FUSARIUM DISEASE OF COFFEE IN COSTA RICA *

By C. Picado T.

The literature on Fusarium diseases of coffee throughout the world is very meagre. Delacroix (**4**)[1] gives *Fusarium coffeicola* as mildly attacking the leaves of coffee in Africa. Averna Saccá (**2**) reports the presence of *F. pallens* in the roots of coffee plants attacked by *Rosellinia* and in some nurseries attacked by *Colletotrichum* sp. in Brazil. It is also found in root lesions produced by nematodes. He reports *F. coffeicola* producing a blackening of the berries and causing their premature falling in Brazil. Fawcett (**5**) reports a *Fusarium* attacking coffee plants in Puerto Rico, destroying the bark at the base of the stem. He believes the fungus follows injuries produced by implements and insects. The disease may be reproduced by inoculating plants with pieces of diseased tissues. Arndt and Dozier (**1**) report *F. martii* following injury by the coffee cricket. As a result of the attack, the cortex becomes black and the trees shed their leaves. A consideration of the above facts establishes the malady under discussion as a new disease caused by an undescribed fungus.

HISTORY

It is very probable that the disease under discussion is none other than the one called "Chasparria" by our farmers. For several years it has been present in the coffee plantations, but has failed to attract attention because its presence was limited to a few scattered plants. For the last two years, the disease has been decidely on the increase and suddenly, most unexpectedly, a severe outbreak occurred after the trees had withstood fairly well a long period of drought during the years 1929 and 1930. The rainfall for the last five years was as follows:

	Meters	Inches
1927	2.233	87.91
1928	2.295	90.35
1929	1.775	69.88
1930	1.153	45.39
1931 (11 months only)	2.420	95.27

Following the beginning of the rainy season of this year (1931)

* A contribution from the laboratory of the hospital.

[1] Numbers in parenthesis refer to literature cited.

the trees, instead of initiating new growth, shed their leaves and the new shoots dried up and blackened. Whole plantations, many acres in extent, were completely defoliated.

The disease seems to have had its beginning in the province of Heredia and gradually progressed towards the East, apparently following the direction of the wind. Later, the disease appeared to some extent in Alajuela and more recently, we have seen isolated cases of it about 15 kilometers from the center of the infestation, irrespective of the kind of soil.

LOSSES

An accurate estimate of the losses as a result of the disease is, at present, impossible. Although many of the attacked plants died, many more recovered but made an abnormal growth. In some places, the disease began before the start of the rainy season and when the berries were maturing. As a result, the yield this year will be greatly reduced. Diseased plants, although recovering, will yield less each successive year until the crop will fail to pay expenses. The culture of coffee will then be abandoned.

Expert growers estimate that the crop in the affected areas will be reduced by 25 per cent. At present prices, this means a gross loss of about $100,000.

SYMPTOMS

During the course of the disease both internal and external symptoms are produced. The external symptoms differ depending on whether the disease develops in the spring or autumn.

In the first case, the plants are attacked severally and the course of the disease is more rapid. With the first rains of the spring healthy trees start their new growth and begin to flower. The heavy rains follow these early showers from two to four weeks later; and with them, active vegetative development ensues. Diseased plants, however, fail to renew active growth with the establishment of the rainy season proper and instead, the young, tender shoots produced with the early rains die and become charcoal black (Fig. 1). In some cases the trees begin to shed their leaves, the new shoots stop growing without blackening, but produce abnormal leaves and branches. New plants pruned half a meter from the ground produce new shoots but these are abnormal. Diseased, abandoned fields have plants with their tops dead but with a proliferation of thin, slender, unproductive branches underneath.

In the autumn, the plants are attacked when vegetative growth

is slow and the berries begin to ripen. There is not the rapid defoliation that characterizes the spring symptoms, but instead the disease appears gradually and its course is much delayed (Fig. 4). The invasion, however, is persistent and finally the fruiting branches are destroyed. The berries from such branches are seedless and only those fruits that mature before the branches begin to dry have a normal appearance.

Besides these obvious symptoms, there are others more difficult to detect such as the splitting of the cortex of green branches; a gradual yellowing of the leaves, beginning along the veins, prior to their being shed; dry, sunken areas of cortex tissue and black necrotic are formed in dead branches.

