FUNGI ASSOCIATED WITH ROOT ROT OF APPLE TREES.

Minor Thesis in Plant Pathology,

Submitted by

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For the Degree of Master of Science.

Virginia Polytechnic Institute, Blacksburg, Va.

April 14, 1916.

Approved

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The term root rot has been rather generally used to designate a number of diseases affecting apple roots. The difficulty of examining the troubles under natural conditions and the vagueness of the symptoms make it very difficult to distinguish some of these diseases in a concise way. There are, however, a considerable number of root troubles which have been studied and described in some detail.

Review of Literature on Root Rot. In New York and other northern states, Grossenbacher[7 & 8] has found low temperature to cause extensive killing of bark and outer wood especially in the region of the crown. The upper lateral roots may be affected.

A root rot of coniferous seedlings is believed by Graves[6] to be due to asphyxiation. However, such a condition does not seem probable in apple orchards.

Citrus trees in Florida are affected by a serious rotting of the roots and crown believed by Grossenbacher[9] to be due to conditions of water supply and temperature, while Fawcett[3] thinks it probable that the fungus Pythiacystis citrophthora is responsible since it causes a rather widespread rotting of trunks and crowns which resembles the foot rot.

Whipple[18] recognizes two distinct forms of root rot of apple in Colorado, one attacking all varieties below ground

and the other attacking only Ben Davis and Gano. The latter, which is the more serious, may extend up the trunk to the large branches causing the bark to die and shrivel up, taking on a marbled appearance. The wood becomes a dark brown color with chocolate spots on the trunk. Fungi are associated with the trouble but their role is not yet understood. Waterlogged soils and arsenical spray have been offered by Paddock(13) as possible causes. Trees are killed in two years' time.

Perhaps the best known root rot of apples is that produced in the Pacific Coast Region by Armillaria mellea. Four forms of the fungus are distinguished by Lawrence(10). The production of large, dark, many-branched rhizomorphs on roots and in the soil and of sporophores which appear at the base of trunk in autumn is distinctly characteristic of this trouble. The disease resembles winter injury to crown in some cases according to Piper and Fletcher(14). Entrance is believed(10) to be through wounds. Rotting is more common where the main roots branch out. The fungus spreads in a fan-shaped growth between the bark and wood. The whitish or dirty brown rhizomorphs spread through the soil some distance, descending to a depth of two feet. When the roots are advanced in decay, the leaves become thin usually in midsummer. Trees live from one to several years after attack.

A root disease described by Wilcox[19] as "A Rhizomorphic Root Rot of Fruit Trees" is apparently quite common in Arkansas, Oklahoma and Missouri. The causative fungus, Clitocybe parasitica, produces rhizomorphs and sporophores very similar to those of Armillaria. In fact, it is held by some to be a species of Armillaria. The rhizomorphs may spread ten feet from diseased wood, and may penetrate the live cortex directly. Entrance is usually through the smaller roots. The fungus spreads through the vascular bundles and medullary rays.

The fungus Thelophora galactina, according to Von Schrenk,[17] has been known as the cause of a rotting of apple roots for thirty years in West Virginia, Kentucky, Illinois, Missouri, Arkansas, and Oklahoma. It produces bright orange red, leathery sheets on roots and around the base of the trunk. The trees show no sign of disease until immediately before death, then they wilt and die within a few weeks. When transferred from oak to young apple trees, the fungus kills them within a year.

In Pennsylvania, Fulton[4] and Orton[11] report apple trees affected at or below the crown by a dying of the bark which may girdle the tree and extend for some distance up the trunk. It progresses more rapidly during the growing season. Recent work by Orton and Adams[12] shows this to be caused by the fire blight organism, Bacillus amylovorus.

