THE SPECIFIC EFFECTS OF CERTAIN LEAF-FEEDING COCCIDAE AND APHIDIDAE UPON THE PINES.

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In the great volume of entomological literature, we find but few references dealing with the specific effects of the attacks of sucking insects upon the tissues of their hosts. Statements describing the appearance of the work to the naked eye are, at most, meager in detail; and there are few records of morphological and chemical study of the affected plant tissue and of histological study of the parts of the insects concerned. The present paper summarizes the results of a year's work in the Entomological and Botanical laboratories of Stanford University on a few species of plant-sap sucking insects living on the needles of the pines and the precise character of the results of their work.

Over a year ago we became interested in finding the cause of certain, peculiar, light spots on the needles of the various species of pine found in California, the presence of which has not heretofore been explained. Also, it was noticed that light, greenish-white areas frequently surround scales of *Chionaspis pinifoliæ* Fitch, the white pine scale, and of *Aspidiotus abietis* Schr. (*A. californicus* and *A. florenciæ* Coleman), giving the leaves of a badly infested tree a mottled, sickly appearance. Later, these spots were observed to turn brown, often killing large numbers of the needles. The purpose of this investigation was to find an explanation for these phenomena.

The study of the insects *in situ* is of primary importance in working out this problem. Two methods suggest themselves: (1) observation of the insect at work and of the wounds made on the plant. The latter should be observed immediately after the insect has stopped feeding and at intervals later to determine the ultimate effect of the sucking upon the metabolism of the plant. Another method, (2), is morphological study of the insect and its work by means of sections through the affected tissues and the beak of the insect. Both of these methods have been used in the investigation here summarized.

The odor of two cubic centimeters of the acid diluted with fifty cubic centimeters of water was easily perceptible to a man at a distance of fifteen feet from the dish.

While my results do not agree with Howlett's, this may be due to the fact that we used different methods, or even to some dissimilarity in the responses of *Stomoxys* in the two regions. One can conceive of the same species of insect attuned to respond to certain odors in one environment, responding to very different odors in another.

CONCLUSION.

The odor of ammonia attracts a varied dipterous aggregation. The species which respond to it are known to spend at least a part of their lives in some form of animal excrement. Practically all animal excrement gives off ammonia during some stage in its decomposition. These flies are probably lured to the manure chiefly by the odor of this gas. The response is not always a simple one, but is sometimes complicated by other factors, as has been shown in the studies on *Musca domestica* L. referred to above.

I am indebted to Mr. J. R. Malloch for the identification of several species of Diptera.

BIBLIOGRAPHY.

Barrows, William Morton—1907. The reactions of the pomace fly, Drosophila ampelophila Loew, to odorous substances. Journ. Exp. Zool., vol. 4, pp. 515-537, (5 figs.)

Chatterjee, N. C.—1915. Chemotropism; influence of kusum oil on insects. Journ. Bombay Nat. Hist. Soc., Bombay, vol. 24, no. 1, pp. 198-199.

Howlett, F. M.—1912. The effect of oil of citronella on two species of **Dacus.** Trans. Ent. Soc. London, pp. 412-418, (pls. 39 and 40).

1914. A trap for thrips. Journ Econ. Biol. London, vol. 9, no. 1, pp. 21-23.

Imms, A. D.—1914. The scope and aims of applied entomology. Parasitology, vol. 7, no. 1, pp. 69-87.

Richardson, C. H.—1916a. A chemotropic response of the house-fly, (**Musca domestica** L.). Science n. s., vol. 43, no. 1113, pp. 613-616.

1916b. The Response of the House-Fly (**Musca domestica** L.) to Ammonia and Other Substances. Bull. 292, N. J. Agric. Expt. Station, 19 pp.

Severin, Henry H. P. and **Severin, Harry C.**—1914a. Relative attractiveness of vegetable, animal and petroleum oils for the Mediterranean fruit fly (**Ceratitis capitata** Wied.) Journ. N. Y. Ent. Soc., vol. 22, no. 3, pp. 240-248.

1914b. Behavior of the Mediterranean fruit fly (**Ceratitis capitata** Wied.) towards kerosene. Journ. Animal Behavior, vol. 4, no. 3, pp. 223-227.

Tragardh, I.—1913. On the chemotropism of insects and its significance for economic entomology. Bull Ent. Research, vol. 4, pp. 113-117.

Verschaffffelt, E.—1910. The cause determining the selection of food in some herbivorous insects. Proc. Sci. k. Akad. Wet., vol. 13, pp. 536-542.

