

## ONION-LEAF ANTHRACNOSE

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The fungus flora of Porto Rico has been the subject of detailed study during the last decade. A number of investigators have worked assiduously on various problems involved and have made very important additions to our knowledge of the subject. The present paper comprises the study of a new anthracnose disease of onions which was discovered by the writer in one of the eastern districts of the Island in January 1924.

The destructive nature of the disease makes its study of interest not only to Porto Rico where onion-growing promises to become an important industry but to other onion-growing countries in the world, in which this crop so far as reports go, is not subject to this malady. There is here presented a brief report on the investigation of this disease and a description of the pathogene. A popular account was published by the writer in 1925 (4). A note on this disease was also given by Cook (1).

### SUSCEPTS

*Allium cepa* L. (Market onion) has been most seriously affected and the false shallot to a lesser extent. Infection has been produced on the latter, both under laboratory and field conditions. Plants of all ages are equally susceptible, although most of the damage appears to occur in plants three months old and during rainy weather. However, that plants of this age happen to be so seriously attacked under the prevailing weather conditions, is no indication that they are at this age most highly susceptible. The outbreaks of this disease here recorded appear to be correlated with the amount of rainfall and the length of the rainy periods.

#### Varietal Susceptibility.

The work of Walker (5, 6, 7) on the resistance to onion smudge, which is also an anthracnose disease, led the writer to undertake a comparative study of the resistance of white, yellow and red varieties to the pathogene in a field of about .60 acre. Seedlings 40 days old were transplanted to plots 4 × 40 feet. Four plots were planted to red onion (Red Wetherfield and Red Bermuda), four to White onion (White Bermuda) and the rest of the field to yellow onion (Yellow Globe and Yellow Bermuda). The disease made its appearance at

about the same time on all varieties. Careful observations showed that red, yellow and white varieties are equally susceptible. The tropical varieties (Bermuda onion) showed no less susceptibility to the pathogene than the temperate-zone varieties tested.

#### DISEASE

We have named this disease the "*Onion Leaf Anthracnose*" to avoid confusion with smudge which is often referred to as "onion anthracnose" and with diseases known as "onion leaf-spot".

#### History and Range.

As stated before in this paper the disease was first found by the writer in January 1924, in the eastern part of Porto Rico. In 1925 it was found in one of the northern districts and in the same farm where it had been discovered in 1924. In 1926 and 1927 material with anthracnose infection was obtained from the northwestern and northern sections. It does not appear to have been reported outside of Porto Rico.

#### Importance.

Data were gathered on the number of plants which were entirely killed by the disease, those which did not attain full maturity and those which were only slightly affected. Comparison of the yield from an uninfected field with conditions otherwise similar to those of diseased plots were made. From our observations it appeared that from 45 to 55 per cent of the crop was lost.

The damage to the bulbs while usually indirect is generally severe. The disease prevents the complete formation of the bulbs when the attack is early. In this way bulbs from diseased plants will be undersized. When attacks are late the disease opens the way for bacterial or other rots.

#### SYMPTOMATOLOGY

##### Morphologic Symptoms.

The disease is primarily of the leaf blade but the bulb scales may also be affected. The characteristic morphologic symptoms on white and yellow varieties are elliptic or oval tiny whitish spots which grow in all directions. During rainy weather the pathogene causes a rotting of the tissues. This destruction extends along the surface of the leaves and down to the bulb scales. Inner-bulb scales are not affected.

The symptoms on the red onions, especially the Red Wetherfield, are similar to those on the yellow and white varieties except that

in the early stages of spot formation a red stain becomes prominent on all the injured tissues. This color soon takes on a violet appearance. This violet stain occurs before any fruiting bodies arise and disappears with age.

The diseased tissues in all varieties become paper-like and brittle when dry. Old spots may be almost colorless, but more commonly they are yellowish. They are sub-elliptic to irregular in shape.

#### Signs.

Conidia are produced abundantly in acervuli in the spots. The spore masses are creamy, flesh-like or pink to ferruginous. Ascospores may be found under certain conditions.

