STUDIES ON THE RESISTANCE OF ARIZONA ASH (FRAXINUS TOULLEYI)
TO THE ROOT-ROT FUNGUS (PHYMATOTRICHUM OMNIVORUM).

by

Nicholas V. Ponomareff.

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INTRODUCTION.

The disease commonly referred to as Texas (cotton) root rot is most destructive in the arid Southwest. It presents a great problem not only to the farmers and ranchers raising agricultural crops, but also to the communities and individuals in towns and cities, for the disease attacks most of the ornamental and shade trees and shrubs. The Arizona ash, one of the most desirable shade trees, is no exception.

The Arizona ash, *Fraxinus velutina* var. *Touneyi* Rehd. (*Fraxinus Touneyi* Britt.) is native to Southern Arizona and is abundant in the mountain canyons and along streams and washes at altitudes of 3000 to 6000 feet. However, it is found occasionally at lower altitudes along the bottoms of dry streams which are fed by seepage water from the canyons. In cultivation the tree grows luxuriantly and develops a large, dense and beautiful canopy for which it is very highly prized as both an ornamental and a shade tree. Unfortunately, it is not immune to the root-rot fungus and many trees die of the
Figure 1.

A healthy Arizona ash tree early in spring with the foliage not fully developed. (University of Arizona Campus).
Figure 2.

Arizona ash trees suffering from Texas (cotton) root rot disease. Note the scant foliage and dead branches. Spore mats of *Phymatotrichum omnivorum* (Shear) Duggar were found in abundance in August of 1935, irregularly covering the ground under these trees. (Early spring 1936, University of Arizona Campus).
disease each year. However, some ash trees known to be affected by the disease remain alive for many years, suggesting the possibility that the species possesses a certain degree of resistance to the disease. (See Figures 1 and 2).

The purpose of the studies described in this paper is to determine: (1) the degree of susceptibility of Arizona ash to root rot; (2) the effect of environmental conditions under cultivation and in nature upon the susceptibility to root rot; (3) the nature of resistance of the ash to root rot; and (4) the effect of changes in environment upon resistance.

CAUSAL ORGANISM.

Texas (cotton) root rot is caused by a fungus first described by Pammel in 1889 as *Oxonium auricomum* Link (8). Shear in 1907 found it to be a distinct species of Oxonium and named it *Oxonium omnivorum* (10). The strands of the fungus are dirty yellow to brown when old, and somewhat lighter to almost white in the very early stage. In most cases it is very readily seen with the naked eye on the root-rot infected roots of plants. Frequently white mycelial tufts arise on the strands which are referred to as pseudosclerotia. The strands consist of hyphae sometimes interwoven and sometimes running parallel for considerable length, in either case anastomosing frequently. From the outermost hyphae of the strands arise short, slender, septate hyphae with perfect cruciform branching and acicular (needle-like) tips. These acicular hyphae give the strands a characteristic fuzzy coating.
The resting stage of the fungus is represented by spindle-shaped to spheroid sclerotia which are formed by the enlargements of the vegetative strands in the soil near root-rot infected roots of plants and on various media in the laboratory. In the early stages of development sclerotia are creamy white and heavily coated with acicular hyphae. With further development they become dirty yellow to buff to a deep brown. When mature only a few acicular hyphae remain attached to the surface.

The conidial stage of the fungus was first reported by Thornber in 1906 (15) and in 1916 Duggar (3) described and named it *Phymatotrichum omnivorum* (Shear) Duggar. This stage is represented by cushion-like mycelial masses on the surface of the soil directly above or in close proximity to the active strands in the soil. These are commonly known as spore mats and make their appearance during the summer months (primarily in July and August in Arizona) a few hours after rain or after an irrigation. In the early stage the spore mat consists of a film of barrel-shaped hyphae. This film rapidly grows in thickness and in diameter. Conidia are formed in the center which becomes light buff in color. When the growth is extended over a few days a well defined zonation occurs. A mature spore mat is about one-fourth-inch in thickness and may cover from a few square inches to a square foot or even more. The dissemination of the spores is sometimes very rapid, so that a day or two after the spore mat has reached maturity only a trace of its presence can be detected.

The root rot fungus is common in the semi arid region of Arizona below 5000 feet, in Texas, Oklahoma, New Mexico, Arkansas, and also has been reported from the southwest corner
of Utah (6). The number of plants susceptible to this disease was determined in 1929 by Taubenhaus, et al. (14) and found to be 274 species of cultivated plants including important field crops, vegetables, fruit trees, berries, and ornamentals, and also of some 244 species of plants not ordinarily cultivated.

EXPERIMENTAL FIELD

The field experiments were conducted at the New University Farm four miles northwest of Tucson, where a two-and-one-half acre plot with an active root-rot infection had been set aside for studies of Texas root rot. The field is somewhat triangular in shape, two short sides of which are bordering on an orchard and alfalfa field and the long side adjoining uncultivated land covered with mesquite and other native plants. The root-rot spot covers about two-thirds of the area extending along one short side as its base and forming a semicircle, the periphery of which includes only a part of the long side and does not reach the other short side. In 1930 about one-third acre entirely within the root-rot spot at one end of the field was assigned for the study of the effect of the root-rot fungus on various species of trees and shrubs. Among these species were a few five-year-old Arizona ash trees used for the studies described in this paper (Fig. 3). Acala cotton had been grown on the remainder of the field and the entire area had been under close observation for the past ten years. The soil is a light sandy loam with a fairly uniform reaction
Figure 3.
One of the five year old Arizona ash trees used in the experiment. (New University Farm, early spring 1936).
which averages about pH 8.5.

In addition some observations and experimental work had been conducted on the adjacent orchard and alfalfa fields which are heavily infected with the disease.

HISTORY OF THE EXPERIMENTAL FIELD.

At the time the field was set aside for study ten years ago, the root-rot spot occupied about two-thirds of the area. From personal observations during the past two seasons and collected data for the previous years, it is apparent that the spot is largely confined to a certain area of the field, going through cycles typical of the root rot, namely, breaking down and renewing its growth from the carry-over spots remaining within the area originally occupied by the old spot (7). Even though there were numerous individual and small groups of plants found to have been killed each season by the fungus throughout the field outside of the periphery of the spot, these individuals and groups had never been observed to originate aggressive new spots and thus take possession of the entire field (Fig. 4).

The portion of the field on which trees and shrubs have been grown since 1930 is entirely within the root-rot spot. However, the disease has not during the last five years killed any plants of a number of species which are known to be susceptible to the disease. Furthermore, cotton planted in spaces between the trees and shrubs in the season of 1935
Figure 4.

The experimental cotton field and the tree nursery. During the last ten years the root-rot spot remained confined to the area within the approximate boundary line as is shown in the sketch. Dead individual and small groups of plants appeared in the rest of the field every year. The portion of the field under trees and shrubs was formerly a part of the root-rot spot but no disease appeared ever since the trees and shrubs were planted, even though some of them were known to be susceptible to the root rot fungus.
Figure 4.

Trees and Shrubs:
1. Inoculated ash
2.  
3. trees
4.  

Cotton:

Root-rot spot.

Scale: 1 inch = 50 feet.
completed its growth without being infected, as revealed by an examination of several hundred roots. It is of interest to note that this section of the field receives more frequent irrigation in summer, namely 4 inches every 10 to 12 days, as compared with 4 inches every 18 to 20 days for the rest of the field; during the winter months less irrigation water is used.

SOIL.

a. Physical properties.

During the excavations for the purpose of studying the roots it was observed that a few days after the field was irrigated the sub-surface formed a crust of firmly cemented soil particles. The thickness of this crust gradually increased; at the time the field was to receive another irrigation it reached one to two feet and could be broken through with a shovel only with considerable effort. Physiological dryness of this crust was so apparent that no water-content determination was considered to be necessary. On the other hand, the same soil during the first three to four days after irrigation was found to be more or less uniformly moist and loose within the first two to three feet of depth; under this layer was a stratum of soil in a water-logged condition.

b. Temperature.

Late in June, 1935 two soil thermographs were installed in an alfalfa field at a depth of ten inches, one on the periphery of an active root-rot spot and the other in non-infected
soil about 30 feet away from the spot. Average monthly maximum temperatures were calculated from the thermograph charts as is shown in Table I.