It has been necessary to develop or adapt several points in microtechnique in order to use the second of these two methods, i. e., morphological study. These points have been discussed in an article* which recently appeared in *Science*.

I. The White Pine Scale, *Chionaspis pinifoliæ* Fitch.

The white pine scale, *Chionaspis pinifoliæ* Fitch, is a conspicuous and widespread pest of pines in North America. It is, as a rule, particularly common on transplanted trees. In the East, the most important host is the white pine, *Pinus strobus*, which it has attacked wherever this pine is grown in North America, often proving a serious pest. It has done great damage to the Monterey pine, *Pinus radiata*, in California.

The white pine scale is entirely a leaf feeder. The flat side of the needles is usually occupied, but in a serious infection the rounded surface is covered as well. As a rule, a single row will be found along a needle, the scales occuring in groups.

As seen in Photograph I, wherever a scale or group of scales occurs, a light colored area with much less chlorophyll than usual is found. It is evident that this is due, primarily, to the sucking of the insects. After many months of sucking, many of the light-colored areas die and dark, reddish spots appear. One of these spots is shown on the upper needle in Photograph I, directly under the scale. Later the needles change from the mottled appearance they have had for some time to a uniform, sickly, yellow color and finally die. Great numbers of the scales are attacked by parasites, but these usually come too late to be of service in checking the damage done by the particular insects they parasitize. It is evident that they partially overcome the enormous increase of which this insect is capable. Lady bird beetles, including *Chilocorus bivulnerus* Muls., the two-stabbed lady bird, are usually of great help.

The amount of damage which a pest does is in direct proportion to its numbers. An insect may be widespread, but it may be held in check so that it is usually of comparatively small importance. Occasionally this species becomes abundant enough to cause great damage. We find that when for any cause the trees are at all weakened, for instance by fire or lack of

*Brown, Kearn B., Microtechnical Methods for studying certain plant-sucking insects *in situ*. Science, N. S. Vol. XLIV, p. 758, 1916.

sufficient water, the damage from the scales is much more noticeable. It is rarely that trees are actually killed by its attacks.

It is not an easy matter to estimate the amount of damage by such a pest, even when it occurs in such countless numbers as on the Monterey pines at Stanford University. The amount of damage may be estimated by determining the per cent of leaf tissue which has lost its chlorophyll by the sucking and the additional percent of leaf tissue lost by the death of the needles long before their normal time for dying. Our records for *Pinus radiata* in the Stanford arboretum indicate that approximately five per cent of leaf tissue is destroyed in these two ways by *Chionaspis pinifoliæ* Fitch. This injury, together with that caused by the other pests of the Monterey pine, such as *Diplosis pini-radiatæ* Snow and *Physokermes insignicola* Craw so weaken the trees that they fall an easy prey to the Scolytid beetles.

A morphological study of a normal Monterey pine leaf shows its structure to be as follows: On the outside is the epidermis, a single layer of cells through which the stomata, or breathing pores, pass to the tissues underneath. Under the epidermis is the schlerenchyma, a strengthening tissue made up of several layers of heavy-walled cells. Next is the mesophyll with characteristically infolded outer walls, the cells of which contain the chlorophyll granules. There are two resin ducts, one located at a point inside each angle made at the union of the rounded and flat surfaces of the leaf. The resin ducts are surrounded by a double row of resin-secreting cells. Inside the mesophyll is the bundle sheath surrounding the stele with its two parallel vascular bundles. All the tissues described above are shown in Photomicrograph No. II.

Examination of the photomicrographs II and III will show what the insect does to the plant. The four sucking and piercing setæ of the coccid, which are the modified mouthparts of this family, enter the needle either by spreading apart the epidermal cells or at an open stoma. Photograph III shows that schlerenchymatous cells have been bored through. The appearance of the cells around the setæ indicates that this insect, in common with some other members of the family and the *Aphididæ*, uses saliva to dissolve a passage through the hard walls. This section shows that a sheath has been formed around the setæ.

This sheath consists of viscous saliva and passes through the whole of the open space that has been formed by the solvent action of the saliva to a cell that has just been attacked and where fresh saliva has just been poured out to dissolve the cell wall and contents. As soon as a cell has been pierced, its turgor, or pressure from the inside, which is several atmospheres, gives away. The cell's liquid contents is partly forced by the pressure of the surrounding cells and partly leaks out into the open space. It is then sucked up into the insect's body.