If the cortex of a diseased plant is carefully peeled off, black streaks or filaments running from the roots upwards are evident along the vascular tissue. Sometimes these streaks extend to the medulary rays and the pith. The cortex from such plants is easily removed around the lower part of the stem and the upper part of the root. A carefully pulled plant will have a very large number of its rootlets partially desintegrated, blackened and sometimes totally necrotic. Often the injury extends to the primary and secondary roots.

ETIOLOGY

The disease is produced by *Fusarium anisophilum,* n. sp. Its perfect stage is *Nectaria anisophila,* n. sp. which develops in diseased shade trees.

ISOLATION OF THE FUNGUS

The fungus has been isolated easily by aseptic plantings of pieces of infected roots or bark on sugar agar.[1] From this medium the fungus has been systematically transferred to Richard's [2] solution for further growth. It has also been grown on rice (autoclaved in an equal volume of water), potato, carrot, kidney bean and autoclaved coconut water previously boiled and filtered.

CULTURAL CHARACTERISTICS

On sugar agar initial cultures 3–5 days old always produce a light rose color which soon changes. Subcultures from these are almost white or orange, intense vermillion or violaceous red. After the second day, unicellular conidia 3 $\mu \times 6\ \mu$ are produced, especially

[1] Sugar Agar: Sugar-cane syrup (Panela) 10 per cent; Peptone 1 per cent; Ammonium phosphate 0.5 per cent; Agar-Agar 3 per cent.

[2] Richard's solution: KNO_3 10 grs.; KH_2PO_4 5 grs.; $MgSO_4$ 2.5 grs.; $FeCl_3$ 0.2 Mgms.; Sugar Candy 50 grs.; H_2O 1000 c.c.

so in the red colonies. In the white or orange colonies are found long, thin, 2, 3 or 5 septate conidia with curved ends, measuring 4 μ × 40 μ.

Since the production of a given color can not be safely regarded as an index to the unity of the species under study, various tests were made to elucidate this point. The various subcultures were grown on Richard's solution to which 1 c.c. of a 1 per cent solution of caffeine sulphate had been added for each 100 c.c. of the medium. All the cultures, irrespective of their original color, produced salmon colored colonies. In a few weeks both micro and macroconidia were produced. When transferred to slants macroconidia were produced in 6 days even by the less sporulating types. The presence of caffeine was equally antiseptic to all the subcultures and the same thing may be said of the presence of lactic acid.

Old mycelium grown in Richard's solution when ground gives an acrid odor. Allowed to oxidize by exposure to the air the odor soon recalls that emitted by bed bugs. *F. cubense,* a species very similar to *F. anisophilum,* specially when grown on rice, when similarly treated produces an agreeable fruit-like odor.

Successive plantings in liquid media with the same fungus were found to inhibit the growth if the same species was repeatedly used as the inoculum. To this effect, the apparently different sub-cultures of *F. anisophilum* and also *F. cubense* were grown in Richard's solution in flasks, so as to expose a large surface of the medium to the growth of the fungus. Every two weeks the solution was filtered and replanted with its corresponding fungus. After the third planting, the medium was no longer favorable for the growth of the fungus even when a proportionate amount of the nutritive salts were supplied to each flask. The different subcultures of *F. anisophilum,* irrespective of their color or characteristics, when interplanted in the culture media on which they have been growing failed to develop, but when any one of them was planted on the fluid medium where *F. cubense* has been growing, they made very good growth. When *F. cubense* was planted in its own culture medium it failed to grow, but when planted in any of the media where the various strains of *F. anisophilum* had been growing it also made very good growth.

This method of specific vaccination of the liquid medium has not been used to differentiate the imperfect forms of fungi, but we look upon it as more promising than the present serological tests.

Within the limits of our cultures *in vitrio* we feel justified in establishing the unity of the species for the following reasons: (1) Mutation or loss of color; sometimes in the same agar slant. (2) Same coloration in the presence of caffeine. (3) Production of mi-

croconidia and macroconidia irrespective of the original inoculum. (4) Equal sensibility towards antiseptics. (5) Production of same odor producing substances, and (6) same reaction towards vaccinated media.