Fulton and Cromwell[5] at the North Carolina station have recently reported a "Black Root Rot of Apple" which is described as occurring in Pennsylvania and North Carolina. In this form a dense white superficial mycelium is produced which later forms a black encrustation. Wound inoculations rotted as much as fourteen

inches in twelve weeks. The wood is made brittle but some time is required for complete breaking down.

A root disease of para rubber trees (Hevea brasiliensis) in Malaya is interesting in connection with the study reported here because of the close relationship of the causative fungus with one which has been found associated with apple root rot by the writer. Brooks[1] attributes this disease of para rubber to Sphaerostilbe repens, though complete proof of its parasitism is yet lacking, while the writer finds a closely related fungus, Stilbella sp., to be the probable cause of the apple root rot in question. The genus Stilbella is placed with the imperfect fungi, being known only in the conidial stages, but the perfect or asci-gerous stages of species of Stilbella, where known, have been referred to the genus Sphaerostilbe of the Ascomycetes. The close relationship between the species of Stilbella from apple roots and Sphaerostilbe from para rubber is therefore evident and the occurrence of a root parasite in the latter genus increases the probability that the Stilbella under consideration may be responsible for the root rot of apple. The external symptoms of two diseases are furthermore very similar, being characterized by a thinning of the foliage and a dying back of the branches, although these might accompany any kind of root affection. Sphaerostilbe repens, according to Brooks, attacks the roots first but often advances into the trunk. No external mycelium is found, but greyish or brownish rhizomorphs spread between the bark and wood. Later the entire wood is penetrated principally by way of the vascular bundles and medullary rays.

Other minor rots of apple roots are caused by Ozonium auricomum, Ozonium Omnivorum, Dematophora necatrix.

Occurrence and Symptoms.

Root rot has become serious in Virginia in recent years especially in the vicinity of Staunton, Winchester and Middletown, covering the upper valley and extending into Piedmont.

The type of root rot found in Virginia is particularly vague in its symptoms. There is little unusual in the appearance of the rotted roots. No rhizomorphs have been noted except on roots advanced in decay at which time many fungi are found in the roots. No fruiting bodies have been observed on roots or in the soil. It seems likely that injuries, due to fire blight, crown gall, woolly aphis and even borers, are frequently ascribed to the indefinite trouble root rot. It is even held that there is no fungus directly responsible for the disease and that low temperature is probably the cause.

There are, however, definite conditions found in typical cases which indicate that the rotting is produced directly by a parasitic fungus. In making field observations in 1914, Dr. H. S. Reed reported the following facts:

"The disease is more common on York Imperial than on other varieties in the Shenandoah Valley. It appears to cause the greatest injury to trees just coming into bearing. The disease usually begins on the roots situated deepest in the soil and works upward. Shortly before the death of the tree injury to the trunk begins to be manifest above ground."

The trees usually show the disease most prominently about midsummer. In advanced stage the upper lateral roots alone may be supporting the tree. Death is sudden when this stage has been reached.(17) The tree is easily uprooted.

The following facts bear especially on the association of fungi with the disease: Trees are more frequently attacked on newly cleared land, where decaying stumps and other dead wood may

harbor wood-infesting fungi. Young trees planted in places where trees have died of root rot are killed in from two to three years, which strongly indicates that the disease is held over in the soil or in decayed roots. Fungi have been isolated which seem to be constantly in association with the trouble. The wood when newly decayed has a rather typical appearance. The bark is soft and brown. The wood is light brown to dark brown and firm. No mycelium is evident between bark and wood. Distinct zonations are usually seen on the surface of the wood. A white velvety mycelium has been observed on the cut surface of typical roots after a short period in closed damp container. When roots have advanced in decay the wood becomes punky usually whitish with darker lines running through it. It seems likely that various fungi are responsible for this later action on rotten roots. Roots at this stage are by no means uniform in appearance. Trees have been observed to die in well defined isolated areas, another fact strongly indicative of the fungus origin of the disease.

Isolation of the Fungi found in Rotting Apple Roots.