Table I. Comparative Soil Temperature of Infected and Non-infected Areas.

<table>
<thead>
<tr>
<th>Field</th>
<th>July (Max.)</th>
<th>July (Min.)</th>
<th>August (Max.)</th>
<th>August (Min.)</th>
<th>September (Max.)</th>
<th>September (Min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infected</td>
<td>92°F</td>
<td>82.3</td>
<td>94.5</td>
<td>80</td>
<td>90</td>
<td>80.5</td>
</tr>
<tr>
<td>Non-infected</td>
<td>84</td>
<td>71</td>
<td>83</td>
<td>69</td>
<td>86</td>
<td>74.5</td>
</tr>
<tr>
<td>Difference</td>
<td>+8</td>
<td>+11.3</td>
<td>+11.5</td>
<td>+11</td>
<td>+4</td>
<td>+6</td>
</tr>
</tbody>
</table>

It was observed that daily minima were reached between 7:30 and 8:30 A.M. and daily maxima between 6 and 8 P.M. The thermograph charts also recorded sudden drops in temperature at the time of irrigation. Thus, on July 20 the water was turned on at about 7 o'clock in the evening. The soil temperature of the non-infected area dropped within 30 minutes from 76 degrees F. to 71, and at the periphery of the root-rot spot from 89 to 75. During the same season supplementary records of soil temperatures at 12 inch depth were taken on the experimental cotton field and tree nursery. The readings were taken with an ordinary rod thermometer at irregular intervals and showed that the temperature of the soil in the more frequently irrigated tree nursery was at all times 5 to 11 degrees lower than in the cotton field as is shown in Table II.
Table II. Comparative Soil Temperatures at 12 inch Depth Within the Root-Rot Spot in the Cotton Field and in the Tree Nursery.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Cotton field</th>
<th>Tree Nursery</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>June 26</td>
<td>3 PM</td>
<td>83</td>
<td>66</td>
<td>- 17 *</td>
</tr>
<tr>
<td>July 3</td>
<td>4 PM</td>
<td>91</td>
<td>81</td>
<td>- 10</td>
</tr>
<tr>
<td>July 10</td>
<td>8 AM</td>
<td>89.5</td>
<td>79</td>
<td>- 10.5</td>
</tr>
<tr>
<td>July 15</td>
<td>8 AM</td>
<td>93</td>
<td>82</td>
<td>- 11</td>
</tr>
<tr>
<td>Aug. 1</td>
<td>3 PM</td>
<td>85</td>
<td>76</td>
<td>- 9</td>
</tr>
<tr>
<td>Aug. 13</td>
<td>3 PM</td>
<td>83</td>
<td>83</td>
<td>- 10</td>
</tr>
<tr>
<td>Aug. 29</td>
<td>9 AM</td>
<td>90</td>
<td>82</td>
<td>- 8</td>
</tr>
<tr>
<td>Sept. 10</td>
<td>8 AM</td>
<td>89</td>
<td>84</td>
<td>- 5</td>
</tr>
</tbody>
</table>

* Taken one day after the entire area was irrigated.

ROOT EXCAVATION, METHODS AND RESULTS.

a. Cotton field.

Excavations were made to obtain roots with different successive stages of infection, from completely decayed roots to those in the earliest possible stages. The last wilted plant in one of the rows was dug out and the roots examined. A trench started from this point, was continued for 8 feet in the direction of advancement of the disease. The trench increased from an initial depth of 2 feet to four and one-half feet at the end. Most of the 22 roots thus excavated were found more or less constricted at places 4 to 6 inches below the surface of the ground, a milder form of deformity described by Taubenhaus, Ezekiel, and Rea (12), for which they proposed a term "root strangulation", and by Hubbard (4) who proposed a more appropriate term "root constriction."

All the taproots up to the constriction and some laterals of the first five plants were found to be completely decayed.
Roots of cotton six feet from the periphery of root rot spot. A, a constricted root with a large lateral the decayed tip of which had fallen off. Dead, blackish, with brown streaks xylem under a perfectly sound cortex on which Phymatotrichum strands were found. The lower part of the taproot was in exactly the same condition and was cut off for the convenience in arranging for photographing. F, growing next to A; sound cortex enclosing heavily discolored xylem a portion of which below the scalpel is completely dead. (Top cut off about three inches below the surface of the ground). No Phymatotrichum strands were found on B.
Phymatotrichum stands were found on both decayed and live parts. Discolorations of various colors from blackish to brown to yellow were present throughout the xylem and continuing from the decayed parts, while the cortex above the decay appeared to be normal. Farther away the decayed parts were found at increasingly greater depths until at the end of the trench only the tips of taproots and of some of the laterals were found decayed. Discolorations as those described above were present in all roots. In some instances the discolorations extended through the entire length of the roots and rootlets having, to all appearances, sound cortex as is represented in Fig. 5. Phymatotrichum strands and lesions were present on some roots and absent on others.

In addition a number of individual plants were dug at random throughout the field outside the root-rot spot. Constricted roots were found in the same proportions, a seemingly general condition for the entire field. In most cases more or less advanced decay of root tips was found with or without lesions on live tissues and with Phymatotrichum strands present only on a few roots. In no instance was the decay found to originate on other parts with tips of roots remaining sound and healthy.

b. Alfalfa field.

A similar trench was dug ten feet long and three feet wide, with depth increasing to five and one-half feet at the outer end. The roots were liberated by carefully loosening
First, second, and third roots of alfalfa from the periphery of the root-rot spot compared with normal root. Note the discoloration in the third root extending from the lateral into the tap root which is only slightly discolored; also lesions in the cortex shown as darkened areas. (All from within first two feet of soil).
Fourth, fifth, and sixth roots of alfalfa from the periphery of root-rot spot (fifth and sixth were cut in two sections each for convenience). The fourth is from the soil within the first two feet, fifth and sixth roots from third and fourth feet. The strands on all six roots shown in these photographs were found only on parts from the first foot of soil.
the soil on the side of a trench. No constrictions were observed on 13 roots thus excavated. The picture of decay of tips of taproots and laterals was similar to that found in the cotton field with the exception of color of discolorations in the xylem. The dead parts were muddy brown gradually becoming light brown to bright yellow as the distance from the decayed tissue increased. The cambium around the streaked xylem was always bright yellow but the cortex remained unchanged. Phymatotrichum strands were found on all six plants within the first five feet from the margin of the spot, on the plant preceding the last, and on no other plants. (Fig. 6, A and B).

As with cotton, a number of plants were dug throughout the field outside of diseased spots but to a lesser depth. In those cases a hole about two feet deep was dug on one side of the plant, the roots liberated by hand within this depth and then pulled out with as much additional length as possible. In general, lesions (whatever their origin) were found far more numerous than on cotton. Out of 25 plants thus excavated 9 had yellow discolorations in the xylem, two had Phymatotrichum strands, and all had lesions.

The conditions of the root systems of infected cotton and alfalfa, observed in the previously described excavations, were found to be directly opposite to those described by Peltier et al. (9) with one exception — they also found constrictions on cotton roots. According to their description for both species the rot was always found near the surface of the ground and progressed downward; they report that the terminal parts of
roots, even those on most of the dead plants, remained sound and healthy.

The depth at which the infection takes place apparently is influenced by the conditions in the soil peculiar to a given locality.

c. Orchard.

Various deciduous fruit trees grown in this orchard are rapidly dying from the root-rot disease. Alfalfa had been grown on the ground as a supplementary crop and a number of active root-rot spots are scattered in certain parts. In the season of 1935 the alfalfa was three years old. It was striking to note that in spite of the fact that some of the fruit trees had been dead for one or more years, the alfalfa growing around and immediately under some of the dead and dying trees remained in healthy condition. Several crops of spore mats appeared during the seasons of 1934 and 1935 in the shade of alfalfa foliage. Examination of the soil to the depth of three feet showed abundant sclerotia. Examinations of roots of two dead trees in different rows revealed that at some time previous to their death the roots were severely attacked by the root-knot nematode. Phymatotrichum strands were found in masses on roots of all sizes. About thirty feet distant from one of these trees was another tree which had just begun to show wilting on a few of its branches. Both the roots and rootlets were found to be entirely covered with nematode knots.
Figure 7.

One of the charts showing relative positions of the inoculated roots of ash trees, their exact distances from the trunks (figures at the middle of the lines), and depths at which the inoculi were placed (figures at the end of the lines).
INOCULATIONS AND RESULTS.

