungous origin. Furthermore, great reliance is not to be placed upon the results of plat soil experiments unless such results are unusually definite, or unless in a large number of similar tests the data all point in the same direction.

In view of the refractory attitude exhibited toward grafting and cross-inoculating experiments by certain of the infectious chloroses it is considered that this type of experimentation has not yet given a conclusive answer to the question of possible infectivity in pecan rosette. In the early experiments a small portion of the diseased buds developed the disease, though in no larger percentage than in contiguous nursery trees worked with supposedly healthy buds in the ordinary commercial propagation. These experiments need repetition on a large scale under controlled conditions and with extreme care in selection both of diseased and healthy buds and stocks. Moreover, the possibility of insect transmission has not been touched upon from the standpoint of experiment.

The disease has not been definitely and experimentally caused by a set of known conditions. Though it is more prevalent and severe under certain environmental conditions, it occurs to some extent in practically every soil type where the tree has been observed, with the possible exception of swamp land. Under these conditions the tree makes very little growth and presents a starved and stunted appearance followed by a general form of chlorosis bearing no resemblance to rosette. Ultimate proof of the cause must account for all cases of the disease.

It is difficult to explain on the soil hypothesis why only a part of a tree may be diseased and why when two trees of the same age stand within a few inches or a few feet of each other the one may remain perfectly normal and vigorous while the other is stunted and dying back with rosette. The recovery of diseased trees when transplanted in the north on the other hand is also difficult to explain, but no more so than the apparent recovery of potatoes from mosaic when carried from Maine to Colorado or tobacco mosaic under certain relations of light or temperature. As previously mentioned, Baur has clearly demonstrated that the contagium of abutilon mosaic is readily killed by subjection to certain environmental conditions such as the withdrawal of light. He found that cutting out the yellow areas as they developed also finally brought recovery. Here, as in many parasitic diseases both of plants and of animals, though the contagium may be carried to remote parts of the body, it is only in certain definite locations and under certain definite conditions that it can reproduce itself and initiate lesions in the host.

If pecan rosette is due to some chemical compound brought in from the soil in harmful quantities one would expect the tissues along the veins to be first and most profoundly affected and that the result would be evident over the whole tree at about the same time. Furthermore, if the yellow mottling is interpreted as due not to a cause operating from the focal centers of the spots but to a lack of sufficient soil nutrients or water brought in by the veins, how are to be explained the lack of chlorosis along the leaf margins and the abnormally increased growth at the periphery of the spots?

It is true that the local application of purely physical or chemical stimuli may locally cause cells to enlarge or proliferate, and their application in lethal quantities may result in injury and final death without the intervention of any parasitic organism, as witness, for example, the more recent experiments of Dr. Erwin F. Smith (74) in the production of plant overgrowths without the intervention of any parasite. However, as in any cytological or embryological study, it is not the single section taken by itself that tells the story, but the sequence of one following the other, the whole series fitting together in an orderly manner to build up the complete picture, so in the chloroses of plants it is not the mere fact of chlorophyll disintegration that will show the type of disturbance present, or that will eventually lead to the determination of the cause in any particular case, but the whole series of events and appearances concerned in the production and manifestation of the derangement.

It is probable that all effects of parasites upon their hosts, when reduced to their ultimate reactions, may be explained in terms of physics and chemistry. It is in the regulation of these physical

and chemical reactions and stimuli, and in their application in a particular manner and at a particular time and place, that the complexity of the final manifestations of infectious diseases differs from the more simply induced changes in metabolism and structure. It is this regulatory effect and this extreme complexity of reaction which make many infectious diseases so difficult to induce by artificial means.

If causes may in any measure be judged from their effects, the histological and cytological evidence points to the cause of pecan rosette as being similar in its general nature to the ultimate causes of the infectious chloroses. Whether in this particular disease the factors responsible for alterations in the normal structure and metabolism must be introduced into the plant from without, or whether they originate within the plant itself, is a question yet to be answered; but whatever the ultimate solution of the problem may be, the cause will undoubtedly not be found in any simple soil or water relation.