Isolation of fungi from rotted apple roots was begun in March 1915 by C. H. Crabill[2]*, who has recently reported the results obtained. He made isolations from a number of roots, getting a species of ~~Hydnum~~ Fusarium in one case and Trichoderma koenigi from a number of sources. He concludes that the latter fungus is probably responsible for the root rot. Results obtained by the writer do not sustain this conclusion.

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*The present study was continued under his direction until September 1915, and has since been directed by Dr. F. D. Fromme.

Isolations were continued by the writer during the summer and fall with the results that are summed up in Table I. Roots were washed and split with a sterile knife. Bits of the wood were picked from the interior with sterile forceps and thrust into plates of nutrient agar. Most of the cultures were made from wood near the border between live and dead wood.

Table I. Isolations from Apple Roots.

Tree No.	Source	Date	No. stabs.	Sterile.	Unidentified.	Bacteria.	No 3	Trichoderma sp.	Stilbella spp.
1	Roseland	7-6-15	10	4				6	
2	Pleasant Valley	"	26	1		11		12	2
3	Fishersville	7-28-15					1	several	
4	Greenwood	7-29-15	8		2		2	1	3
5	"	8-7-15	16			2		14	
6	Staunton	8-10-15	2				2		
7	Middletown I	8-25-15	21		6	3			12
8	" II	"	9			1		8	
9	" III	"	10		10				
10	" IV	"	5	2		2		1	
11	" V	"	15	2	1	1		1	10
12	Winchester I	"	6		1	2		2	1
13	" II	"	6					1	5
14	" III	"	9		1				8
15	Harrisonburg I	10-20-15	11		5		1	1	4
16	" II	"							
17	Staunton	11-10-15					2		
			154	9	26	22	8	47	45

The fungus designated as No. 3 is an unidentified fungus described later in the pure culture study of the various fungi. In trees Nos. 3 and 17 this fungus was isolated from firm wood which had died only a short time before. The root from No. 6 was whitish and punky. It is possible that these fungi may be different species since the appearance in culture has little distinctive about it. The other two fungi which occur with sufficient frequency to be considered are Trichoderma sp. and Stilbella sp. In a total of one hundred fifty-four stabs, forty seven were Trichoderma and forty five were Stilbella. The roots Nos. 5 and 8 which yielded abundance of Trichoderma were advanced in decay and punky. Root No. 3, which yielded a considerable number of Trichoderma was still partly alive. It will be noted that the high yields of Trichoderma were obtained early in the season before the root rot is seen to be active in the field. In the latter part of the season attention was given largely to the margin between the dead and live wood. As shown in Table I the Stilbella predominates in this region at the season of year in which root rot gives most evidence of activity. The first six isolations are proportionately more condensed since no record was kept as to the number of roots in each lot.

Cultural Features.

The fungi shown in Table I were grown on various media in order to observe their distinctive characteristics and to note their reaction to certain agents which have been recommended as treatment for certain forms of root rot. Table II gives the growth of the fungi in centimeters in seven days.

Table II. Growth of Fungi in centimeters in 7 days.

Name	Starch agar	Czapek agar	Bean agar	Beef peptone agar	Starch agar & CaO. 1 g. per liter.	Starch agar & $CuSO_4$	Cellulose agar	Non-nutrient agar
Trichoderma	11.2				1.1	0.8		
Hydnum	3.0	3.0		0.7	0.6		0	0
No. 3	1.75	1.5	0.5	0.3	0.9		0	1.2*
Stilbella I	0.75	1.0				0.8		
" II	0.60	1.0	0.6	0.3	0.6			
" III	0.75	1.0	1.0	0.7	0.7	0.5	1.6**	1.6**
" IV	0.75	0.75	.8	0.3	0.3			
" V	0.8	0.7	0.5	0.5	0.8			

* The growth of No. 3 on non-nutrient agar seemed to be produced by the bit of agar transferred from the preceding culture.