FRUITING BODIES

After diseased plants have lost their leaves and their branches begin to dry, small epidermic vesicles are found, particularly behind the leaf scar, which contain the sporodochia. These produce either macroconidia or microconidia. Generally, the conidiophores have four segments. If these are thick and short, they will develop into the microconidiophore at the end of which one microconidia usually is present, although rarely there may be two (Fig. 9 *a*). Sometimes the conidiophores are not so uniform and divide into two or three branches.

With *F. cubense,* microconidia and macroconidia are successively produced from the same sporodochium; while in *F. anisophilium* the conidiophoresare differentiated into either one or the other type of conidia.

The microconidia are ovoid, uniloculated, and average $3\,\mu \times 7\,\mu$ in size. The macroconidia are in general almost straight, with the ends bent, and average $4\,\mu \times 40\,\mu$. Some are 6 or 7 septate and measure $7\,\mu \times 60\,\mu$. Intermediate forms with one septum, measuring $4\,\mu \times 20\,\mu$ are also found.

The conidiophores are not always produced immediately, under the epidermis; frequently they are found beneath the cortex directly on the vascular tissue.

Intercalary chlamydospores, spherical and rarely elliptical, are generally formed in sugar agar plates and old carrot slants. When grown on kidney beans, macroconidia are always produced within 8 days irrespective of the type of inoculum used, the medium on which it has been growing previously or its ability to produce microconidia in such medium.

The easiest procedure to obtain conidia is by making an emulsion of the finely ground mycellium in sterile water and inmersing young coffee shoots in it. Within two or three weeks conidiophores bearing conidia with their silky paraphyses are abundantly produced.

THE PERFECT STAGE

The perfect stage of *F. anisophilum* was found in the lesions of diseased shade trees. We have classified it as *Nectria anisophila* n. sp. Its description is as follows:

Perithecia isolated or in colonies, globular, simple or with projections arising from the vesicles covering their walls, deep orange almost vermellion in color measuring 270 μ — 340 μ in diameter; asci cylindrical 70 μ — 90 μ × 12 μ —14 μ; spores 8, monoseptate, usually incline, uniseriate, 14–15 μ × 6–7 μ, hyaline, smooth, elliptic or fusoid; paraphyses filiform, branching, twisted (Fig. 9).

This species differs from *N. inga* Chardon, (**3**), by having larger perithecia and by the presence of branched and twisted paraphyses. On germination *N. inga* failed to produce the form *F. anisophilum*. The codinial stage of *N. anisophila* reproduced in all its details the cultural and pathogenic reaction produced by *F. anisophilum*. Ascospores cultures of *N. anisophila* are able to reproduce the perfect stage when grown on coffee shoots in a moist chamber provided the source of the ascospore has been *Inga* trees and not coffee trees. Subcultures made from said fungus growing on coffee shoots fail to produce the perfect stage when reinoculated into coffee shoots in a moist chamber. *By passing thru the coffee plant as a host the fungus losses its ability to produce its perfect stage.* This explains our failure to obtain the ascigerous stage when making cultures of the fungus isolated from diseased coffee plants. The Nectria form may be obtained from any of the cultures isolated from coffee by growing them on branches of *Inga* in a moist chamber.

SECRETIONS OF THE FUNGUS

In order to study the hosts reaction towards the secretions of the fungus, cultures of *F. anisophilum* were grown in 500 cc of Richard's solution in one liter flasks. *F. cubense* was similary grown for use as a check. At the end of one month the culture fluids were filtered and diluted in various proportions. Young coffee shoots and 12 day old kidney bean plants cut under water were inmersed in the various solutions. In every case, corresponding checks were made by heating the various fluids for 5 minutes at 100°C.

The results warrant the following conclusions:

1. The fungus secretes a thermolable diastase capable of digesting the tissues of coffee or kidney beans in contact with it.