#### Histologic Symptoms.

The effects on the diseased tissues are those characteristic of necrotic diseases. As has been already stated the first indications of the disease are minute spots. These lesions enlarge. Hydrosis is the first histologic symptom which accompanies the penetration of the pathogene, which establishes both intra- and inter-cellular relationship. This is followed by the disorganization of the protoplasts and discoloration of the cell walls. This brings about a collapse of the cells, the first to succumb to the effects of the pathogene being those of the palisade layer. The fungus penetrates farther down into the outer parenchyma cells, desintegrating them and then into the vascular bundles where it runs through and between the cells. Toward the later stages all that is left of the tissues is a skeleton of epidermis and broken cell walls.

#### ETIOLOGY

##### Name, History and Classification of the Pathogene.

The pathogene under consideration was first thought to belong in the genus *Gloeosporium*. Later setae were found, which places it in the genus *Colletotrichum*. Since the fungus appears to be new to science it is given the name *Colletotrichum Chardonianum* in honor of Carlos E. Chardón, the present Commissioner of Agriculture and Labor of Porto Rico.

##### COLLETOTRICHUM CHARDONIANUM spec. nov.

Mycelium is hyaline, white gray or dark and even greenish, depending on the medium in which it is growing. It is 1.41-6.63  $\mu$  thick, septate and profusely branched. (Pl. I, Figs. 10 and 11.)

Acervuli appanate, scattered, or gregarious (Pl. I, Figs. 30-31). These arise under the epidermis and break through it. They vary

in color from ferruginous to brown, sometimes flesh-colored. The setae are brown, septate, slender, acute. in a few cases blunt at the tips,  $98-170 \times 4.8$  u (Pl. I, Fig. 1). Conidia are oblong, in the majority of cases have both ends rounded. In some cases only one end is rounded while the other tapers to a blunt end. In other cases they are slightly curved,  $6-80 \times 1.7-7.0$  u (Pl. I, Fig. 14). The conidiophores are simple, cylindric, terete or with rounded base and pointed tip,  $7.9-79.5 \times 1.72-3.7$  u (Pl. I, Figs. 12 and 13). A few perithecia were once found in culture, but were not produced later. The writer does not feel justified in making a description of the perfect stage from the insufficient material on hand and his failure to obtain it repeatedly.

#### CULTURAL CHARACTERISTICS

Pure cultures of the fungus were obtained in the usual manner and a study was made of its behavior in different artificial media. From pedigree cultures, poured plates of saccharose (3 per cent) agar, glucose (3 per cent) agar, lactose (3 per cent) agar, potato-dextrose agar, corn-meal agar and oatmeal agar, were inoculated. Measurements of colony growth in centimeters were made. Conidia were also measured.

##### On Potato-dextrose agar.

Neutral gray discoloration of the substratum; aerial mycelium in dense tufts, gray, growth profuse; acervuli salmon or ochraceous salmon.

##### On Oatmeal Agar.

Medium unchanged in color, mycelium hyaline or whitish, greenish masses here and there, acervuli numerous, ochraceous salmon or flesh-ocher, in distinct zones, but later filling up all the inter-zonal region.

##### Corn-meal Agar.

Substratum underlying acervuli brown to black; aerial mycelium scanty or none,<sup>1</sup> when present consisting only of short threads, hyaline; acervuli numerous, flesh-ocher to apricot buff, in zones or scattered.

##### Glucose Agar.

Substratum underneath acervuli mummy brown; aerial mycelium dull white and in some places smoky or dark, acervuli large, flesh-

<sup>1</sup> The pedigree cultures used were under one month of age when fruiting is heavy. Older cultures show less fruiting.

ocher or apricot buff to cinnamon-rufous, in zones; setae present, numerous.

#### Saccharose Agar.

Substratum Dresden brown underneath the acervuli, ochraceous-buff elsewhere; aerial mycelium profuse, dark, over old growth, white; acervuli numerous, large, rufous to ferruginous, zones not well-marked, setae few.