** Growth was sparse and produced little aerial mycelium.

The cellulose agar is prepared by adding pure cellulose to non-nutrient agar.

The lime and copper sulphate were added to the media just before pouring the plates. When these materials are added before sterilizing, the media are softened and the lime turns the medium a dark brown. By adding the materials after sterilizing, change in the medium is avoided. Sulphur dust was added to plate cultures as dust spray when the cultures were three weeks old. After three days little inhibition of growth was evident.

The Hydnum sp. on plate cultures produces a rather rapid pure white felty growth of aerial mycelium which runs over the sides of the dish. There is a tendency to form indistinct sectors on

the plate. On rotten roots placed in damp chambers (by C. H. Crabill) the fungus produced typical Hydnum sporophores, (photograph No. 1). The spike-like needles from these sporophores when thrust into agar gave pure cultures of the Hydnum.

The fungus designated as No. 3 in culture seven days old showed a loose white cottony growth slightly more dense one centimeter from the center. In older cultures there are usually several of these zones. Clamp connections are rather numerous in the dichotomously branched mycelium. It is about 15 microns in diameter. Photograph No. 2 shows trees girded just below the surface of the ground. A felt of mycelium extended up over the live bark to the surface of the ground. The shredding of bark was produced in digging.

The Trichoderma grows rapidly with a loose, scant white mycelium, (photograph No. 3). Soon after the plate is covered fruiting begins, at the outer margin if grown under bell jar, near the center if the plate is left uncovered. Clusters of conidiospores 1/2 to 2 mm. in diameter bearing conidia are usually produced. These are at first white, later light green, due to the color of the conidia in mass. These fruiting clusters often occur in concentric rings. The conidiophores are several times two-to three-branched. The conidia adhere to the tip of the conidiophore in a mass. They are globoid or slightly angular from compression in mass, and measure 0.2 micron in diameter. Upon germination they produce one to two germ tubes which anastomose freely. This fungus has been determined by Mrs. Flora W. Patterson as Trichoderma koeningi Oudemans.

The fungus designated as Stilbella sp. has been tentatively placed in that genus. Four isolations of this fungus have been kept separate because of greater or less differences. The fifth, (Table II)

possesses such marked points of difference from the others as to call into question its identity with that group. The following description applies to the first four forms: The mycelium is at first a downy white, later turning greenish from the center outward, finally becoming almost black. The growth in an irregular wavy form as shown in Photograph No. 4, which shows culture 10 days old. The aerial mycelium is abundant and rather closely compacted. Conidia are borne on club-like compacted bundles of hyphae called synemata, which vary in length from ½ to 4 cm. in the different forms mentioned above. These synemata possess a more or less definite head on which conidia are produced sparingly. Their stalks have a similar texture to that of the stipe of the mushroom. The conidia are hyaline, 4x8 microns, fairly regular, elliptical.

Stilbella No. I, (Table II), from Middletown, Virginia, and No. III from Winchester, Virginia, (photograph No. 5), are very similar. No. I produces pigment on starch agar somewhat more slowly than No. III. The latter produces only slight pigment in 14 days. These two forms produce synemata in clusters usually. The base of the synema is brownish while the upper portion is grayish, and often branched. The head is usually considerably larger than the stalk and pure white. As in the other three forms the head is covered with loose hyphae which extend in every direction. The stalk is enclosed in a more compact mat of mycelium.

No. II from Pleasant Valley, Virginia, (photograph No. 6) is similar to No. III in pigment production. The numerous synemata are usually single and much longer than those found in the other forms, especially in tube culture, and there is less

branching. The stalks are brown almost throughout and are about equal in diameter to the head which is white. There is a slight constriction just below the head.

No. IV from Greenwood, Virginia, produces more zones on plate and more pigment in a given time than do I and II. The zones are narrower, making the total growth about the same. The synemata average about 1½ cm. in height, with brownish stalk and white head, the neck being constricted as in No. II. Synemata are produced only in old cultures grown in tubes or bottles.