Inoculations of four-year-old ash trees on the nursery plot were made on November 9, 1934. Fresh, naturally infected roots of Acala cotton from the root-rot spot of the adjacent cotton field were used as inoculum. Three or more pieces of infected cotton roots were tied together with a fine copper wire and used for each inoculation. The inoculi and the tags bearing date and successive numbers of inoculations were fastened to the exposed roots and covered with soil. The trees were previously numbered and a sketch showing their positions on the plot was prepared. To expose the roots holes were dug, one by one, and the roots inoculated. Relative positions of the inoculated roots, their exact distances from the trunks, and depths at which the inoculi were placed were carefully measured and recorded on a separate chart for each tree. (Fig. 7).

On June 1, 1935, when root rot had been active for some time in the nearby alfalfa field, a first series (one root from each tree) of inoculated ash roots were dug out for examination. Parts of roots together with the remnants of inoculum were cut off and taken to the laboratory. Upon examination the inoculum was found to be pulpy with a seemingly greater amount of Phymatotrichum strands on the papery epidermis than when placed in the soil. Some fine strands of Phymatotrichum were found clinging to ash roots which showed no lesions or other injuries. Some had signs of healed scratches inflicted during the process of inoculations.
A second series of inoculated roots was dug out on June 11, when the first few cotton plants were found wilting on the area which was known to have been within the root-rot spot. Examination revealed the same conditions as in the first series. Further excavations of inoculated roots during the season revealed no infection.

On June 14, 1935, six inoculations with 8-day-old, pure cultures of *Phymatotrichum omnivorum* were made as follows: A hole was dug to reach the root; the root was cut and the end attached to the tree was pushed into the test tube with a pure culture of the fungus; then the hole was filled up with the soil. On the same day two holes were dug under two ash trees and the roots thus exposed were covered with 25 to 30 fresh, naturally infected alfalfa roots. Only those having the most abundant growth of *Phymatotrichum* were selected from a great number of carefully examined roots.

On July 31, 1935, three of the six test tubes were dug out and the parts of ash roots in them examined. Only traces of *Phymatotrichum* strands were found adhering to the inner sides of the test tubes. A few small rootlets of ash roots sprouted and grew within the tubes and into the watery mass of agar remnants. No signs of infection with the root-rot fungus were present. On the same day the roots of one of the two ash trees, covered with a layer of *Phymatotrichum*-infected alfalfa roots, were exposed and examined. Only a pulpy mass of alfalfa roots remained. Numerous new rootlet of ash grew through this mass.
No growing mycelium of Phymatotrichum and no signs of infection on ash roots were found.

The other three test tubes and the second hole with buried alfalfa roots still remain in the ground.

FIELD EXPERIMENTS WITH ASH AND COTTON SEEDLINGS.

Seeds of ash and Acala cotton were germinated in the seedbed in the greenhouse; a few weeks after germination a number of both kinds of seedlings were transplanted into medium sized flower pots, two seedlings in each.

On June 29, 1935, when they were about four months old, six ash seedlings and six cotton seedlings were transplanted into the soil two feet ahead of the periphery of the root-rot spot where the Phymatotrichum spore mats were in abundance on this date. For transplanting, holes were dug near the roots of alfalfa plants and the contents of the flower pots were placed in the holes. To avoid disturbance of the seedling root systems the flower pots were carefully broken into fragments and removed. The depth of the holes was planned to be three to four inches greater than the depth of the flower pots.

Two cotton plants showed signs of wilting before the end of the day. A week later they were carefully dug out and the roots examined. No injuries of any kind were found, while several buds on parts that were covered with a surface layer of soil were found to be partly opened. The plants apparently suffered from sudden exposure of their crowns to the direct
One Acala cotton plant (A) and two ash seedlings (B) transplanted on June 29, 1935, from flower pots into the soil two feet ahead of the advancing periphery of the root-rot spot where Phymatotrichum spore mats were in abundance at that time. The photograph was taken on September 20-th. Note the periphery of the spot which by this time had advanced about seven feet. In December, these as well as the other ash transplants used in this experiment were transplanted into the greenhouse where they came out of dormancy and at present are in the state of vigorous growth.
sunlight, rather than from any other causes.

Three of the remaining cotton plants were dug out at intervals for examination of their roots during the season; in all cases they were found to be sound and healthy. The last one was dug out late in December together with all six ash seedlings, a few weeks after the leaves were shed. The roots of all were examined in the laboratory and found to be healthy and normally developed. The margin of the root-rot spot by this time had passed the transplants and progressed about ten feet. All ash seedlings were placed in the seedbed in the greenhouse where they came out of dormancy and at present are in a state of vigorous growth. (Fig. 8).

On July 31, 1935 twenty ash seedlings five months old were transplanted from flower pots into rows of cotton within the active root-rot spot. During the week prior to the date of transplanting, the seedlings in flower pots were daily taken out of the greenhouse and exposed to direct sunlight. The duration of exposure was gradually increased from 30 minutes on the first day to 9 hours on the last. This precaution was taken to avoid injuries to aerial parts which might be caused by sudden change of environmental conditions from the shade and humidity of the greenhouse to the strong sunlight and aridity of the outdoors.

The seedlings were transplanted into the rows of cotton 5 to 8 feet ahead of the advancing disease, and as close to the roots of cotton plants as possible, to insure contact of the root systems of transplants with those of cotton.
Figure 9.

Two of the ash seedlings transplanted into active zone of root rot in cotton field on July 31, 1935. This photograph was taken on September 20-th. The disease was advancing from right to left. All cotton plants are dead with the exception of one on the extreme left which was in the stage of wilting. These ash seedlings were dug out and examined ten days later. Strands of Phymatotrichum were found on their otherwise sound and healthy roots.
All the seedlings continued their growth and development without any noticeable effects from transplanting.

Some of the seedlings were dug out as soon as all the surrounding cotton plants were killed by the disease (Fig. 9). Phymatotrichum strands were found on the roots of all, but no lesions or any other signs of infection. Cross-sections of parts with adhering strands were prepared with a sliding microtome and examined under the microscope. No penetration of the fungus into the tissues of roots was found.

At the end of the season, in November, practically all cotton plants had been killed by the disease but the ash transplants remained alive, although the latter were in a state of dormancy with all leaves shed. They were allowed to remain in the field until spring of 1936. Late in March, when these seedlings already had newly developed spring foliage they were dug out and the roots examined. Some fragments of Phymatotrichum strands were found in four out of twelve roots, but all plants were sound and healthy. After examination the seedlings were planted in the greenhouse where they are still growing.

PURE CULTURE ISOLATIONS OF PHYMATOTRICHUM OMNIVORUM.

a. From diseased roots.

Numerous attempts to isolate the fungus by methods suggested by Taubenhaus and Killough (14) and Peltier, King, and Samson (9) were unsuccessful. Even though the growth of Phymatotrichum was obtained in a few instances, it was con-
taminated to such an extent that no transfer was possible. In all cases the Phymatotrichum hyphae were killed before they had a chance to outgrow other fungus and bacterial organisms of which the latter were predominating. After three months of tedious and discouraging attempts a trial was made as follows:

On December 2, 1934 the roots of cotton freshly infected under field conditions were dug and all parts above and below lesions were immediately cut off. The selected parts of the lesion were wrapped up in a clean moist cloth and taken to the laboratory where, without any delay, they were thoroughly washed in running tap water. After washing, one-fourth-inch sections containing margins of lesions were cut out and sterilized in 1:1000 mercuric chloride solution for 30 to 45 seconds. The solution was poured off and distilled water poured over the sections. Three five-minute rinses were used, after which the material was allowed to remain in the fourth wash for about one hour. Prior to this 36 Petri plates each with two sheets of filter paper, were sterilized in an electric oven for several hours. While the inoculum remained in the last wash, the filter papers in the plates were moistened with sterilized, distilled water. Then bits of roots were deposited on the filter paper, five to each plate, and the plates put into the incubator. The material was examined daily and small amounts of water were added about every third day to keep the filter paper moist. By December 14 light brownish mycelial strands were noticeable in some plates. Microscopic examination made it certain that the fine strands were those of Phymatotrichum.
The next step was to pick off terminal portions of the strands with the end of a flamed needle and the aid of a hand lens; the pieces of strands were then transferred to slanted tubes of potato-dextrose agar. All 24 slants thus inoculated soon showed bacterial contamination. Three of the slants, however, contained one or more growing hyphae which were detected under the microscope. On the fifth day after inoculation, when the tips of hyphae were a few millimeters outside of the contaminated area, an unsuccessful attempt was made to transfer the culture to fresh media. Since it was difficult to make a transfer from slants, fresh terminal portions of strands were selected from the original plates with filter paper. This time 12 Petri plates with the same agar media were used, five transfers to each plate. On the third day some transfers remained dead or dormant with or without contamination; some produced contaminated hyphal growth; one had uncontaminated hyphae. The last one was immediately transferred to a fresh plate and later proved to be a pure culture of *Phymatotrichum omnivorum*.