2. There is a blackening of the coffee tissues immersed in the fluid.

3. Both of these reactions are greatly inhibited by heating the fluids.

Similar tests were made using emulsions of the ground mycelium. Coffee branches immersed in this fluid without previous heating reacted in a similar way as diseased plants in the field; the leaves

wilted, became clorotic beginning at the veins, soon dropped and the branches died. *There is, therefore, a toxic action effective at a distance capable of inducing a chlorotic condition and shedding of the leaves.*

INOCULATIONS

Direct, soil and field inoculations were made. Direct inoculations of the leaves and branches were never successful; the inoculum used was a sporulating culture a few days old. Inoculations into thick roots of old trees also failed and the wounds healed normally.

In pots, with sterilized soil, which was inoculated with the fungus, plants in the butterfly stage, i. e., with only the cotyledons expanded; and plants one year old having six pairs of leaves were planted. Three plants were set in each pot, 2 were left uninjured and the third one was punctured in the root crown. Checks were similarly treated. The uninjured plants in the butterfly stage did not seem to contract the disease but the injured ones are greatly retarded in their growth, a reaction not evident in the check plants (Fig. 4). On the other hand, the one year old plants were immediately attacked by the fungus which destroyed the cortex from the base of the stem upwards (Fig. 5), whether or not punctured at the time of planting.

The resistance of the small seedlings towards the disease might be explained on the basis of the antiseptic action of the caffeine towards the fungus, since they contain practically all the alkaloid present in the seed.

Keeping inoculated plants at a temperature of 18°–22°C. seems to greatly diminish the activity of the pathogene or increase the resistance of the plant to its invasion. The year old plants in inoculated soil may, at that temperature, remain apparently healthy for at least 5 months. The same plants taken outdoors (45°C. under sunlight) begin to defoliate in about 5 weeks and by that time the cortex of the branches has begun to dry. They behave as if inoculated at the time of transferring them to the open.

In our experimental plot, 25 two year old plants brought from areas not infected were set in 5 rows, 2½ meters apart and inoculated as follows: The middle row was inoculated at the time of planting with pieces of diseased roots around each ball of earth. The remaining rows were allowed to establish themselves and two weeks later the soil around them was inoculated with cultures of the fungus. One row was not inoculated to serve not as an absolute but as a relative check. At the end of a month, the row inoculated at the time of planting had 15 branches completely defoliated and begin-

ning to blacken while the remaining 4 rows had a total of 16 defoliated branches. Two weeks later the row inoculated at the time of planting had 18 defoliated branches; those inoculated fifteen days later had 7, 10 and 16 defoliated branches respectively and the check row had only 4 defoliated branches. By this time the fungus had spread through the soil to the check row and could be isolated from the soil around it. At the end of two months the plants were showing typical symptoms of the disease; the stems had gray colored cracks surrounded by black tissue (Fig. 6) and the new shoots in the base of the branches died back, showing that although defoliation occurs from the lower branches towards the top ones, death follows afterwards from the top towards the base.

Proliferation of rootlets followed the destruction of the roots by the fungus (Fig. 7). The fungus was recovered from these disease roots and sporodochia were present in the defoliated branches.

As previously stated inoculations performed in the thick roots of old trees failed to reproduce the disease for 5 months and the inoculum failed to enter the host through the injury. After this period, however, plants were infected. A close examination revealed that the inoculum spread through the soil and later attacked the young, fine roots.

OTHER HOSTS

Kidney Beans.—By an accidental contamination in the laboratory this legume was found to be susceptible to *F. anisophilum.* Repeated inoculations demonstrated that the black kidney beans were resistant, but the white and red varieties highly susceptible. The roots were destroyed and cankers developed at the base of the stem as a result of the infection, but the vascular tissue was not penetrated (Fig. 8).

This plant was used in the field as an index host to study the spread of the fungus in the soil. It was found that in two weeks the fungus spread 2½ meters through the soil.

Lima Beans.—A small planting of this legume growing near our inoculated plots was attacked also by the fungus.

Flame Tree (Ponciana regia).—A natural case of the disease has been found on this tree under which diseased wood was piled. The fungus *F. anisophilum* was isolated from the diseased tissues.

Shade Trees.—In view of the fact that most coffee shade trees are legumes, a close examination of them was made. Lesions and symptoms similar to the ones produced by the coffee disease were found. Tissue plantings of affected roots and branches gave cultures of *F. anisophilum.*

Gliricidia maculata, Inga sp., and *Erythrina* sp. were found to contract the disease. The perfect stage of the fungus, *Nectria anisophila* was found in the dead branches of these hosts.