Plateau* has shown that the saliva of *Hemiptera* has the power to change starch to sugar. We have proven that this coccid is no exception by the tests made on cells that had just been pierced by the insect. We get negative results with iodin as a test for starch, but Fehling's solution, a test for sugar, gives positive results. The presence of proteid-dissolving and cellulose-dissolving enzymes in the saliva is indicated by the destruction of the cells of the plant by its work. Most of the nutriment is obtained from the mesophyll tissue. This is shown by the number of cells broken up and destroyed or partially destroyed in all of the many sections examined. So we place *Chionaspis pinifoliæ* Fitch in the second class of sucking insects described by Busgen,† "those piercing into the parenchyma by boring through the cells."

It is not possible to say positively whether there is an actual poisoning to the plant by one or more of the enzymes that are injected into its cells. The following evidence points toward such conclusion: The mesophyll tissue is first discolored, then browned and killed, as shown in photomicrograph IV. The killed and discolored mesophyll cells are those shown darkest in the photograph. The mesophyll region near the upper side of the illustration would appear exactly the same as the corresponding area near the lower margin, if the scale had not been sucking in the vicinity of the darkened cells, thereby killing them. This dead tissue extends for a distance much greater than the beak can pierce.

It is not likely that these cells are merely dried out and starved by withdrawal of liquid coming up from the roots,

*Plateau, Recherches sur les phenomenes de la digestion les insectes. 1874. Bruxelles.

†Busgen, M., Der Honigtau, Jenaische Zeitschrift fur Naturwissenschaft, Bd. 25, p. 381, 1891.

because, so far as it is possible to see, the fibro-vascular bundles are not in any way injured. It is probable that the xylem functions in an infested leaf as in a normal one, supplying water and raw materials.

Our research goes to show that one effect on the pines of the work of various insects is only accidental. This is the piercing of the resin duct or of the cells so close to it that the resin becomes infiltrated through a part of the leaf, giving it a white, spotted or banded appearance. That this occurs but rarely in the case of *Chionaspis pinifoliæ* Fitch is explained by the common location of the insects in a single row along the needles, with the beaks entering the leaf tissue at points remote from the resin ducts. In the section shown in photographs II and III, the saliva has dissolved tissue not more than two or three rows of cells distant from the resin duct.

II. *Aspidiotus abietis* Schr.

Another of the economically important *Coccidæ* affecting the conifers is commonly known as the black hemlock or pine scale, *Aspidiotus abietis* Schr. (*A californicus* and *A. florenciæ* Coleman). Observation of trees badly affected by this scale shows that it does great damage at certain times. At Campo Seco, Calaveras, Co., California, we* found it in 1915 the most abundant scale of the region, stunting and killing large numbers of digger pine, *Pinus sabiniana.*

The appearance of *Aspidiotus abietis* Schr. on the trees and the damage it does is shown in photograph V. The damage, a mottling of the needles followed by browning and dying, is similar to that of *Chionaspis pinifoliæ* Fitch. Great numbers of the leaves fall. The remaining leaves are often so sickly looking, or so many are browned, that whole groves of affected trees may look as if a fire had singed the leaves. One noticeable difference from the work of *Chionaspis pinifoliæ* Fitch is the large number of spots which have lost all chlorophyll. These spots are often one-fourth of an inch in length and extend, in some cases, around the needle. These spots will be discussed later as "infiltrated spots."

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*Brown, Kearn B., Rpt. of Entomologist and Pathologist on Pests and Diseases of the Campo Seco, California, Region. 1915.

Morphological study of infested needles shows that the manner of sucking is similar to that described above for *Chionaspis pinifoliæ* Fitch. Photograph VI of the setæ *in situ* in a needle of *Pinus sabiniana* gives an idea of the first stage of the work. Owing to the size of the scale and the shape of the needles of many of the hosts, this scale frequently pierces the resin ducts accidentally. Credit for this discovery is due to Mr. E. A. Cornwall, graduate student in Entomology at Stanford University, who, in preliminary work in this investigation, cut sections showing this. The "infiltrated spots," caused by this tapping of the resin duct, are similar to those due to aphid attacks and shown in photograph VIII. A section of digger pine needle, showing killed cells infiltrated from broken resin duct is shown in photomicrograph VII.