#### Lactose Agar.

Substratum flesh-colored with mummy brown discoloration under old acervuli; aerial mycelium in short, whitish threads, scanty; acervuli numerous, large, flesh-colored to ferruginous, zoned at first.

#### Dextrine Agar.

Substratum unaffected in color, aerial mycelium scanty, short, white threads; acervuli numerous, large, rufous to ferruginous, not zoned.

TABLE I

SEVEN-DAY-OLD GROWTH OF *C. Chardonianum* n. sp. IN DIFFERENT ARTIFICIAL MEDIA

(Diameter of growth in centimeters)

Culture	Potato dextrose agar	Oat-meal agar	Corn-meal agar	Glucose agar	Saccharose agar	Lactose agar	Dextrine agar
1 -----	8.0	7.8	7.0	8.0	8.1	7.7	7.4
2 -----	8.0	7.8	7.0	8.0	8.0	7.6	7.5
3 -----	8.3	7.8	7.8	7.8	8.0	7.7	7.5
4 -----	8.3	7.8	7.8	7.7	8.0	7.8	7.5

#### Pathogenicity.

Inoculation experiments were made as follows:

(a) A small portion of a healthy plot of onions (24 square feet) was sprayed with a suspension of conidia obtained from fresh lesions. This was done at 4 P.M. on a day in January. The plants were not protected from the sun and they received no watering. However, the air was warm and moist. It was conducted during a rainy period. Symptoms as already described (small whitish spots) appeared in from 3 to 5 days. Out of 34 plants so sprayed 31 showed symptoms at the end of 5 days.

(b) Strong, vigorous plants in clay pots were similarly treated. In this experiment the plants were covered with bell jars lined with moist filter paper. Symptoms appeared in from 3 to 5 days.

These two series of experiments prove beyond any possible doubt the power of *C. Chardonianum* to produce infection of onion plants.

**Life History.**

The life history of this pathogene probably involves only the asexual or conidial stage, although the sexual stage may also occur in the field.

*The primary cycles* occur as lesions on leaves of full-grown transplants in the field.

**PATHOGENESIS****Inoculation.**

Infected debris from the previous season is probably the chief source of the inoculum. It seems possible that the mycelium of the fungus is carried over to succeeding seasons in these old plant fragments. When the rainy season approaches, coincident with maximum development of the onion plant, it appears that a conidial crop is produced from this hibernating mycelium. Spattering rain brings about the transfer of the inoculum (conidia or perhaps ascospores) from this debris to onion leaves. Here the primary cycles are started, which by subsequent production of pink or creamy conidial masses furnish a constant source of inoculum for the secondary cycles.

**INCUBATION**

Germination of conidia was studied in Van Tieghem cells in the usual manner, at a temperature of 21° C. The first evidence of protrusion of germ tubes appeared at the end of three and one-half hours and germination was at its maximum at the end of four and one-half hours.

The spores of this species like those of most species of *Gloeosporium*, *Colletotrichum* and *Glomerella*, become septate prior to germination. Germ tubes grow from the ends of the spores (Fig. 18, 20, 23), although not infrequently from the sides (Fig 16). Germ tubes are of various lengths and in almost every case an appressorium is borne at the tip (Figs 17-22) of each tube. The plasmic contents of the spore creep into the appressorium and a septum is laid, thus cutting it from the germ tubes (Figs. 17-23). Primary appressoria germinate by sending out penetration tubes which enter the susceptible and develop into a mycelium or by production of secondary or tertiary appressoria. It is evident that appressoria may be subsequently produced again and again from primary or initial appressoria depending on environmental conditions. The spore upon falling on the infection court probably makes a first attempt to germinate, sending out a germ tube whose elongation may be arrested by want of moisture. An appressorium is formed at the end of the short

tube. This is able to withstand unfavorable conditions. When better conditions of humidity are restored the appressorium in turn attempts to germinate in the same manner as the conidium. In this way several appressoria may result from one conidium before entrance or penetration is effected.