CONTROL

Mechanism of Infection.—Taking into consideration the prolonged periods of drought and the fact that the disease appeared within a a few kilometers of the capital at a time when the increased use of electricity for heating and as a substitute for other sources of fuel, brought about a decrease in the price of timber, we are led to conceive the origin of this disease in the following manner. In the old days, it was a regular practice among coffee growers to prune their coffee shrubs and shade trees, and to sell the waste wood so obtained, there being a ready market and quite a big demand for it. This demand has fallen off and there is no market for this wood. The practice of pruning is gradually disappearing and those growers who still practice it, pile up the twigs and branches either in the coffee plantations or in places nearby. Every one of these piles has become a breeding place for millions of *Nectria* which have scattered and invaded the surrounding leguminous trees and coffee shrubs. The spread of the disease following the light rains, and its increased virulence during the rainy season is thus explained.

Once the soil becomes infested, the fungus attacks the small rootlets and the fate of the plant depends upon the severity of the infection and upon the fertility of the soil. In those cases where the infection is severe, even though the plant produces new roots the fungus will make much headway, and the plant will become seriously affected. In poor soils where root development is checked, even slight infections will prove disastrous to the plantation. On the other hand, in good soils the plants will tolerate and withstand a slight infection.

Defoliation may be ascribed to two reasons: Not enough water to supply the demands of transpiration and photosynthesis, and the toxic action of the enzymes or catabolic secretions of the organism. Coffee seedlings planted in pots filled with inoculated soil thrive very well for some months at least, provided they are kept under cover, in a humid atmosphere, and are watered regularly. These seedlings soon loose their leaves after they are planted in the open in spite of our efforts to gradually accustom them to the effects of direct sunlight. The roots of those plants which are grown under shade, and

of those grown without shade are injured. Plants grown in pots continued to live although the dead tissue in the cortex extended as far as the woody cylinder. They continue to live even if the bark at the base of the stalk is stripped off.

The presence of the fungus in the branches is due to secondary infection. In spite of repeated inoculations and spores sprayed on the branches of healthy plants, we have not been able to produce the disease. Everything tends to prove that initial infection takes place through the roots. Cultures from root material have always yielded the *Fusarium anisophilum* only, while culture from branch material show other genera of fungi also.

Some years ago, several *Poinciana Regia* tres were imported from the island of Puerto Rico, and planted along a road leading to the Insane Asylum near our laboratory. One specimen was planted near a shed which is used for storing timber. Since this timber came from an infested zone, we asked our collaborator Mr. Elías Vicente, to examine it for *Nectrias*. Not only did he find plenty of them, but he also called our attention to the fact that the *Poinciana* which was growing nearby, was beginning to show symptoms of a disease similar to that attacking coffee and *Gliricidia,* namely: defoliation, drying up and blackening of the branch tips, and black streaks along the vascular region. We proceeded to dig out and wash several roots. Upon examining them we found that they exhibited bark lesions. Out of 20 cultures made in sugar agar Petri dishes, 17 of them showed a *Fusarium* which had all the characteristics of *F. anisophilum.*

Infection was possibly due to the proximity of the tree to the infected wood pile, since the other *Poinciana* trees are still healthy.

Control Measures:

Studies on the life-history of the pathogene makes us advocate the following control measures:

1. In infested regions, the seed beds should be made in as poor a soil as possible with the idea of obtaining by natural selection, only those plants which survive because of unusual resistance.

2. In new plantings, the use of shade trees susceptible to the disease, should be discontinued.

3. Prune and burn all dry twigs and branches, from coffee shrubs and shade trees alike.

All these protective measures should be adopted simultaneously, and legislation to that effect should be passed. Any source of in-

fection which is not destroyed might result in the spread of the disease by the wind, and the infection of the soils.