III. *Aphididæ* on Pines.

Several species of *Aphididæ*, or plant lice, infest the pines, including, at Stanford University, *Lachnus pini-radiatæ* Davidson and *Essigella californica* Essig. As but little is known of the effects of conifer-feeding *Aphididæ*, we have made careful, daily observations of these species for a period extending over six weeks. We aimed to find out their habits and to determine, if possible, whether there is any relation between the aphid attacks and three abnormal conditions found on infested needles. Examples are shown in Photograph VIII. They may be described as:

(1) minute white spots;

(2) gumming, apparently from small leaf punctures;

(3) infiltrated spots similar to those described above on page 418. Some of the infiltrated spots show a reddish area in the center of one side, either as a small red mark or as large spots of dead tissue.

Lachnus was found to be a comparatively sedentary species. It seldom removes the beak from the tissues, often remaining in one position for weeks at a time, even in stormy, windy weather. It is conspicuous, usually being found in colonies of two to six apterous females, the progeny of one stem-mother, on one side of the needle. The whole colony is not over an inch in length. The part of the leaf where they are working is covered with gray, waxy secretion. The leaves finally

get a coating of black, sooty mold, which grows in the honeydew excreted by the aphids. Both the gray secretion and the sooty mold fungus greatly injure the appearance of infested Monterey pines. Frequently, when a colony of *Lachnus* is rubbed off, small, discolored areas where the beaks have penetrated are disclosed. A large specimen which has been parasitized and killed is shown in Photograph IX. This also shows the final effects of the work, a discoloration of the needle where the insect had sucked. The reddish area extends entirely around the needle. A light green area is shown on either side of this red spot.

Essigella was found to be an active species, the individuals sucking for a day or two in a place, retreating to a protected position at the base of the needle bundles at the slightest disturbance, and also remaining hidden there in wet, windy weather. Detailed, daily observations of all individuals on a number of selected needles were made for a period of six weeks.

Selecting examples at random from our records of *Essigella*, we find that punctures were made during this comparatively short period in 83 places on one leaf, and 74 on another, though the number of aphids on a leaf at the time the observations were made was, on the average, five. Conclusions drawn from the observations are as follows:

(1) Out of about 6400 feeding positions observed, but few left any mark that was visible to the eye, except sooty mold. This was removed at the end of the observation period to make it possible to find any markings there might be beneath.

(2) *Two* feeding positions, in each case of one day only, were followed by an exudation of gum. We made sections to find the cause of the exudation. Both were shown to be caused by a puncturing of the resin ducts. This is apparently accidental, as they would be as poor feeding ground as could be found in the leaf.

(3) *Three* cases of infiltrated spots occurred. One was accompanied by gumming. All were the same as other infiltrated spots found on aphid- or coccid-infested pines.

(4) There were *ten* small, light colored spots which, in every case, followed a period of sucking of two or more days.

Experiments were tried to determine whether infiltrated spots could be produced artificially. Selected digger pine

needles were pricked in the region of the resin ducts with Minutien-Nadeln, which are very small needles, commonly used for pinning tiny insects. Effects of the punctures were noted as follows: Out of 20 pricks, *four* showed an exudation of gum. After two weeks two of these gave evidence of infiltration. At the end of the second week, the spots were small. They continued to grow larger.

IV. Conclusions.

We can say, therefore, that the damage to the needles of pine trees by aphid feeding is:

(1) From the honeydew fungus, which makes the trees unsightly and interferes with the process of photo synthesis, or food manufacture, in the needles by shutting off part of the light;

(2) By discolored areas from which food has been taken and which have been whitened by admission of air to the cells;

(3) By the making of conspicuous, infiltrated spots and causing gum exudation;

(4) Inconspicuous damage by the great number of piercings and suckings. This, though usually invisible, is probably more important than the first three taken together.

The damage to pines by the coccids studied is due to sucking and the enzyme action of the saliva injected to assist in this process. The damage consists of the destruction of chlorophyll-bearing cells with a subsequent whitening of the needles. This is followed by the death of the needles in some cases, or, occasionally, by the production of infiltrated spots that, while conspicuous, are not of great importance.

EXPLANATION OF PLATES XVIII AND XIX.

Fig. 1. Effect of *Chionaspis pinifoliæ* Fitch. On needles of Monterey pine. (x3). On needle above, the red area (very dark in photograph) shows where the beak has punctured. A light green area extends for some distance on either side of this spot. Several of the scales shown in the needle below have been parasitized.

Fig. 2. Photomicrograph of section of Monterey Pine Needle (x60) showing beak of *Chionaspis pinifoliæ* Fitch *in situ* (in open space in upper right hand corner).