This method was again successfully repeated during June, 1935.

b. From sclerotia obtained in the field.

During September, October, and November sclerotia were found in abundance in the margins of root-rot spots in the alfalfa field and under the dead trees in the orchard, but none were found in any place within or outside the root-rot spot in the cotton field. Many hundreds of sclerotia were unsuccessfully used during these months in the attempt to obtain pure cultures. In no instance was the growth of *Phymatotrichum* pro-
duced on any of the culture media, such as moist filter paper; sterilized or unsterilized white sand and soil; living and autoclaved potato tubers, carrots, the roots of a number of woody plants; and various agar media. Different methods of surface sterilization of sclerotia were used. Some lots were thoroughly washed in sterilized distilled water and others were dipped into 1:1000 solution of mercuric chloride, alone or in equal parts with 50 per cent alcohol for periods ranging from 15 seconds to four minutes. No growth of any kind was obtained from sclerotia sterilized from three to four minutes in either solution; bacteria and fungi other than Phymatotrichum grew from all others.

Early in June, 1935, the examination of soil for the presence of a fresh crop of sclerotia was begun and the first group of sclerotia were found coincidentally with the formation of spore mats in the orchard and in the alfalfa field on June 27. Later in the season the sclerotia were found in the cotton field again coincidentally with the formation of the first spore mats. Both sclerotia and spore mats were for the first time observed to be present in this cotton field. Examination of soil samples from the field revealed that the milky white, immature sclerotia were only directly under the spore mats, which did not hold true for the alfalfa field or the orchard. In the alfalfa field they were found in the center of root-rot spots, within the periphery, and up to three feet outside the spots; in the orchard they occurred only immediately above the dead roots of dead trees (three trees were examined).
Again, as in the previous season, all attempts to obtain pure culture from these sclerotia throughout the summer were unsuccessful.

c. From sclerotia grown in jars.

Pure cultures of Phymatotrichum were obtained from sclerotia grown in Mason jars. Two methods were used for the inoculation of soil with roots of cotton and alfalfa, which were freshly infected with the root-rot fungus under field conditions. The first consisted in bringing both the soil and the diseased roots to the greenhouse where the roots were placed in two-quart jars and covered with the soil. In the second method the jars were taken to the field where the roots were placed in them and covered with soil immediately upon removal of the latter from the ground; the soil used was taken from the very spots in which the plants grew.

The first method for growing sclerotia proved to be unsatisfactory, for a very few sclerotia formed in only one of the jars with cotton root as inoculum. In the second method six jars were inoculated with cotton roots and six with alfalfa roots. Numerous sclerotia were produced in all jars with cotton roots but none appeared in jars containing alfalfa roots.

It is of interest to note that, as examination revealed, sclerotia were not produced in any place within the soil in jars; they formed in spaces between the glass and the soil.

Pure cultures were obtained without difficulty from all sclerotia grown in jars. However, some had to be separated from
Figure 10.

One of the boxes with plate glass sides used for study of growth and development of mycelial strands of root rot fungus on roots of ash and cotton seedlings. (Photographed at the end of experiment).
mixed contamination with Rhizoctonia and Fusarium spp. No bacterial contamination took place in any of these cultures.

INOCULATIONS OF SEEDLINGS IN THE GREENHOUSE.

Several series of inoculations of young and mature plants of cotton and seedlings of Arizona ash in the greenhouse were studied during the year 1935 and the first quarter of 1936.

The roots of plants in the seed beds, in Mason jars, and in specially constructed wooden boxes with glass sides were inoculated with pure cultures growing on various media and with naturally infected roots of cotton and alfalfa.

a. Cotton and ash seedlings growing in boxes.

These boxes were designed by Dr. R. B. Streets, Department of Plant Pathology of the University of Arizona. They had a capacity of about five gallons each. They were provided with two opposite plate glass sides which tapered downward from about 10 inches apart at the top to four inches at the bottom, as shown in the photograph (Figure 10.). This was done in order to expose practically the entire root system for observation. The boxes were first filled with the soil brought from a field free from root rot and then adjusted at an angle to prevent the soil from falling out when the upper glass sides were necessarily removed in planting and other operations. The roots of three-week-old seedlings of cotton and ash were carefully deposited on the exposed sides of the soil blocks.
in boxes and the glass plates were put in their places and fastened with small wooden strips at their edges. Three ash and two cotton seedlings were thus transplanted under the glass sides of ten boxes. All seedlings withstood the transplanting and were growing vigorously at the time of inoculation on March 29, 42 days later.

Pure ten-day-old cultures of *Phumatotrichum omnivorum* growing on autoclaved carrots in 750 cc. Erlenmeyer flasks were used for inoculi. The glass sides were removed, holes were made to receive the entire contents of a flask for each side, and the inoculum was deposited. Then the cover plates of the boxes were put back and fastened. This arrangement offered a splendid opportunity for observation of the progress of growth, both of the fungus and of the roots of seedlings. Two of the ten boxes were reserved as checks.

On April 2, the white mycelial growth had progressed up to three inches from inoculi, in all instances coming in direct contact with the roots of one or more plants. On April 9 one cotton seedling was found completely wilted. Typical Phumatotrichum strands were plainly visible on the soil and on most of the roots. The roots of wilted cotton visible through the glass side, appeared to be normal in color and shape. Close examination in the laboratory showed that there was a slight wrinkling of the surface tissues of the cortex throughout the entire root system without the presence of individual lesions. The strands and young hyphae did not follow the lengths of the taproot and rootlets, but crossed them in the direction which the
fungus naturally grew from the inoculum. Sections were cut out from different parts of the root system, divided in two, and one series of halves was used for obtaining cultures and the other series was killed for sectioning.

On April 16 a second cotton plant and on April 29 a third, died in the same box. The procedure and results of examinations for both were identical to those of the first one. All the cultures obtained from surface sterilized sections were mixtures of Phymatotrichum, Rhizoctonia, Fusarium, and bacteria. Because no pure culture of Phymatotrichum had been produced by any of the halves of sections, all the corresponding halves were considered to be of no value for the study of the Phymatotrichum fungus within the tissues and were discarded.

No more plants died in any of the boxes and the surviving plants were reinoculated in June with freshly isolated pure cultures of the root-rot fungus. Since no plants contracted the disease by the end of October, it was assumed that an inactive strain of the fungus had been used. Through the courtesy of Mr. C. J. King of the U. S. D. A. Experiment Station, Sacaton, Arizona, sclerotia-forming pure culture on autoclaved cotton roots was obtained and fresh transplants were made on the similar media in eight-ounce, wide-mouth bottles. A vigorous growth of Phymatotrichum was initiated overnight and in a few days sclerotia were forming in abundance in all bottles. These cultures were used for inoculations at the time the sclerotia were turning brown in color. Roots of plants in five of the original eight boxes were inoculated; roots in three boxes
Figure 11.

Roots of one year old ash and cotton plants inoculated with a pure culture of Phymatotrichum fungus bearing mature sclerotia. This photograph was taken on April 16, 44 days after inoculation. Note the extremely abundant growth of mycelial strands of the fungus in a radial direction from the culture.
Figure 12.

A close view of one of the roots of cotton seen in Fig. 11. The root is almost completely hidden by the mass of Phymatotrichum strands growing from inoculum. (51 days after inoculation)
boxes used for other experiments; the original checks were untouched. It was necessary to frequently remove the flowering parts of cotton and occasionally parts of foliage during the entire period of experimenting for two reasons; first, to prolong their vegetative growth and second, to prevent excessive development of root systems with reference to the capacity of boxes.