The germ tubes enter through the stomata of the leaves or through wounds, but penetration may occur through the cuticle probably by mechanical pressure as reported by Dey (2) for *Colletotrichum lindemuthianum*. The germ tube upon effecting entrance or penetration undergoes a change into a thicker hypha or primary mycelium, Leach (3). Further development of the pathogene brings it into a closer relationship with the susceptible parts manifested in a series of changes which constitute infection.

#### Infection.

The primary mycelium makes its way into the epidermal cells, which it soon destroys. It runs between the cell wall and the plasma membrane. After reaching this stage the mycelium sends out any number of hyphae which grow in every direction, penetrating and killing the cells. These hyphae branch again and again and soon the tissues are traversed by the infecting hyphae. The necrotic symptoms which accompany the infection of the tissues are the result of the reaction of the susceptible cells to the substances produced by the invading hyphae. Toxic substances are probably produced soon after invasion since the latent period of infection appears to be short (2-4 days) and furthermore, the succession of changes from hydrosis to complete cell disorganization, seems to come with considerable rapidity. Under favorable conditions of atmospheric humidity, acervuli soon arise over the surface of the lesions. They are formed in breaks of the epidermis and may grow together in masses. Conidia make up the greater bulk of these masses which are pinkish or creamy. These spores constitute the inoculum for the cycles.

#### SAPROGENESIS

All observations tend to show that the mycelium in the necrotic areas of the leaves, lives over in a saprophytic manner until the coming season. Conidia are produced during the saprogenesis stages and furnish the inoculum for primary cycles. Whether one or more crops of conidia are produced by the mycelium in this stage is not known.

*Secondary cycles* constitute the most important phase in the

disease. They are initiated continuously by conidia produced in the primary cycle and are in all respects similar to the primary cycles.

#### EPIPHYTOLOGY

That stage in the life of the suscepi, just before or during bulb formation, seems to furnish the most favorable conditions for the attack of the pathogene.

#### Temperature.

Spore suspensions of the fungus were incubated at different temperatures. It was demonstrated that spores fail to germinate at 15° C and below. The following are the results of germination in tap water at different temperatures: At 18° C., 39.15 per cent; at 21° C., 41.92 per cent, at 25° C., 92 per cent; at 27° C., 86 per cent; at 30° C., 80 per cent; at 33° C., 64 per cent. Parallel trials conducted with distilled water showed that germination is equally good in distilled water as in tap water. It is seen that germination is at its maximum at 25° C. but is poor at lower temperatures of 21° C. and 18° and at a higher temperature of 33° C. In the field the range in which the disease seems to appear more commonly is 23° C.-30° C. In cultures the optimum temperature seems to be 27° C. See table II. In general, in the three media growth was best at 27° C. with maximum production of conidial masses.

TABLE II  
SEVEN-DAY-OLD GROWTH OF *Colletotrichum Chardonianum* IN  
CENTIMETERS IN DIAMETER

Temperature	Potato dextrose agar	Corn-meal agar	Oat-meal agar
6° C.-----	0	0	0
9° C.-----	0.1	2.3	1.0
12° C.-----	2.2	4.5	3.6
15° C.-----	2.7	3.8	5.2
18° C.-----	5.5	6.0	6.3
21° C.-----	7.5	7.5	7.6
25° C.-----	8.5	7.8	7.8
27° C.-----	9.0	7.8	7.8
30° C.-----	8.0	7.0	7.0
33° C.-----	7.8	7.0	6.5

We do not feel justified in assuming temperature to be the most important factor in epiphytotics although coupled with moisture it may have great influence in limiting their extent and destructiveness.

Moisture probably plays the most important role in determining



the nature and severity of outbreaks of this disease. It seems essential in effecting the dissemination of conidia. The occurrence of the disease in the field and the production of secondary cycles is dependent to a large extent on precipitation.

#### CONTROL

It is quite doubtful whether we can resort to any eradictory measure in the control of this disease. The removal of diseased leaves and plants to prevent further spread of the pathogene sounds logical, but general practice tells us it is highly improbable that the average farmer will gain anything by that method. The production of conidia under field conditions in such great numbers curtails the possibilities of the application of this eradictory practice, since secondary cycles will occur with the same ease.