So far as worked out, the infectious chloroses in general exhibit, like pecan rosette, a simultaneous and deep-seated derangement both in morphogenesis and metabolic processes. Structural derangements in diseased leaves may show abrupt local change. That is, in many cases, entirely different types of tissue derangement, such as reduction in size of cells and number of cell divisions or enlargement of cells and increased number of cell divisions, may occur side by side, not only in the same leaf but even in the same part of the leaf. Along with these structural changes occur fundamental functional derangements concerned with the assimilation and translocation of carbohydrates and with nitrogen metabolism. As is well stated by True and Hawkins (79) in considering spinach blight:

It would seem to be indicated that the cause of carbohydrate accumulation should be sought in the deeper lying metabolic processes in connection with which carbohydrates are utilized. . . . Accumulation is due not to a breakdown of digestion but to some partial failure in the subsequent metabolic processes in connection with which carbohydrates are used.

Moreover, not only restriction in chlorophyll development occurs, but often abnormal and irregular groupings and a reduction in size and number of chloroplasts. In the later stages disintegration of the plastids and other cell contents often follows, and finally a shriveling of the entire cell ensues.

It is typical of the group of infectious chloroses that only meristematic tissues are morphogenetically affected. There is always a rather definite incubation period for each disease, and external signs of the disease gradually progress from the point of entry of the contagium. In some instances the contagium appears not to reach all parts of the plant, as in the case of potato plants secondarily infected with mosaic or leaf-roll where often only a part of the

tubers give rise to diseased progeny. Furthermore, the fact that many of these diseases are transmissible by insects shows them to be entirely different in nature from those chloroses due to soil or climatic conditions. In some of these diseases where the insect relations have been most carefully worked out it is indicated that a definite incubation period must also elapse after feeding upon a diseased plant before an insect becomes infective.

Except in the most general way, the infectious chloroses exhibit no pattern as related to the leaf. The factors controlling morphogenesis of the leaf in many cases seem to have run riot. In the mosaic type the spots, irregular in themselves, are also irregularly distributed over the surface of the leaf. In many cases, it is true, they avoid the larger veins, but in other instances they are distributed irrespective of venation.

There is nothing to show a difference in kind between those mosaics transmissible by expressed plant juices and those transmitted only by grafting, such as the infectious mosaic of Abutilon. The causal contagium in the latter case may be compared to a more obligate parasite which is greatly restricted in the conditions necessary to its life activities and reproduction.

The fact that later investigation has shown some of the filterable contagium diseases to be due to microorganisms is at least presumptive evidence in favor of the organism theory of infectious chloroses. Furthermore, Allard has shown that with a fine enough clay cup it is possible entirely to filter out the infective principle from the expressed juices of tobacco plants affected with mosaic.

The crucial difference, however, lies in the power of self-reproduction possessed by the contagia of all the infectious chloroses. To give a concrete example, starting with a mosaic-diseased tobacco plant and in each case using a drop of the expressed plant juice diluted 1 to 1,000 in water, it is possible to cause the disease in an indefinite series of plants successively inoculated one from the other at proper intervals, and the juice from the last of the series will possess as high a power of infection as that from the first. In a case like this it might be mathematically shown that if reproduction had not taken place a drop from the last plant of the series must contain less than one molecule of the originally injected material. No such power of self-reproduction has ever been demonstrated for a definite chemical compound. The true enzymes are formed by living organisms in response to certain stimuli and have never been shown to be capable of self-reproduction.

Assuming the contagia of infectious chloroses to be nonliving substances, their effects may be compared up to a certain point with various phenomena of bacterial toxins and immunization. However, the fact of reproduction in these contagia is in no way elucidated by

any such comparison. Diphtheria toxin, for instance, if injected into a susceptible host may cause all essential signs of the disease, but it takes living bacteria to produce more of the toxin so that the disease may be transmitted down the line through a successive series of individuals.

On the other hand, the "Contagium vivum fluidum" theory of Beijerinck (17) in some modification is at least worthy of serious consideration as a working hypothesis. It is not impossible that decomposition processes may be propagated by methods entirely unlike reproduction by division as known in living organisms and so as to imitate self-reproduction. If some such course of events could be demonstrated it would perhaps explain the known facts, including the gradual progress of the disease from the point of entry, equally as well as the organism theory. However, though it is well to keep an open mind on all questions still in the realm of theory, no such course of events is yet known to chemistry. On the other hand, most of the known facts concerning the so-called filterable virus diseases, so it seems to the writer, conform to the known results of invasion by parasitic organisms.