Since no infection had occurred in the third series, the roots of plants in one of the check boxes were inoculated, on March 2, with the contents of two bottles of cultures, which contained matured (four-month-old) sclerotia and still active mycelium. The inoculum with a heavy radial spread of mycelium growing over some of the visible roots is shown in Fig. 11. A close view of one of the roots of cotton, which is almost completely hidden under the mycelial strands of P. omnivorum, is shown in Fig. 12. Up to May 5, no infection had taken place.

On the 23rd of July three boxes which had not been re inoculated in June, were used for inoculations with naturally diseased roots of cotton and alfalfa. One side of each box received roots of alfalfa with active mycelium of P. omnivorum and the other side similar roots of cotton. Each side received six to eight such roots deposited equally distributed over the roots and rootlets of the plants. Up to the end of November no infection had taken place. It was observed that the strands of Phymatotrichum were produced by all root-sections of cotton and none by those of alfalfa; also, that while the cotton root sections remained solid the alfalfa root-sections became pulpy masses.
One of the methods used in inoculation experiments. Part of the root system was forced into the bottle with a pure culture of Phymatotrichum omnivorum and covered with sterilized soil which then was moistened. This experiment was performed with the plants growing in boxes with detachable glass sides. Note the pulpy remnants of alfalfa roots through which the roots of inoculated cotton grew freely. These were originally blocks of root-rot-diseased alfalfa roots used as inoculum in the earlier experiment. (Reassembled for photographing after examination).
On November 24 the roots of plants previously inoculated with alfalfa roots were exposed in two boxes for trial inoculation with sclerotia-containing pure cultures of Phymatotrichum grown on autoclaved roots of cotton in widemouth bottles. One of the cultures was a first transplant from the original King's culture and the other was obtained from sclerotia grown in one of the Mason jars in the laboratory. The following method of inoculation was used:

One of the exposed rootlets of cotton in a box was carefully raised and forced into the bottle containing the culture; and bottle was then filled with a sterilized mixture of soil and coarse sand; then a sufficient amount of sterilized tap water was added to just moisten the mixture. The inoculated bottle was then pressed into a space previously provided for it in the soil of a box whose plates were then replaced and fastened. On February 15, a little over three months later, the bottles, together with the entire root systems of the inoculated plants, were removed for examination. Although the strands of Phymatotrichum were present throughout the soil in the bottle, rootlets in the bottles remained sound and healthy and had made considerable growth. Fig. 13 illustrates the above described experiment.

b. Inoculations of seedlings in seedbed.

The seedlings of Arizona ash and Acala cotton were grown from fresh seeds in alternating rows, both seedlings and rows having a seven-inch spacing. A series of inoculations of roots
with naturally infected roots of alfalfa and cotton and with pure cultures of Phymatotrichum was made repeatedly throughout the season of 1935, beginning in June and ending in November. A number of seedlings were dug out periodically for examinations of their roots. Not one of the seedlings of either species has contracted the disease up to the present.

INOCULATIONS OF SEEDLINGS IN MASON JARS.

For this experiment 36 two-quart Mason jars were used. The jars were charged with the media as follows: Pads of cotton about three-fourths inch thick were put into jars and saturated with tap water and then covered with about an equal layer of bits of healthy cotton roots. Then the jars were autoclaved twice, 30 minutes each time with a 24-hour interval. Pure cultures of Phymatotrichum omnivorum were introduced into 26 jars which were allowed to remain in the laboratory for 20 days. At the end of this incubation period, on January 10, 1936, all the jars were examined and contaminated ones, two in number, were discarded. The fungus was growing abundantly in the rest of the inoculated jars and on some strands the thickenings characteristic of forming sclerotia were visible. Both the inoculated and sterile jars were divided into two lots, one for cotton seedlings and the other for ash, and sterile jars served as checks. Since it was found that there were nematode knots present on some rootlets of ash all the seedlings of both species were given a hot water bath for 30 minutes at 122 degrees F. Then two seedlings were placed in each jar and their roots cov-
ered with steam-sterilized soil. Cotton plugs were adjusted in
the mouths of jars around the stems of seedlings. In the mean-
time three boxes were prepared to receive the jars in an elec-
trically heated seedbed. The spaces in between the jars then
were packed with the raw cotton for insulating purposes to
maintain an even temperature of from 85 to 90 degrees F.

Periodical examinations of jars showed that the fungus was
growing vigorously in all inoculated jars. At the end of the
first week one of the check cotton plants and one of the inocu-
lated plants wilted and they were allowed to remain undisturbed
for further observation. On January 20, ten days after inocu-
lation two more cotton plants, both in one of the inoculated
jars wilted and two days later one more of the checks in another
jar of cotton wilted. On this day all wilted plants were pull-
ed out and examined. The tips of all the roots were found dead
and blackish brown in color, the discolorations extending upward
within the tissues of woody cylinders up to about three inches
from the dead area. No lesions or any other injuries to the
roots were found. Likewise, there were no strands found, pro-
bably because the pulling the roots through the soil rubbed the
strands off. No more plants wilted after this in any of the jars.

The general appearance of the roots of wilted plants was
such as to leave no doubts in the mind of the investigator
that the roots suffered from hot water treatment followed by
abnormal conditions in jars without drainage rather than from
any other causes.

On April 30 six of the jars, three with cotton and ash
plants each, were reinoculated with masses of pure culture sclerotia. The results up to the present date, May 7, are negative.

INOCULATIONS OF ACALA COTTON SEEDLINGS IN THE TEST TUBES.

Two different methods for inoculations of cotton seedlings grown in large test tubes were used. In the first method the amounts of water, to bring the soil to its water holding capacity in each test tube, were determined and poured into the tubes. The strips of bark from healthy roots of cotton, one to each tube, were suspended down to the bottom and the tubes filled with soil. The upper ends of bark strips were left uncovered. The test tubes were then plugged with cotton and autoclaved twice, 30 minutes at 15 pounds' pressure each time, with a 24 hour interval. Upon cooling after the second sterilization 8 of the 12 tubes were inoculated with pure cultures of P. omnivorum grown in large test tubes on small bits of autoclaved roots of cotton. The inoculi were placed on the exposed ends of the bark strips thus insuring the direct contact and penetration of the fungus into the soil. All tubes were placed in the incubator maintaining a constant temperature of 85 degrees F. On the third day, when there was no doubt that the inoculi produced good growth of mycelium, the seeds of Acala cotton were surface sterilized and placed two in each tube. The seeds germinated within two days, were reduced to one to each tube, and were allowed to remain in the incubator
until the hypocotyls became too crowded and somewhat coiled in the spaces between the soil and the cotton plugs. Then, with aseptic precautions, the plugs were removed, the seedlings straightened out, and the plugs in a loosened condition replaced around the stems. In this procedure one seedling was broken and the tube discarded. The test tubes now were kept in the laboratory where the temperature fluctuated from 65 degrees at night to 80 degrees F. in the day time. Sterilized tap water was added when needed. At the end of the first week numerous rootlets were visible through the glass sides of the tubes. The growing tips of many in both check and inoculated tubes were turning dark brown in color. Typical Phymatotrichum strands entirely covered the top surfaces of soil in inoculated tubes and the downward growth of mycelium progressed to about 2 inches from the surface. During the following daily examinations it was observed that in both series the rootlets whose growing tips were browned ceased to grow and gradually darkened throughout their visible portions and became water-soaked in appearance. Two weeks after the seeds were planted the strands of Phymatotrichum were abundant throughout the soil in all inoculated tubes. The strands and rootlets in the tubes were examined under a low power microscope, with the light directed on the object from above. In many instances the rootlets of seedlings grew through the strands breaking them at points where they crossed the path of growing tips. At this time the number of browned, water-soaked in appearance, and ap-
pearance, and apparently dead rootlets was great in both inoculated and check tubes. However, it should be emphasized that not in one instance there was observed any accumulation of mycelial growth on any parts of rootlets, either abnormal or normal in appearance in inoculated tubes. Likewise, in no instance the mycelium exhibited a tendency to envelope the rootlets or any of their parts. The strands that appeared to have been growing on the rootlets showed under the microscope that their course was an independent one and only coinciding with that of rootlets. One inoculated tube with the greatest number of dead rootlets was selected, the aerial parts cut off, and the soil was removed from it by a stream of water. The roots, liberated from the soil were placed into the sterilized beaker with sterilized distilled water and thoroughly washed in three changes of water. One section from each dead rootlet was cut off with the flamed scalpel under the water and deposited on the potato-dextrose-agar slants. After this was done the main root with the remnants of rootlets was examined. Eleven of seventeen rootlets were found dead, all the way to the main root. The main root itself was found sound and healthy.