#### Protection.

Spraying and dusting with suitable liquids or dusts will eventually come into use more and more. Three factors should be considered in the general application of these protective methods, namely, the cost of application as compared to the gains from it, the adhesive power of the dusts or liquids and the general effectiveness of these fungicides.

Preliminary laboratory trials have been conducted with various dusts to find the effect on the spores of the pathogene. Those tried were: Kolo-dust, dusting sulphur, copper lime dust, Banks' Colloidal copper, corona colloidal copper, copper hydroxide and Killtone copper dust.

The following results were obtained in triplicate trials with spore counts of 4,000 and using the dusted-slide method employed at Cornell University.

Corona colloidal copper.....	no germination
Banks' colloidal copper.....	36.47% germination
Cu(OH) <sub>2</sub> .....	37.40% germination
Killtone copper dust.....	9.00% germination
Copper lime dust.....	62.84% germination
Kolodust.....	57.47% germination
Dusting Sulphur.....	60.50% germination
Check.....	59.50% germination

#### General observations of the germination processes.

In Banks' Colloidal copper brown appresoria were abundant, produced successively from the same germ tube; germ tubes are very long and normal in growth, soon giving rise to a cobwebby

mycelium. In copper hydroxide no appresoria were observed and germ tubes were very short; this was also the case with Killtone copper dust. Kolodust: appresoria abundant and germ tubes long. Dusting Sulphur: appresoria present; germ tubes extremely long, much more so than in Kolodust. Copper lime dust: appresoria present; secondary conidia also produced in large numbers; germ tubes very long and profusely branched. The failure of sulphur dusts to inhibit spore germination is in line with our general knowledge of the non toxic properties of the sulphur fungicides to anthracnose fungi.

The data and observations given above on the germination of conidia of *C. Chardonianum* in various fungicides suggest a rather effective action of Corona colloidal copper, Killtone copper dust, Banks' Colloidal copper and copper hydroxide. Apparently the first two are more promising than any of the others. However, it must not be overlooked that the adhesiveness of these dusts to the waxy surface of the onion leaf has to be tested before any more definite steps to their application in the field are attempted. So far the writer has been unable to test the adhesiveness and relative efficiency of these fungicides under field conditions.

#### SUMMARY

(1) An anthracnose disease of the leaves and bulb scales of the onion is described.

(2) Market onion (*Allium cepa* L.) and a multiplier variety of *Allium cepa* L. often referred to as false shallot are affected.

(3) There does not exist any resistance in the yellow, red or white varieties to the disease.

(4) Tropical varieties of onion are as susceptible as the temperate-zone types.

(5) Plants of all ages are equally susceptible.

(6) The symptoms on the leaves may be described as elliptic or oval spots. On red varieties a red stain appears on young lesions.

(7) The pathogene is described as a new species, *Colletotrichum Chardonianum*.

(8) Growth of the causal fungus in carbohydrate media was excellent. It was especially good in starch-containing media.

(9) Infection experiments under control conditions demonstrate the ability of *C. Chardonianum* to produce disease. Infection occurs primarily through the stomata but the germ tubes may penetrate through the cuticle.

(10) Conidia germinate in water at a temperature of 21° C. in 3½ to 4½ hours. During germination of conidia, primary, secondary and even tertiary appressoria are produced. Also secondary conidia. Temperatures of 18° C. and 33° C. seem to inhibit germination, while conidia germinate readily at 25° C. In synthetic culture media the fungus grows best at a temperature of 27° C. In the field the disease appears during seasons when temperature is 23° C–30° C.

(11) The pathogene apparently lives over from one season to the other as mycelium in debris on the soil.

(12) The conidia seem to resist the action of the sulphur fungicides. An effective action of copper fungicides, particularly colloidal copper, has been observed in laboratory experiments.

This investigation was begun in the Insular Experiment Station but the bulk of the work was done in the Laboratory of Plant Pathology, Cornell University, Ithaca, N. Y.