All these fungi fruited best in cultures prepared by pouring about 100 cc. of starch agar into a wide mouthed bottle and plugging with cotton.

No. V Stilbella from Harrisonburg, Virginia, produces a wavy growth very like the others, but it produces little aerial mycelium on plates and no pigment. In tubes it produces a loose white mycelium with some pigment in old cultures. On plates small white, horn-like projections have been put up to a height of a few milimeters.

Inoculation Experiments.

The fungi under study were tested as to their parasitic qualities by inoculation on live apple roots in sterile damp chambers, and on roots of young trees.

Several roots were washed in alcohol to sterilize the tissue alive. Part of the bark was stripped off and they were inoculated with the Trichoderma, a little nutrient agar being added to give an initial growth. The fungus did not seem to be able to advance beyond the nutrient medium and growth ceased as soon as the agar was exhausted.

Six trees about two years old growing in tubs of

composted soil were inoculated with pure cultures of Trichoderma by C. H. Crabill in the spring of 1915. One of these examined six months and another a year after inoculation appeared to be in perfect health. No injury to roots could be found.

The Hydnum sp. was inoculated into a live root in chamber through a wound. After four weeks there was slight discoloration in the immediate vicinity of the wound. On another root after three weeks there was, as in controls, no effect whatever. On a section of root only recently dead the wood was discolored for two to three centimeters proximally and distally and to a depth of four tenths centimeter.

The Fungus No. 3, Table I, from Fishersville, Virginia, was inoculated into live roots in chamber and roots of young trees with negative results.

No. 3, Table I, from Staunton on a live root in moist chamber after four weeks had advanced 1-1½ cm. in the cambium region but had not penetrated the wood to any extent. Young York trees inoculated with pure cultures this fungus in wounds at the crown healed the wounds normally. Three trees were inoculated by placing pieces of the crown shown in photograph 2 near the roots. One small lateral root which had been wounded showed open wounds after 3½ months in the greenhouse. The wound seemed to be slowly girdling the root in one instance. Plates were made from this root. Six stabs were sterile, one gave bacteria, and three others produced a Penicillium and two unidentified fungi.

Roots were inoculated in moist chambers with Stilbella I and Stilbella III (Table II) in the same manner as described above.

On one set of roots no wounds were made. Here No. II was used instead of No. III. After the transferred medium had been entirely spent and dried out razor sections were made of the region beneath the point of inoculation. The mycelium had not penetrated the bark. Other roots were wounded by notching with a sterile knife at the center. Ten days after inoculation drops of brownish watery fluid appeared around the margin of the fungus advance. After six weeks the roots were removed and split through the center. The wood was turned brown to a distance of 5 cm. in one case proximally and distally, (photograph No. 7), and to a depth of 1/2 cm. In another root the discoloration extended to the center a distance of one centimeter. Three roots were inoculated with each of the fungi and all showed similar results. No. I seemed to advance somewhat more rapidly than No. III. That the roots remained alive was evidenced by the buckling of the bark around the margin of the infected area and the breaking of the epidermis elsewhere by live tissue. On the surface of root the fungus spread as a white mat. This later became a black hard encrustation. No mycelium is apparent between bark and wood in the early stages. Distinct zonations appeared on the wood beneath the bark. Mycelium was quite abundant in the vascular bundles near the margin of the live wood. Twenty thrust cultures from these roots yielded 5 sterile, 4 bacteria, 1 mold and 10 Stilbella, apparently pure cultures. The mycelium is found in large tufts in the vascular bundles but it is not abundant in the medullary rays. One root cut transversely showed a white fluffy mycelium on the end in 24 hours in moist chamber.

A young apple sprout was potted in a glass jar in greenhouse soil, and inoculated with Stilbella III without positive re-

sults.