The inoculi from dead rootlets remained sterile until the agar media became dry and were discarded.

On the 27th day from planting two inoculated seedlings were found dead and the remaining 5 in an advanced wilting condition. All check appeared to be normal, with the exception of some browned rootlets. The first two were removed from the tubes in the same manner as described above and the agar slants were inoculated with sections from the main roots which were dead and
covered with strands of Phymatotrichum, later pure cultures were obtained. On the next day all the rest of the inoculated seedlings were dead and were allowed to remain undisturbed for further development. About three weeks later a few sclerotia were formed in one of the tubes.

On the 35th day from planting one of the checks wilted and within the following 6 days the remaining 3 were dead. The rootlets of one were found slimy to the touch, later some bacteria were cultured from them. The roots and rootlets of the rest of the checks were water-soaked, light to dark brown, and with cortical tissues peeling off in flakes.

The second method was intended to study the difference in the aggressiveness of the fungus when its growing hyphae meet the growing rootlets. For this experiment small bits of healthy roots of cotton were placed on the water-saturated cotton plugs in the tubes and autoclaved twice as in the first experiment. Upon cooling 8 of the 12 tubes were inoculated with pure cultures of Phymatotrichum. A dry mixture of soil and white sand was sterilized in the electric oven for 12 hours and poured into the tubes. The sterilized tap water was added to all tubes in the amounts previously determined. The seeds of Acala cotton were surface sterilized and germinated on the sterilized filter paper in Petri plates. The radicles were one to two centimeters long when the seeds were planted in the tubes prepared as described above.

In the duration of experiment the development of both rootlets and fungus were exactly identical with those in the
first experiment with the exception of simultaneous death of all seedlings within fourth week after planting in both inoculated and check tubes. Cultures of bacteria were obtained from one check and two inoculated samples. To all appearances the bacterial contamination was general in the entire lot. The experiment was considered a failure and no significance was attributed to it.

Entirely different behavior of both Phymatotrichum fungus and watermelon seedlings was recorded by Butler (2). In his experiment the fungus enveloped the rootlets on fourth day after planting. The browning of rootlets was observed only after ten days and was attributed to the fungus which penetrated the tissues of the rootlets.

In his paper he also describes the behavior of the fungus attacking the rootlets of watermelon on agar media. To quote: "... (hyphae) had started to grow around and on the roots after 8 to 16 hours. After 24 hours the entire root often was surrounded by the fungus, especially near the root-tip." "... The roots of seedlings that had been in contact with the culture of the fungus for 48 hours were completely surrounded by mycelium". "Actual penetration had taken place in many instances after 48 hours." He also states that: "The fungus was even more rapid and severe in its attack on Acala cotton seedlings grown in the same manner. Hyphae enveloped the roots very rapidly, then advanced up the stems onto the cotyledons."

It is easily seen from the above records that the resistance in the seedlings of cotton (as well as watermelon)
varies in different environmental conditions.

**PURE CULTURES.**

The best results in growing pure cultures were obtained with autoclaved roots of cotton and a decoction from them added to two-and-one-half per cent agar. Addition of two per cent of dextrose slowed down the growth of the fungus considerably. The fungus grew well on the liquid decoction from cotton roots and produced rather firm mats up to 15 mm. in thickness with about 90 per cent of it in the liquid.

An optimum hydrogen ion concentration was found to be between 7.5 and 8.0.

The formation of sclerotia on the above mentioned solid media was more usual than exceptional. The sclerotia are readily produced in Petri dishes when the agar media are protected from drying too quickly. This was accomplished with the aid of bell jars and cans with tight lids. Some bottles with freshly inoculated autoclaved roots of cotton were divided in two lots. One kept in the incubator with constant temperature of 85°F. and the other in the laboratory with temperature ranging from 65 to 75°F. The sclerotia were formed in both lots, but much slower at the lower temperature. Another test was made with the series of bottles kept in the incubator until the presclerotial swellings began to form. Some of the bottles were removed from the incubator and kept at the room temperature of 65 to 75 degrees F. The sclerotia formed in both lots simultaneously. Therefore it was concluded that the formation of
Figure 14.

Chlamydospore-like bodies from pure cultures on autoclaved cotton roots. Found only at base of pseudosclerotia and occasionally on surface of pseudosclerotia. Chlamydospore-like bodies formed: (a) from base cell of acicular hyphae; (b) from lateral branch of acicular hyphae; (c) terminal on hyphae; (d) intercalary and terminal; (e) terminal in chains. (f) Body germinating?

(Drawn with aid of camera lucida. X980).
An unusual vegetative form of *Phymatotrichum omnivorum* produced in pure culture on potato-dextrose agar medium. If it were not for the septate character of hyphae this sphere could easily be confused with oogonium of some of the Phycomycetes. Note the collapsed cells of hyphae the contents of which were withdrawn by the enlarging spherical cell. (Magnified about 350 times).
sclerotia was retarded by lower temperature but not inhibited. However, after sclerotal formation was initiated lowering the temperature down to 65 F. did not hinder its growth.

NEW VEGETATIVE FORMS OF PHYMATOTRICHUM OMNIVORUM.

Two heretofore undescribed vegetative forms were produced in pure cultures. One resembled chlamydospores as is presented in a series of camera lucida drawings shown in Fig. 14. This form was found in a 4-month-old pure culture growing on autoclaved roots of cotton in an 8-ounce wide-mouth bottle. Only one plug was found to contain them. They were in relative abundance on all strands immediately surrounding pseudosclerotal outcrops. A pure culture of Phymatotrichum was obtained from all samples transferred on agar media. No germination of these chlamydospore-like cells took place in hanging drops.

The second form was observed on potato-dextrose-agar in a Petri plate. These consisted of swollen cells of hyphae and resembled the oogonia of some Phycomycetes. (Fig. 15). An examination of some freshly inoculated Petri plates revealed that in one of the plates the mycelial advance from the centrally placed inoculum had stopped half way on one side of the plate. A brown zone about 2 mm. was formed all along the line where the fungus had terminated its advance. This brown zone was examined under the low power microscope and the spherical bodies were found. At first they were considered to belong to some foreign organism but by tracing back some of the hyphae bearing spheres it was found that they were growing from...
strands of Phymatotrichum. The microscope was trained one of the largest spheres with the intention to study its development. About two hours later it was found collapsed. The same happened to the next one studied. Late in the afternoon the microscope was trained on one just beginning to show swelling. On the next morning it was found in the form of a small sphere with collapsed cells of hyphae on both sides, a condition which was immediately found to be general, indicating that the enlarging cells drew the protoplasmic contents of the neighbouring cells. Early in the afternoon the sphere reached its maximum size and collapsed. A further study disclosed that the brown color of the band where the fungus stopped its progress forward was caused by the discharged substances from the spheres.

Pure cultures of Phymatotrichum were obtained from bits of agar containing spherical bodies. It is possible that this form was identical to that mentioned by Taubenhaus and Killough (14), which they supposed to have been asci which failed to mature.

EXPERIMENTS WITH MYCELIAL AND SCLEROTIAL EXTRACTS.

The experiments described below were inspired by the study of enzymatic action of *Rhizoctonia solani* causing damping off of seedlings. The procedure was outlined by Dr. J. G. Brown, Head, Department of Plant Pathology, University of Arizona. Pure cultures of *Rhizoctonia* grown on agar media in Petri plates were
Figure 16.

Four-week-old Acala cotton seedlings after 15 days in the sterilized distilled water (A) and in 0.5 per cent solution of mycelial extract from aerial mycelium of pure culture of Phymatotrichum omnivorum (B). The seedlings in both media remained healthy. (See also Fig. 17).
The roots of seedlings from the bottles shown in Fig. 16. Note the growth of new shoots in both series. The darker color of hairy rootlets of seedlings kept in the mycelial extract solution (right) was caused by the deposit of sediment from the extract.
scraped off with a flamed scalpel and placed in a sterilized mortar. A small amount of heat-sterilized white sand and 25 cc. of sterile distilled water were added and the whole was ground for 40 minutes. After grinding 75 cc. more water was added, the material thoroughly mixed, and filtered through the sterile filter paper. The filtrate was poured into three vials in which were placed a few one-week-old seedlings of Acala cotton, Douglas fir, and ponderosa pine. Checks were placed in vials with sterile distilled water. All the seedlings in the vials with mycelial extract were dead within 24 hours while the checks remained unchanged.