Acknowledgments.—Before bringing this paper to an end, the author wants to express his indebtedness to the laboratory staff for their helpful collaboration; to Mr. Ingo H. da Silveira-Grillo for his valuable information about the *Fusariums* attacking coffee in Brazil; to Mr. Bienvenido Matienzo of the Department of Agriculture and Commerce of Puerto Rico for his helpful suggestions in gathering the bibliography; to D. Elías Vicente for his devoted help; to Mr. Arturo Roque, Assistant Phytopathologist of the Insular Experiment of Puerto Rico for translating the manuscript into English and to the Insular Experiment Station of Río Piedras, Puerto Rico, for publishing the paper.

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Explanation of Plates

PLATE XLIX

Fig. 1. New shoots die back producing abnormal forms.
Fig. 2. Abandoned tree, top dead, abnormal branches arising from below.
Fig. 3. Plant attacked while bearing its crop.

PLATE L

Fig. 4. Plants in "butterfly" stage. Plant at extreme right was punctured. Note retarded growth.
Fig. 5. One year old plant on inoculated soil showing lesions along stem.
Fig. 6. Splitting of the stem.

PLATE LI

Fig. 7. Root proliferation following destruction of normal roots by *F. anisophilum*.

Fig. 8. Inoculated kidney beans showing canker. Check in the middle.

PLATE LII

Fig. 9. (*a*) Conidiophores bearing microconidium and macroconidia. (*b*) Perithecia, (*c*) asci, paraphyses and (*d*) spores of *N. anisophila*.