I owe an acknowledgment to Dr. Mel. T. Cook, Chief Pathologist, for helpful advise during the early studies on this disease, to W. R. Fisher of the Department of Plant Pathology at Cornell University, who kindly made some of the photographs, and finally to Prof. H. H. Whetzel of the Department of Plant Pathology, Cornell University, for valuable suggestions and criticisms and for reading the manuscript.

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## PLATE I

Fig. 1 = Setae of *Colletotrichum Chardonianum* n. sp.  
 Fig. 2 = Two fertile hyphae conjugating.

Fig. 3 = Fertile hypha with terminal conidium.

Figs. 4 and 7 = Fertile hyphae.

Figs. 5, 6, 8 and 15 = Types of fertile hyphae with terminal conidia.

Fig. 9 = A fertile hypha with cells giving rise to one-celled conidiophores which bear conidia at the tips.

Figs. 10 and 11 = The mycelium of *C. Chardonianum*.

Figs. 12 and 13 = Conidiophores of *C. Chardonianum* bearing conidia at the tips.

Fig. 14 = Conidia of *C. Chardonianum*.

Fig. 16 = Germinated conidia conjugating.

Figs. 17 and 22 = Conidia germinating in water, giving rise to primary, secondary and even tertiary appresoria.

Figs. 18 and 20 = Two conidia germinating by a short tube and an appresorium.

Fig. 19 = A conidium germinating and producing primary and secondary appresoria.

Fig. 21 = A germinating conidium with primary appresoria.

Fig. 23 = Conidia germinating into a long tube bearing at the tips secondary spores.

#### PLATE II

Fig. 24 = A longitudinal section through a healthy onion leaf (outlined with aid of camera-lucida).

Fig. 25 = A section through a lesion of a diseased leaf. Note the hyphae penetrating through and running between cells. The destruction of the palisade cells is evident.

Fig. 26 = Another section through a diseased leaf showing destruction of palisade cells and also hypha penetrating through cells of vascular bundles.

Fig. 27 = View of surface of onion leaf showing a few spores germinating and penetrating through the stomata. Note the production of appresoria. (Outlined with aid of a camera-lucida.)

Fig. 28 = Section through epidermis showing an appresorium germinating and the tube penetrating through a stoma. The very early stages of a pathological condition: branches of the primary mycelium penetrating the susceptible cells.

Fig. 29 = Mycelial threads (hyphae) as seen within and between cells in the vascular bundles (highly magnified). (Outlined with aid of camera-lucida.)

#### PLATE III

Fig. 30 = Shows lesions on the leaves of onion. The leaf on the left shows the early stages of the disease; that on the right the more advanced stages with masses of acervuli.

Fig. 31 = Enlarged base of the onion plant of Fig. 32 showing the acervuli of the fungus arising on the surface of the leaf bases. Notice poor root system from imperfect nutrition.

Fig. 32 = Typical case of onion plant affected with *C. Chardonianum*. Notice the diseased leaves and the bases of leaves already destroyed.

Fig. 33 = Culture of *C. Chardonianum* on hard potato dextrose agar.