An extract from the mycelium of pure cultures of *P. omnivorum* was prepared in a similar manner to that described above. The concentration of the extract in this experiment was about 0.5 per cent by volume. The seedlings of Acala cotton were four weeks old. No reaction in seedlings had taken place during the two weeks and new rootlets grew out in both check and extract bottles as shown in Figures 16 and 17.

The experiment was repeated with extracts from a pure-culture sclerotia. Two and nine-tenths grams of sclerotia from one 4-month-old pure culture of *Phymatotrichum omnivorum* were ground and a 2.9 per cent extract prepared. Twenty cc. of this extract was reserved for later use. Three cotton and 3 ash seedlings, six and three-weeks-old respectively, were placed in the bottle with 2.9 per cent sclerotial extract and the same number in the bottle with sterilized distilled water. The cotton seedlings in the extract started to wilt within 24 hours.
Six-week-old seedlings of cotton and three-week-old seedlings of ash kept for four days in the sterilized distilled water (left) and in 2.9 per cent solution of sclerotial extract (right). Note dried up cotton seedlings and just wilted ash seedlings in the right plate.
At the end of 48 hours all seedlings were dead but the checks remained unchanged. The ash seedlings at this time just began to show signs of wilting and after 4 days from the beginning of the experiment they were dead. All checks remained unchanged. (Fig. 18).

Dilutions of 0.029 per cent and 0.0029 per cent were made from the reserved sclerotial extract which had been kept in the refrigerator for 5 days. One-half of each dilution was brought to the boiling point, cooled, and the seedlings of cotton and ash were placed in them in bottles. The unboiled portions of the sclerotial extract were used for controls. All seedlings whose roots were placed in the unboiled dilutions of the extract wilted and the seedlings reacted in the same manner in both dilutions. The cotton seedlings wilted at the end of the third day and the ash seedlings at the end of the fifth day. All seedlings in the boiled fractions of the sclerotial extract dilutions remained healthy and their roots produced a number of new branches. Study of the cortical tissues of the roots of wilted seedlings disclosed plasmolysis of the protoplasmic contents.
DISCUSSION.

Due to the failure to obtain freshly root-rot-infected tissues of ash roots either under natural conditions with the root rot in the field or through the artificial inoculations with cultures no studies as outlined were possible. Therefore, an advantage had been taken to study the environmental conditions under which the ash trees and cotton plants were grown. Since the cotton plants were used as inoculated checks in all the described experiments, and the ash trees were grown in the root-rot spot of the cotton field only this field will be discussed.

It is very well known that any organism causing the disease in plants depends upon the environmental conditions, and, likewise, the degree of predisposition (or resistance) in the host plants depends upon the environmental conditions under which the plants grow. All the attempts to produce the disease in ash seedlings and young trees, as described in this paper, gave negative results. The fact that the abundant mycelial growth of Phymatotrichum on the roots of those plants failed to cause an infection could not be considered as either a proof or an indicator neither of immunity nor of high resistance. It should be remembered that none of the cotton plants which are highly susceptible to the root rot, used as inoculated checks had contracted the disease. Apparently these plants as well as the
healthy cotton plants surrounding the scattered diseased individuals and small groups, and undisturbed stands of healthy alfalfa growing for three years in the areas bearing abundant crops of sclerotia (under the dead fruit trees), were not predisposed to allow the invasion of the parasite.

Since no enlightening information on this subject was found in the surveyed literature, the investigator takes a liberty to offer his theory based upon the results of his personal experimentations and the field observations, and those reported by other workers.

The diseases in plants do not just happen. They are the result of a definite and specific aggregation of the biological factors governing the growth and development of the host and the parasite causing the disease.

According to Appel (1) the infection of plant by a parasite is possible only under the following conditions:

1. The parasite must be present.
2. Internal qualities of the host must be such as to make the infection possible.
3. Coincidence of the infection period with the susceptible condition of the host.

In the inoculation experiments the parasite was present. The internal qualities of both hosts were such as to make the infection possible (they were susceptible; ash trees and seedlings are known to have been attacked and killed by the root.
rot). The parasite was in active infection period (it infected and killed all the plants that were naturally growing around the transplants; in the greenhouse sclerotia were used as inoculum). But, apparently, the hosts were not in the susceptible condition.

The seedlings had escaped the infection even though they were raised in the soil taken from the same field, and were transplanted into the same soil, and exposed to the same environmental conditions under which the naturally growing plants had been attacked and killed by the root rot. This fact had suggested that the plants growing in the field with the active root rot become predisposed to the infection at some period before the actual infection takes place. The seedlings transplanted into the field after this critical period had passed, remain immune to the advancing infective strands of P. omnivorum, which were present on the roots of examined transplants. In this, perhaps, is the explanation of the inconsistencies in the behavior of the fungus as described by King (5). He states that the alfalfa plants which recover in the centers of the root-rot spots are killed in the next season by the retroactive invasion of the fungus. In the same paper he states: "In 1918 the writer had under observation several diseased spots of Hairy Peruvian alfalfa in which the inside of the areas had been replanted with Grimm alfalfa. During the entire season none of the plants of the Grimm variety showed any infection.
On July 12, 1922, another spot was spaded up and planted to cowpeas. No root-rot infection occurred during the season, and the cowpeas grew luxuriantly. The writer of this paper is inclined to believe that reestablished plants had gone through the "critical period" again early in the season and thus became predisposed to the infection, while Grimm alfalfa and cowpeas had escaped the infection because they were planted after the "critical period" had passed and the plants retained their natural resistance to the parasite.

Particular importance had been attributed by the writer to the hardening and physiological dryness of the subsurface soil during the intervals between the irrigations, very sudden changes of soil temperatures near the surface when the irrigation is applied, and the water-logged condition of the subsoil for a few days following the irrigation. The direct result of the hardening of the subsurface soil is the development of a peculiar deformity of the toproots of cotton. Taubenhaus et al. had investigated this trouble in Texas, Arkansas, and Mississippi and proposed the term "root strangulation". In their paper published in January, 1931 they stated: "It seems to occur only in flat, poorly drained, heavy clay soils, which are compacted by continuous rains or irrigation, and then further hardened in the absence of cultivation, by continuous hot, dry weather. The direct cause of the trouble is apparently that the upper portion of taproots and laterals of the young seedlings are caught early in the season in a subsurface layer
of hard, dry clay in which further development is prevented. Affected plants die when the constricted areas in the hot, dry soil are killed, or when the moisture supply which can be transported through the constricted areas becomes too greatly inadequate for the requirements of the plants." ... "Affected plants are usually noticed first when they wilt suddenly and die without any preliminary yellowing of the foliage. Plants wilting in this way resemble those dying from Phymatotrichum root rot. However, when plants with strangulated roots are pulled, it is found that instead of the decayed roots characteristic of root rot, the roots are deformed but sound". One year later Hubbard (4) reported the same condition in California and proposed a more appropriate name of "root constriction". He stated: "The soil in which these plants were grown is a light sandy loam that becomes very hard when dry, and the constricted plants were found in each case in plots that had not been irrigated after planting". Thus the above workers had furnished indisputable proofs as to the nature of the constrictions on the roots of cotton plants. Yet a number of investigators had associated the root constrictions with the Phymatotrichum disease. It should be recalled that Pammel (8), the very first investigator of the cotton root rot, considered the constriction to be one of the symptoms of the disease. He used term "enlargement", referring it to a swollen zone on the taproot above the point of constriction. He stated: "It is caused through the shrinkage of the tissue below the point of attack, and the stor-
age of elaborated material in the enlargement." ... "New root-
lets are thrown out at the enlargement, and under favorable con-
dition the plant is then capable of maintaining itself." In
1923, Taubenhaus and Killough (14) in the legend under the Fig.
3 in their paper pointed out the constrictions in the two photo-
graphed roots of cotton as one of the symptoms of the root-rot
disease. There were eight more photographic presentations of
the diseased cotton roots and all of them had the constrictions.
In 1926, Peltier, King, and Samson (9) describing the types of
the root-rot infections in cotton plants stated: "In the first
type the strand hyphae first accumulate at the point of the
root just below the constriction between the root and the stem
and the depressions are formed in this zone." ... "Plants in-
fected in this manner always show a pronounced constriction of
the taproots just below the collar. This point of infection is
the one usually found on cotton plants in root-rot spots".