Four trees were inoculated October 1, 1915, by spreading agar from pure cultures of Stilbella I and III on wounds of small roots. After six months they were uprooted. The wounds were standing open although they had not enlarged during the winter to any considerable extent. In some cases the bark was buckling up in an attempt to heal the wound. In others it was loose and frayed in appearance. Stilbella was obtained from these trees in four thrust cultures out of ten.

Trees in tubs were inoculated in April 1915 by C. H. Crabill with soil and roots from a diseased orchard at Middletown. Four of each of these lots were examined April 1, 1915. In all except one tree inoculated with soil, there were numerous irregular splotches in the bark which occasionally extended through to the cambrium. Dark strips transversed the bark just beneath the epidermis. Many wounds made at planting were still open and part at least were enlarged. One root 2 cm. in diameter, which had been cut off in planting, had died back 12 cm. and browned lines apparently wood bundles extended ahead several centimeters. Zonations appeared beneath bark. Lesions appeared in the bark ahead of the general decayed area. Fifty four stabs were made from the trees in this lot. Of these, thirty nine were sterile, seven produced bacteria, and eight yielded unidentified molds and other fungi.

Conclusions.

Isolations made from a number of apple trees affected with root rot from several localities in Virginia produced cultures of the following fungi: Hydnum sp., Trichoderma koenigi, an unidentified Basidiomycete, and Stilbella spp. Of these, T. koenigi and Stilbella spp. were the only fungi found in any considerable number of cases.

As the result of inoculations with pure cultures of these fungi on live apple roots in moist chambers, it appears that the first two named are merely saprophytes while the unidentified Basidiomycete is at best a feeble wound parasite. Stilbella spp. on the other hand is evidently an active wound parasite and may be responsible for the greater part of the root rot found in Virginia.

Since roots inoculated with soil from a diseased orchard show symptoms of disease, the causative organism must be capable of persisting for some time in the soil.

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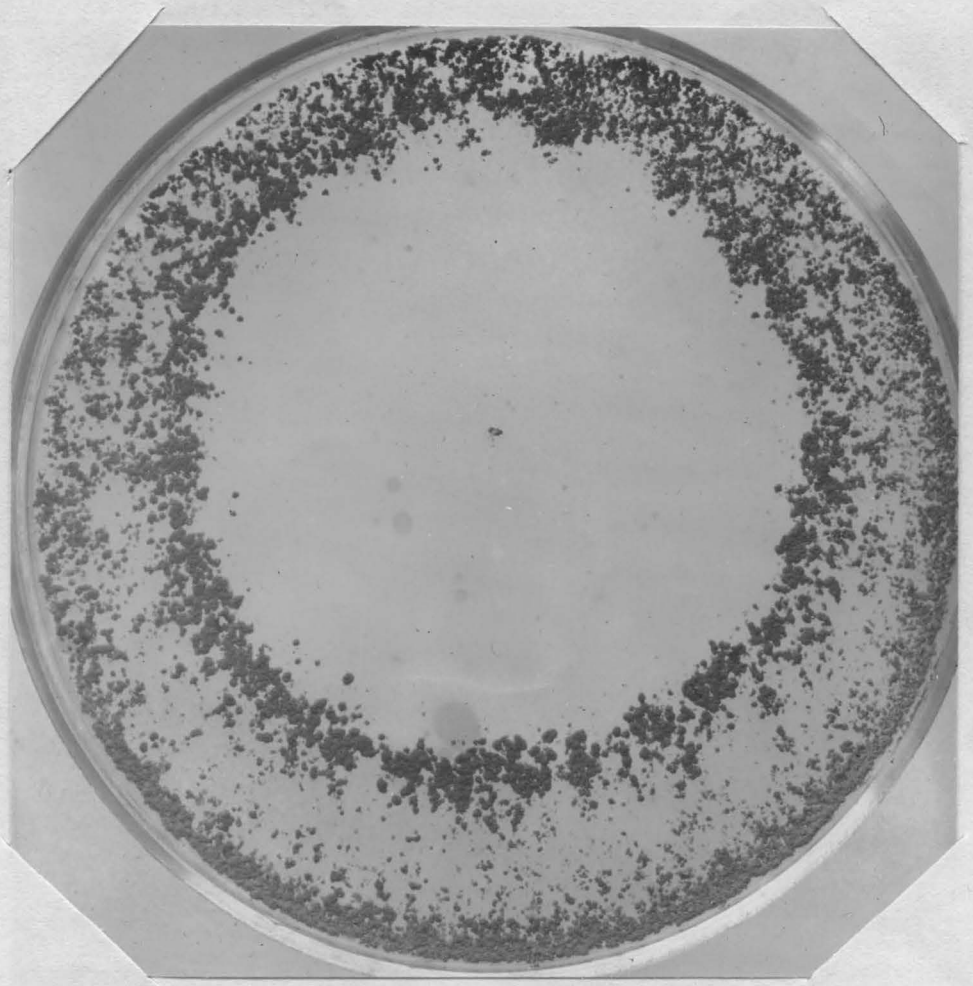
Photographs.