#### SUMMARY.

As a class, the chloroses due to soil or atmospheric conditions are rather general effects which are more or less comparable to starvation, overfeeding, or direct poisoning. There are not the profound or strictly localized derangements in both metabolic and anatomical development, and it is a question in regard to many of these phenomena whether, in the restricted sense, they should be regarded as diseases at all.

In the specific chlorotic diseases of an infectious nature fundamental derangements in both physiological and structural development are simultaneously brought about. Although all effects of parasite upon host when reduced to their ultimate reactions are probably to be explained in terms of physics and chemistry, it is in the regulatory effect and in the extreme complexity that these and many other infectious diseases differ from the direct effects of nonliving substances.

The histological and cytological evidence suggests that pecan rosette in its specific sequence of signs and in the complexity of the structural and physiological derangements bears far more similarity to the known infectious chloroses than to those caused by soil or climatic conditions. Whether in this particular disease the factors responsible for alterations in the normal structure and metabolism must be introduced into the plant from without, or whether they originate within the plant itself, is a question yet to be answered; but whatever the ultimate solution of the problem the cause will undoubtedly not be found in any simple soil or water relation.

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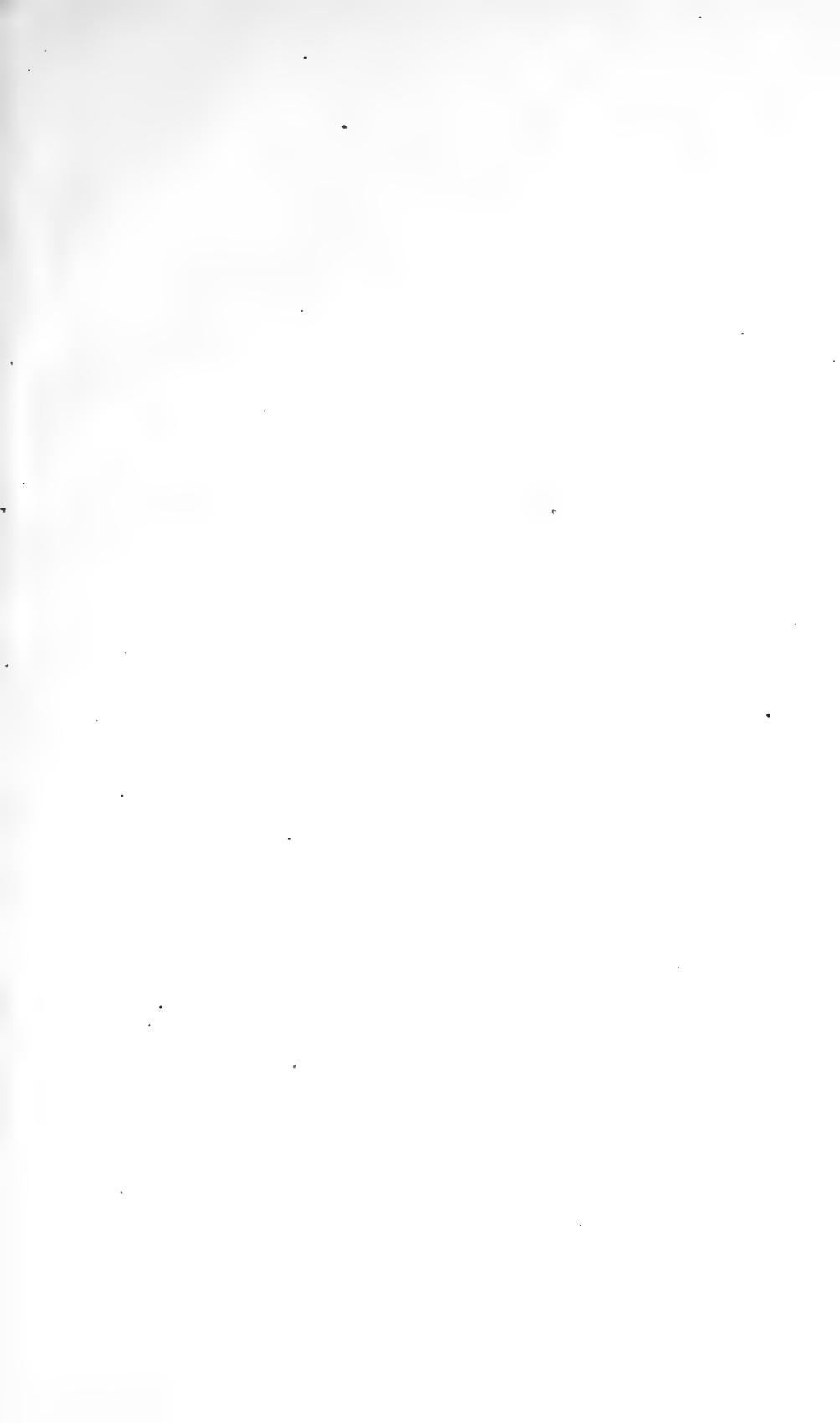
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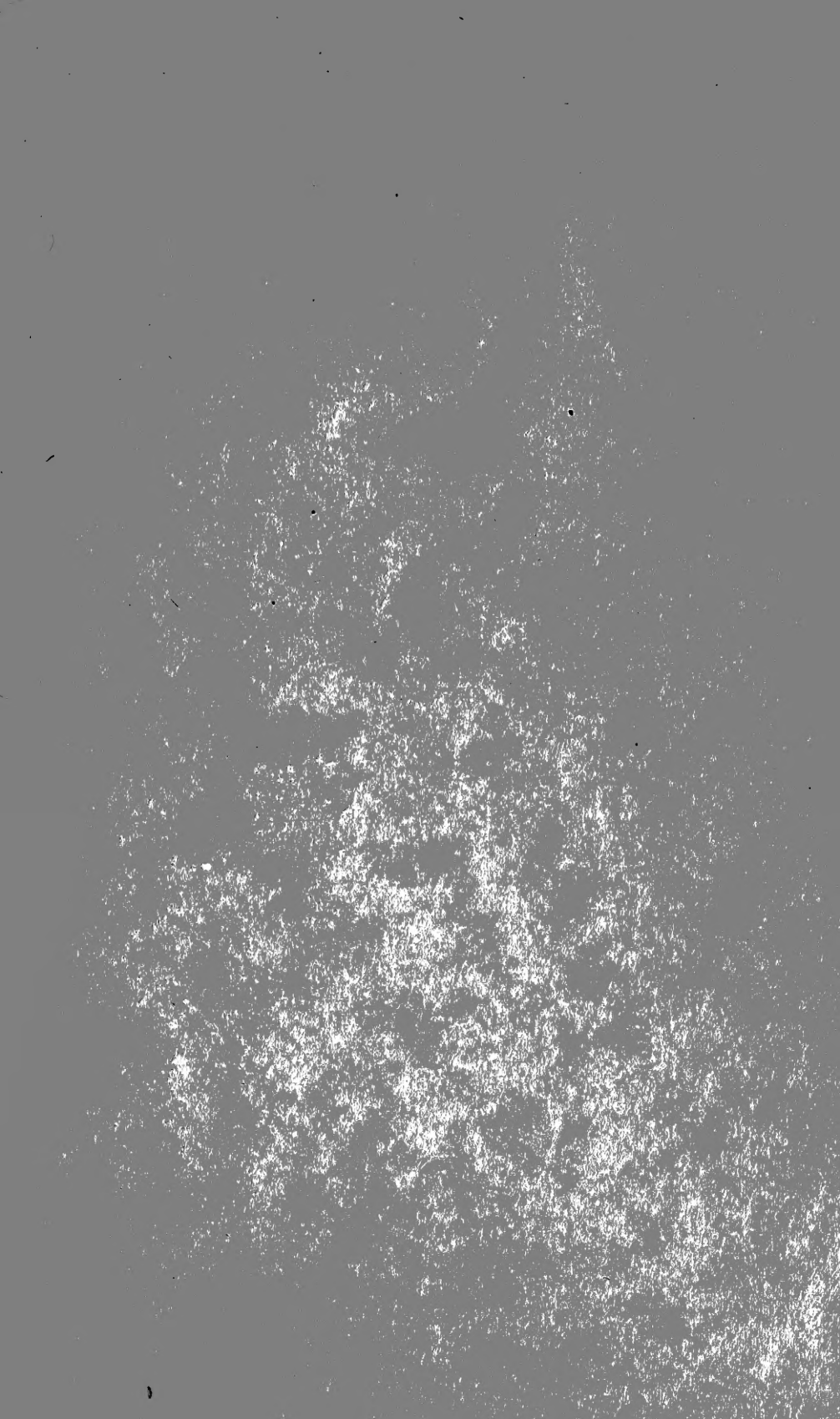
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