PLATE XLIX

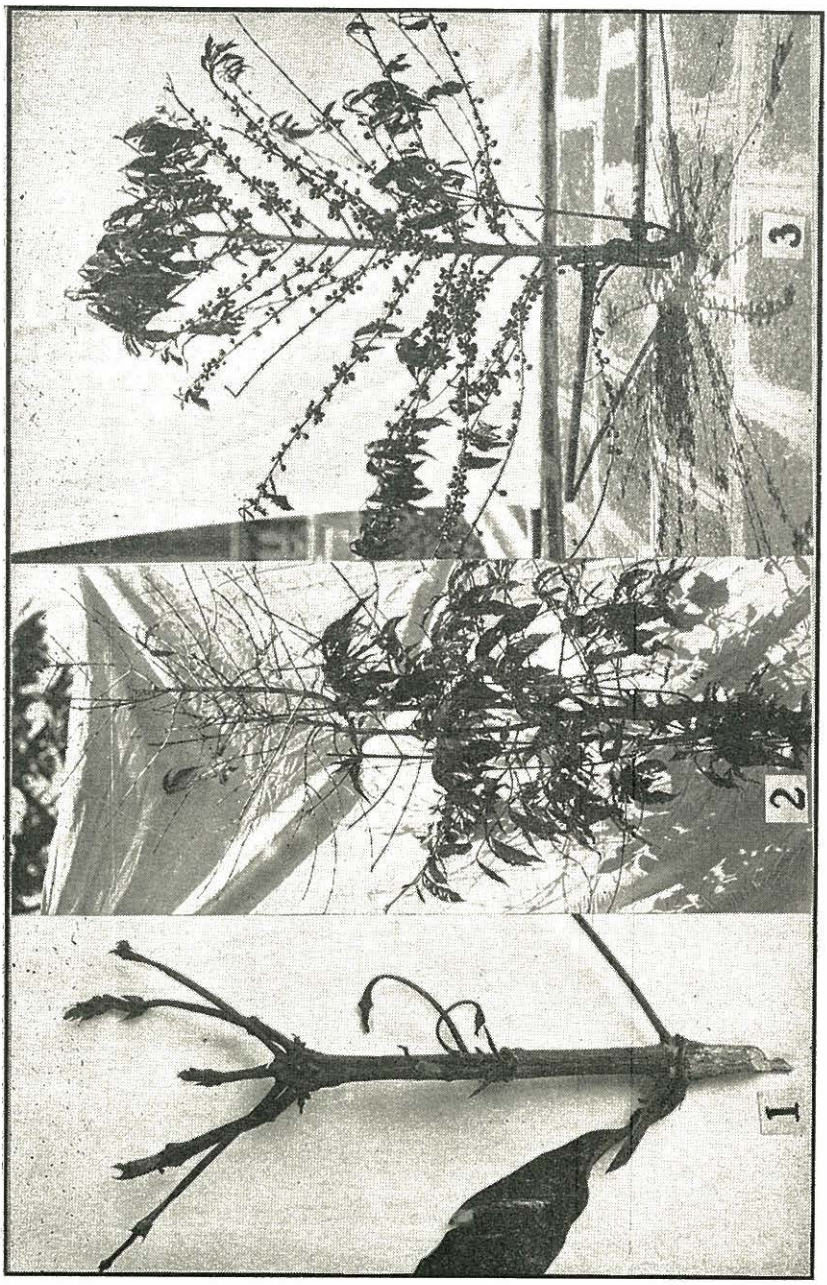

PLATE L

PLATE LI

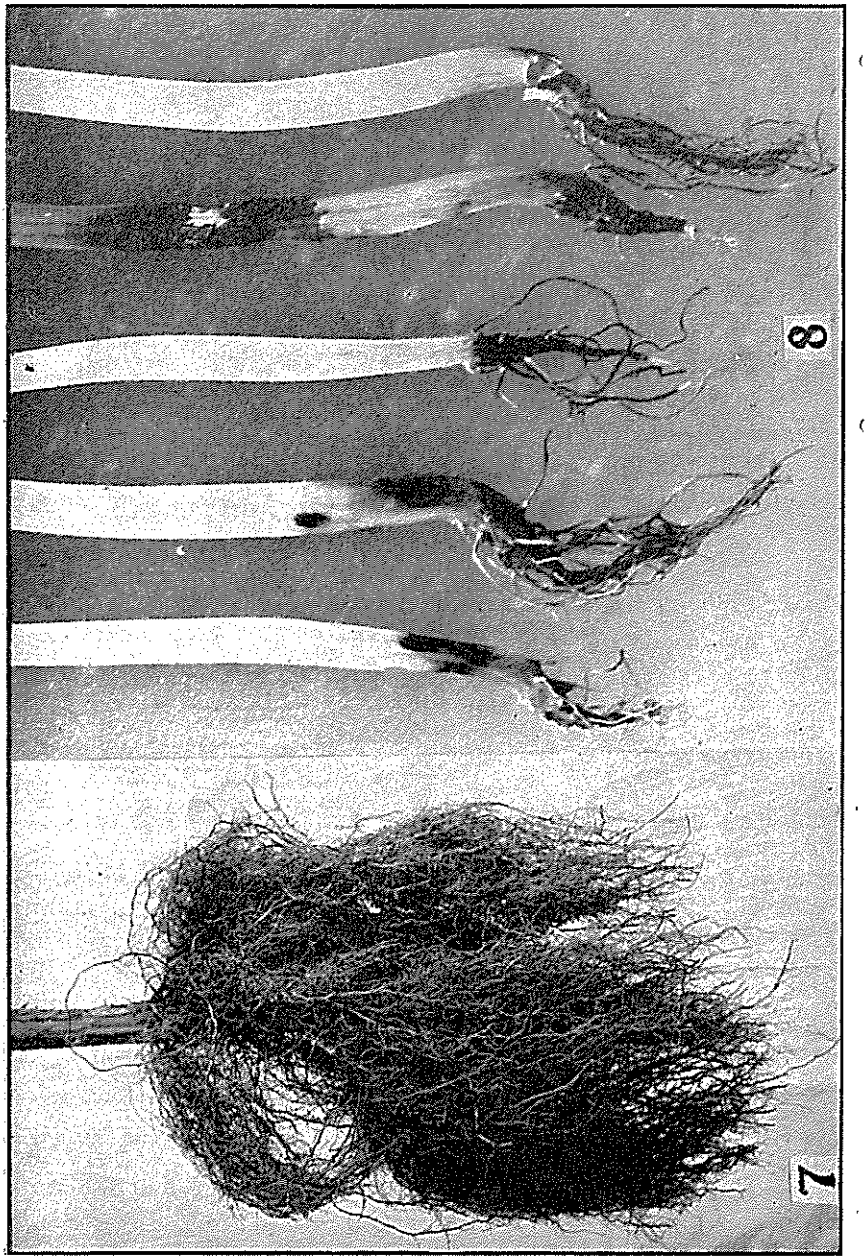

PLATE LII