Considering the factors referring to the root rot and the
root constriction and their possible interrelation as found in
the studied cotton field it is necessary for the writer to make
a choice. The constriction should be treated as one of the
symptoms of the root rot (8, 9, and 14), or as a symptom of the
soil condition unfavorable for the cotton plants and responsible
for inducing the predisposition (or decreasing the degree of
the natural resistance) in the plants, and thus to allow the
root-rot fungus to enter the root tissues.

In this connection the reader is requested to recall that
the disease failed to appear in the plot within the active root-rot spot of cotton field where the trees and shrubs are grown. The soil had received no disinfecting treatment, the change was only in the frequency of irrigation which was almost doubled. Consequently, the periods and the degree of hardening and the dryness of the subsurface layer of the soil were shortened, and the drop in the soil temperature was not as great as in the rest of the field when irrigation was applied.

A verbal statement of Dr. A. H. Finch, Associate Professor of Horticulture, University of Arizona, based upon his personal experience and observations seems pertinent. He stated that in the Yuma Valley pecan trees were grown on some fields that became unsuitable for the other crops because of the heavy infestations with the root-rot disease. After the pecans were planted, the fields were irrigated very heavily to meet the requirements of the trees. No infection with the root-rot disease had taken place for a number of years, twelve years in one case. At the age when the trees began to bear nuts it was found necessary to decrease the amounts of water and the frequency of irrigation to improve the quality of the nuts and to increase the crops. The root rot has reappeared during the first season in all the fields to which these measures were applied.

On the strength of the results of his own experiments and field observations and those reported by other workers (4 and 13), collaborated with the verbal information, the writer believes there is a wide variation in the resistance to the disease be-
tween the normal plant and the one actually dying as a result of unfavorable conditions, of which, in this case, the constriction on cotton roots are a symptom (4 and 13). Therefore there must be a certain "critical period" at which the fungus, *Phymatotrichum omnivorum*, when present, becomes capable of overcoming the decreased resistance and causes infection of the tissues with which it is in contact.

There is no reason to believe that the ash trees do not respond to the environmental conditions in the soil. The resistance in ash trees to the root-rot disease is very high when the trees grow under favorable soil conditions. The fungus may grow and develop on the root surfaces without being able to affect an entrance. As soon as the soil conditions become unfavorable for the normal functions of the root tissues the natural resistance is decreased and the fungus affects the infection.
The Arizona ash, *Fraxinus velutina var Toumeyi* Rehd. (*Fraxinus Toumeyi* Britt.), is native to Arizona and is highly prized as an ornamental and shade tree. It is not immune to the root-rot disease, but there are diseased trees which are known to remain alive for many years, thus suggesting the possibility of being resistant.

The causal organism, *Phymatotrichum omnivorum* (Shear)Duggar was described in its three stages, vegetative, sclerotial, and conidial.

The physical conditions of the soil as influenced by the irrigation were studied and described. The soil temperature in the diseased and healthy areas at ten-inch depths of both cotton and alfalfa fields were taken and the differences recorded and tabulated.

The trenches in the cotton and alfalfa fields were dug outward from the margins of the root-rot spots. The excavated roots were examined and the conditions in which they were found described.

Healthy stands of alfalfa were found growing for the past three years under and around the trees killed by the root rot.
The soil in which these plants grew was found to be heavily infested with the sclerotia of the root rot fungus, and the spore mats had appeared repeatedly under the healthy alfalfa crowns. The plants are still in a fine condition.

Many times repeated inoculations of the five-year-old ash trees growing in the experimental field, and of the seedlings four to eighteen-month-old growing in the greenhouse were unsuccessful. Negative results were also obtained in attempts to produce the disease in the ash and cotton seedlings by transplanting them into the active root-rot zones, both in cotton and alfalfa fields. Cotton plants were used in all experiments as inoculated checks.

In the greenhouse the inoculated plants were grown in the specially constructed boxes with two opposite glass sides, in two-quart jars, and in the seedbed. Pure cultures in various stages of development and grown on various media, as well as the naturally infected roots of cotton and alfalfa, were used as inoculum.

The seedlings of Acala cotton were germinated in the freshly inoculated soil in large test tubes. No infection had taken place even though the roots grew through the growing strands of the root-rot fungus. Two methods of inoculating soil in the test tubes were described.

Experiments with the mycelial and sclerotial extracts of the Phymatotrichum fungus were made.
Two new vegetative forms of the root-rot fungus were produced in the pure cultures and described. One was resembling the chlamydomospores, the other was of oogonia-like swellings of the cells of hyphae. Both intercalary and terminal swellings were observed.

Experiments with the mycelial and sclerotial extracts of the fungus were made. The young seedlings of both ash and cotton were not disturbed after their roots were immersed in the 0.5 per cent solution of the mycelial extract. The solutions of the sclerotial extract of 2.9, 0.029, and 0.0029 per cent dilutions had caused the death of all seedlings. The reaction exhibited by the cotton seedlings was much more rapid than that exhibited by the ash seedlings. The seedlings, the roots of which were immersed into the boiled solutions of the sclerotial extract remained unchanged.

In the "Discussion" it was attempted to show that "root constriction" of cotton is not caused by the Phymatotricum fungus. Instead, it should be considered as a symptom of very unfavorable soil conditions during the early life of cotton plants. These unfavorable conditions of the soil are responsible for bringing about in plants a predisposition to the root-rot fungus. The writer has failed to find any evidence demonstrating that the fungus is capable of entering the host tissues when the host is growing under favorable conditions. The writer has expressed his belief that the plants become infected by the
Phymatotrichum root rot only at a certain "critical period". This "critical period" ordinarily occurs early in the seasonal growth.
CONCLUSIONS.

1. Arizona ash is highly resistant but not immune to the root-rot fungus.

2. No diseased trees growing naturally and under natural environmental conditions were found by the writer or were reported to him. The trees under cultivation become susceptible to the disease when their physiological requirements are neglected. This is usually when the trees are grown in an unsuitable soil (such as caliche), when underirrigated, or both.

3. Due to the failure to produce the disease by inoculation, the nature of resistance in the tissues of the roots could not be determined.

4. The ash trees and seedlings grown in soil under favorable conditions remained immune to the disease. If this is not an exceptional case peculiar to the locality, but is true in general, then the control measures may be confined to the elimination of factors creating conditions unfavorable to the root system of the ash trees.

5. No particular significance was attributed to the two new vegetative forms so far as the disease is concerned. In the case of spherical, oogonia-like swellings of the hyphal cells there is a possibility that some toxic substance was present in the medium around which the fungus was building up a barrier.
6. The experiments with diluted extract of Phymatotrichum sclerotia suggest that the fungus, at least in its resting stage, possesses a toxin or toxins which are capable of affecting host plants; also that the toxic substance is not thermo-stable. The rate with which the different species of seedlings react to the sclerotial extract possibly may indicate the respective degree of resistance (susceptibility) to the attack of Phymatotrichum. For instance, it is obvious in the experiments referred to that cotton seedlings are much more sensitive to the toxic action of the extracts than are the ash seedlings placed in the same containers. Negative results with the mycelial extract may possibly be due to the use of aerial hyphae rather than hyphae in contact with the roots of the host plant. The experiment deserves further attention.

7. The results of the experiments with the inoculations were analyzed and the following conclusions were made:

(a) The resistance in seedlings is nil when they are growing under the extremely abnormal conditions such as on agar media in the test tubes.

(b) The resistance is slight when the seedlings are growing under very abnormal environmental conditions in the soil in the test tubes.

(c) The resistance is considerable when the seedlings are growing under abnormal conditions with their physiological requirements favorably satisfied.
(d) (Deduction). The resistance in the seedlings and in mature plants is great when they are growing under their optimum environmental conditions.
LITERATURE CITED.