Fig. 3. Photomicrograph of beak of *Chionaspis pinifoliæ* Fitch, in section of Monterey pine needle (×360). Same as Photograph II, more highly magnified. The setæ, which form a sheath of saliva as soon as they enter the leaf, are shown in position, also the effect on the leaf tissues. The setæ have entered the leaf on the upper side, passed through the epidermal and schlerenchymatous cells, and entered the mesophyll region. The open space shows that most of the cells have been dissolved, with some of the cell walls not yet completely destroyed, while those on the extreme left of the photograph that are not in direct contact with the saliva are in normal condition. During the preparation of the section, the setæ were accidentally broken from the body of the insect near the point where they entered the leaf tissues.

Fig. 4. Photomicrograph of a longitudinal section of a leaf of Monterey Pine (×60); the mesophyll tissue near the upper margin of the photograph has been killed and discolored by *Chionaspis pinifoliæ* Fitch.

Fig. 5. *Aspidiotus abietis* Schr. on needles of digger pine, showing adult and young scales *in situ* (×3). The light-colored areas on the leaves are the effect of the sucking.

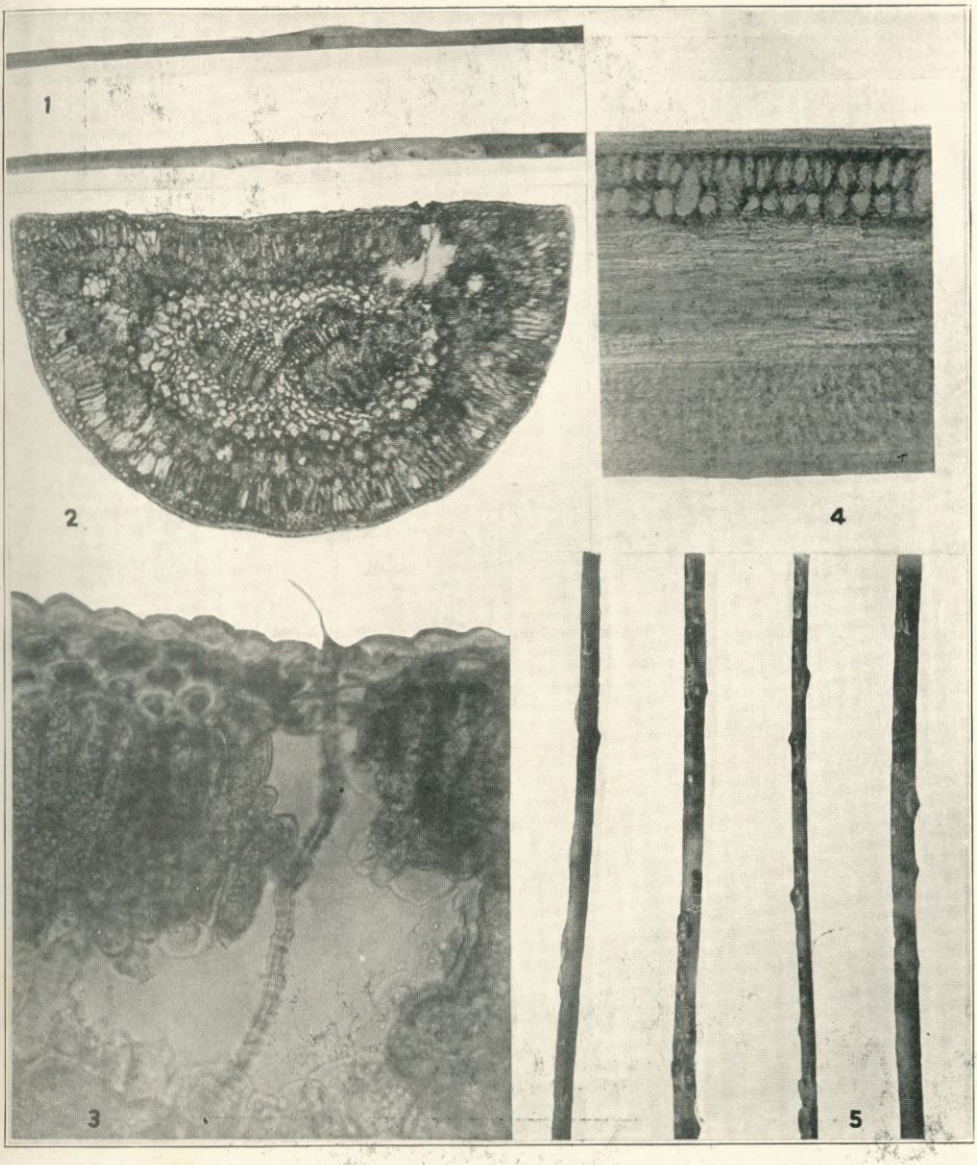
Fig. 6. Photomicrograph of stained section of digger pine leaf (×360), showing beak of *Aspidiotus abietis* Schr. *in situ*. The sucking setæ have just entered the mesophyll tissue, hence no large open space has been dissolved. One cell has been killed. The cell from which the coccid was sucking at the time it was killed and sectioned is partially filled with saliva. A drop of the saliva may be seen near the distal end of the setæ.