1. Hydnum sp.
2. Rotted crown from Staunton.
3. Trichoderma koenigi.
4. Stilbella sp. on plate. (No. I).
5. Stilbella in bottle culture. (No. III).
6. Stilbella in bottle culture. (No. II).
7. Root Inoculated with Stilbella (I).

No. 1.

No. 2.



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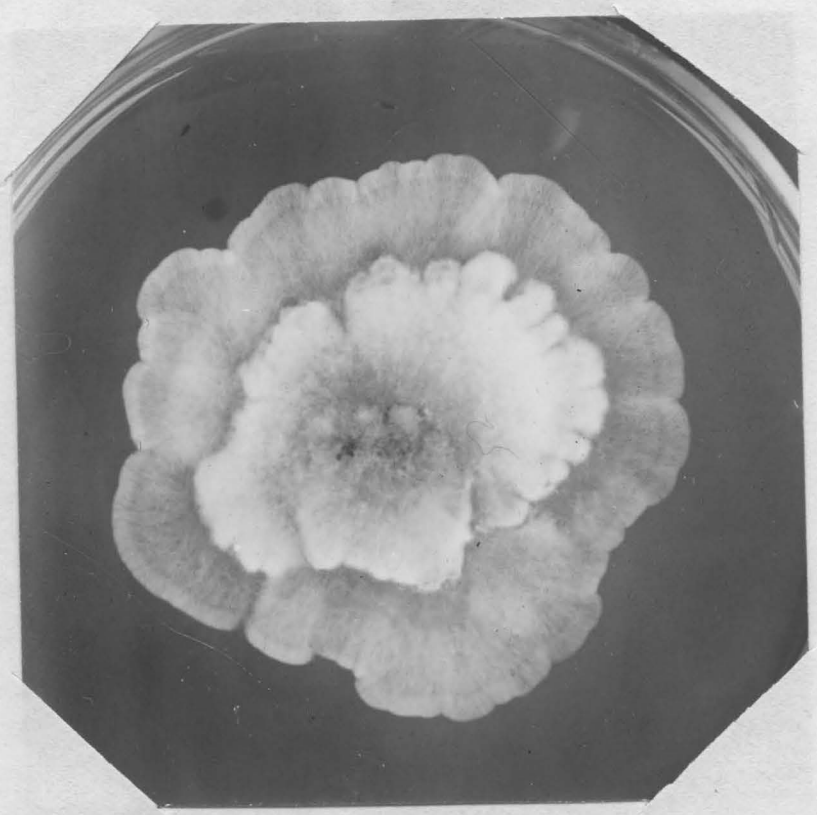
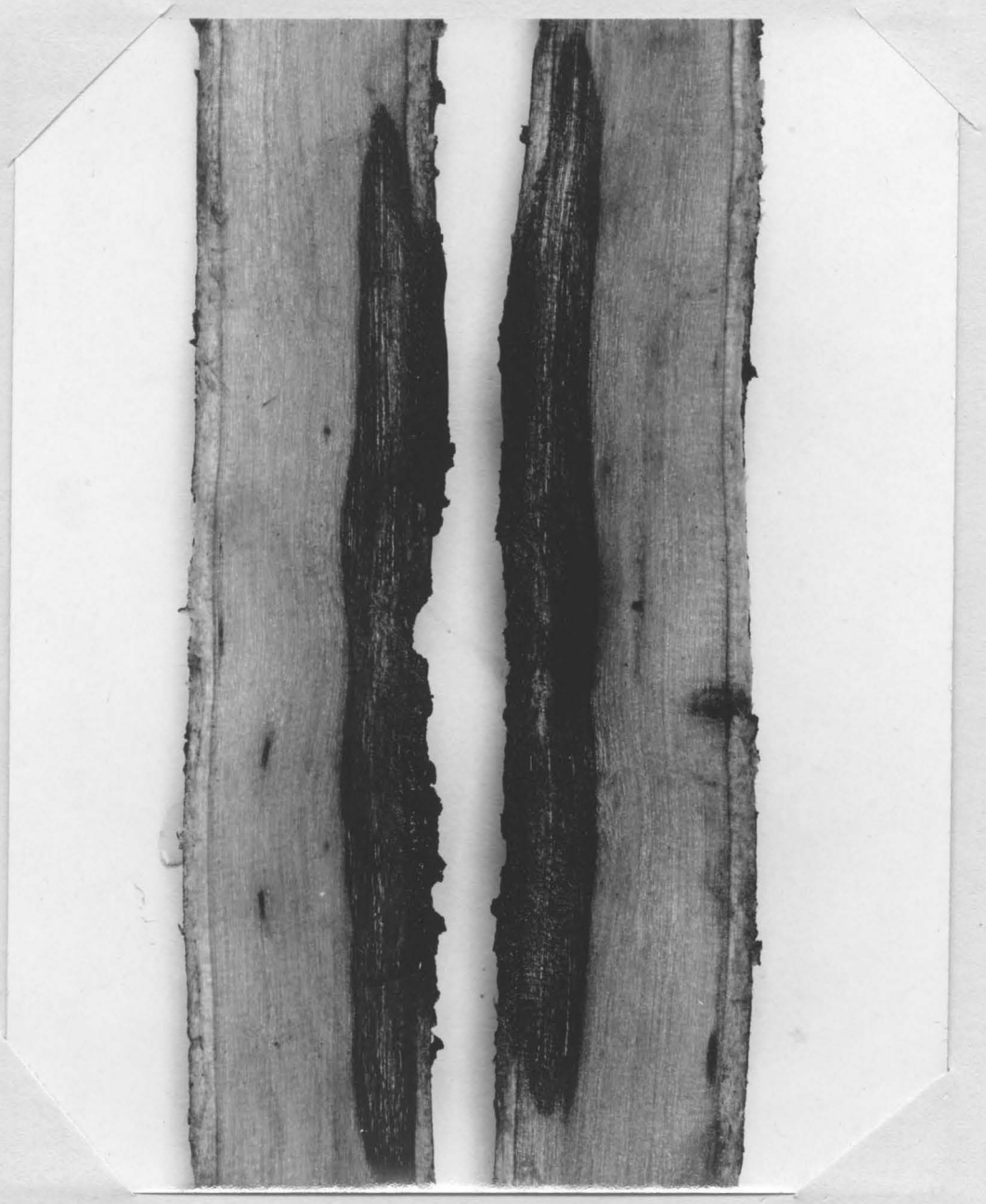


No. 4.

No 5

No 6



No. 7.