PLATE I

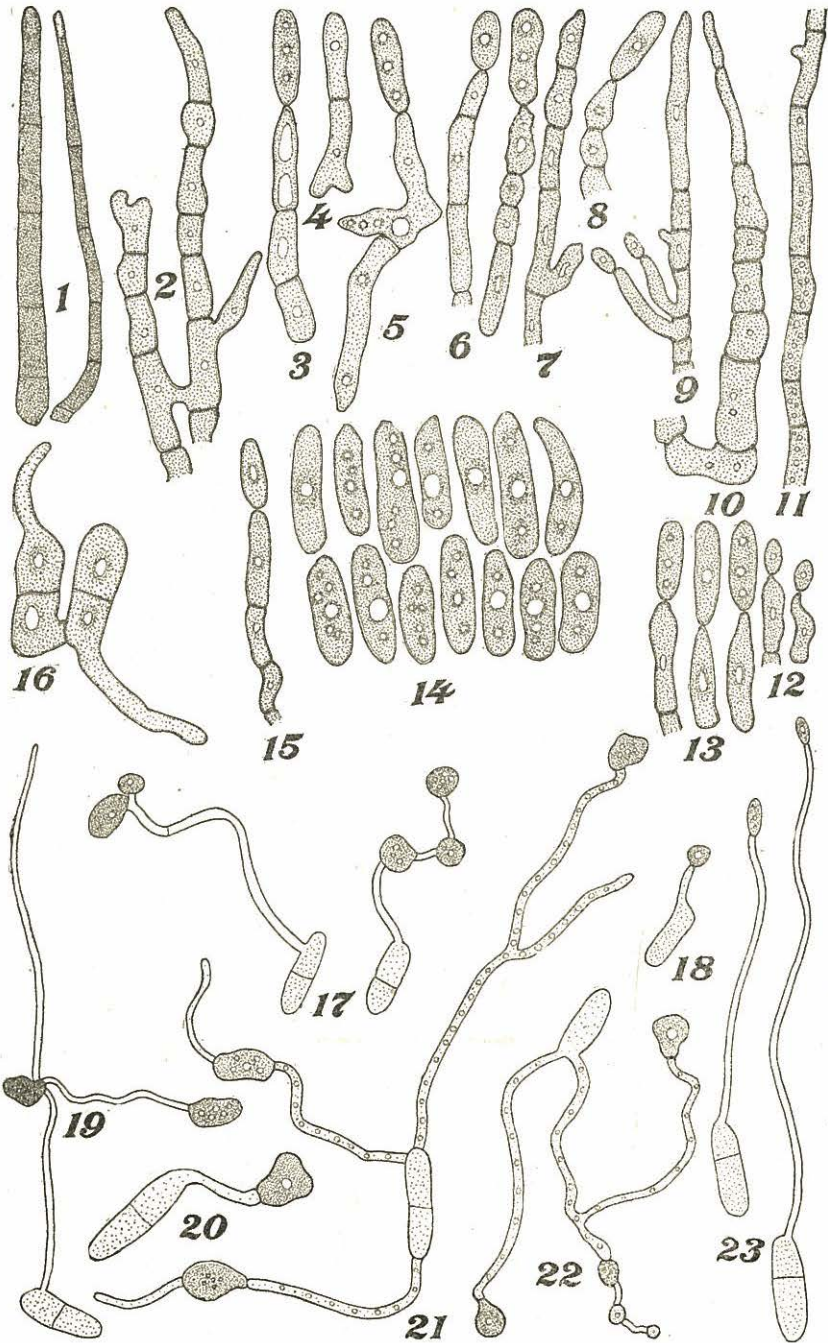


PLATE II

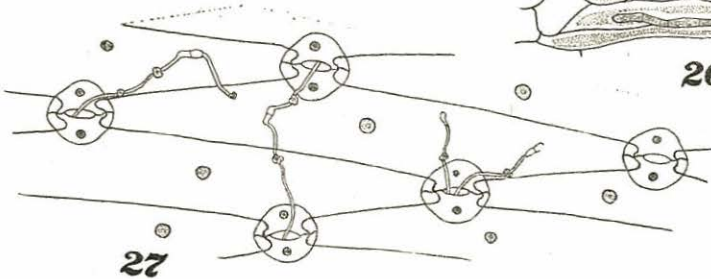
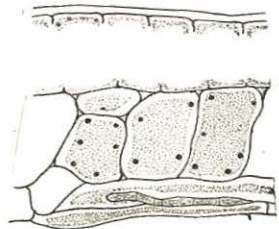
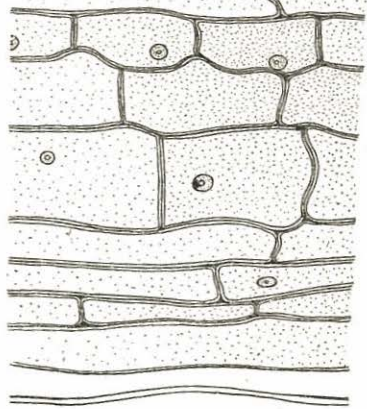
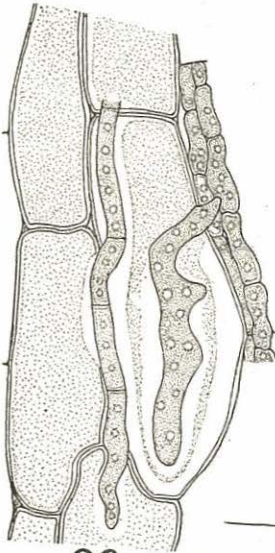