Fig. 7. Photomicrograph of a section of digger pine needle (×120) showing killed cells infiltrated from broken resin duct, the large open area a little to the right of the part of the section photographed. All the mesophyll cells above and to the right of the duct have been infiltrated. There is a distinct line separating them from the non-infiltrated area.

Fig. 8. Effects of aphid sucking on digger pine (×3). Needles 1 (on left), 2, 5, and 6 show small, white areas; 2, 3, 4, and 6 show infiltration. The spot on needle 3 is without exudation, but has a red center.

Fig. 9. Parasitized *Lachnus* on Monterey pine needle, with results of sucking. (×3).

NOTE: In making the plates the author's photographs have been slightly reduced, hence measurements given above are only approximate.



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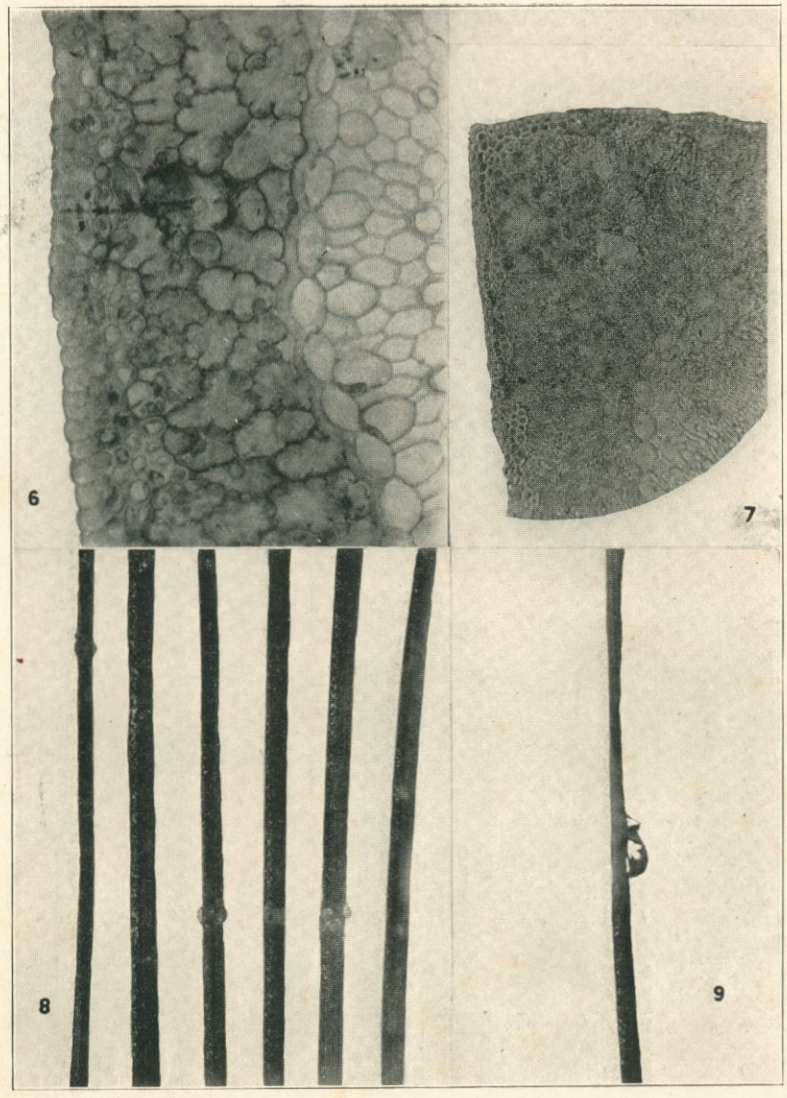

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