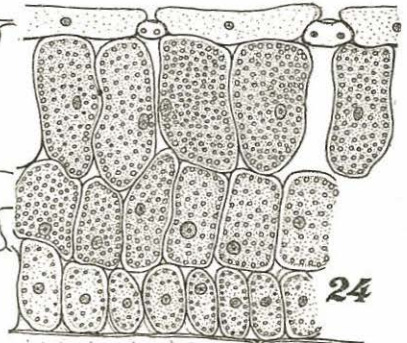
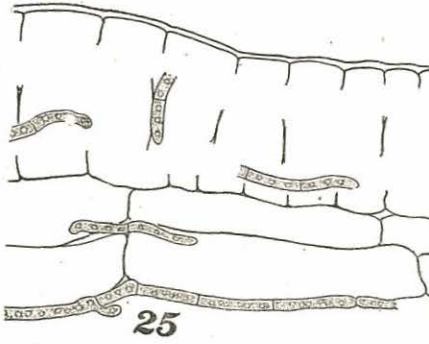
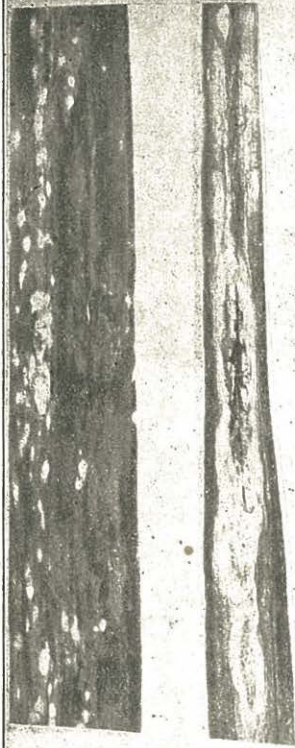
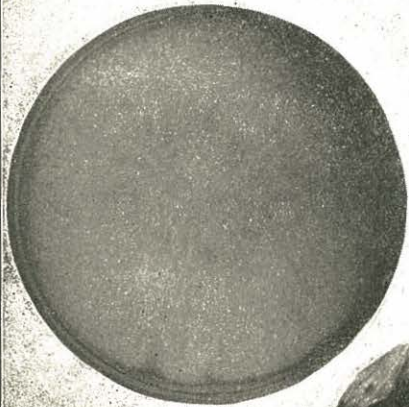


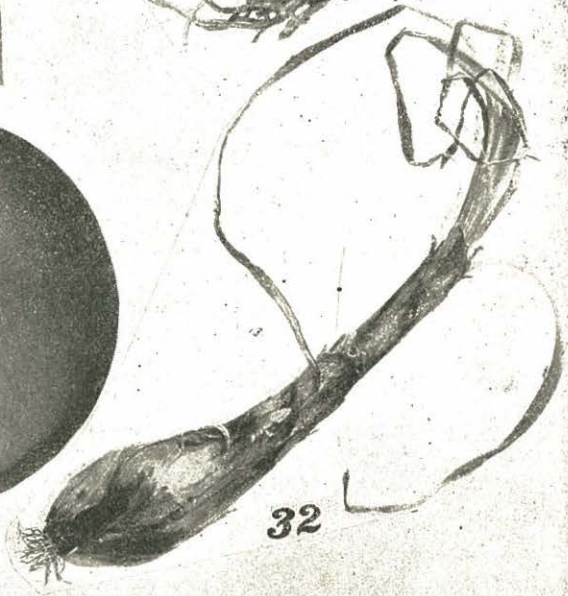
PLATE III



31



33



32