Host-parasite relationships in the pink-root disease of Allium cepa L

William Alexander Kreutzer

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HOST-PARASITE RELATIONSHIPS IN THE PINK-ROOT DISEASE
OF ALLIUM CEPA L.

by

William Alexander Kreutzer

A Thesis Submitted to the Graduate Faculty for the Degree of
DOCTOR OF PHILOSOPHY
Major Subject Plant Pathology

Approved:

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In charge of Major work

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Head of Major Department

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Dean of Graduate College

Iowa State College
1939
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INTRODUCTION

Pink-root is one of the most serious maladies of the onion. Its causal agent is present in the soils of much of the land in this country in which onions are grown. Consequently, the disease is an ever present menace to the onion industry. A review of work conducted during the past two decades emphasized the conflicting views as to the identity of the pink-root pathogen, as well as the confusion of the trouble with other onion disorders. In addition, more knowledge was needed to clarify a possible relationship between the causal agents of pink-root and bulb-rot in inducing the latter. Further, too little was known pertaining to the parasitism and host range of the organism causing pink-root, as well as the influence of environmental factors on the pathogen and the degree of severity of the disease.

The present study was designed to, (a) determine the identity of the causal agent of pink-root of onions in Colorado, (b) investigate the symptoms of the malady with the view of establishing reliable indices by which it could be recognized, (c) study the parasitism and virulence of the pathogen and its possible influence on the incidence of bulb-rot, (d) determine the effect of temperature variations on the growth of the organism and the degree of severity of pink-root, and (e) investigate the host range of the pathogen, and its prevalence in the soils of Colorado.
PERTINENT LITERATURE

Taubenhaus and Johnson (41) first reported the disease known as pink-root of onions in 1917. The malady was observed first, according to Taubenhaus (42), by F.W. Mally in 1915 in Webb County, Texas. In his first report, in which no etiological agent is named, Taubenhaus (41) described the symptoms of the disease as follows, "The roots of the affected sets in the seed bed or the plants in the field turn pink in color, then shrivel and die." In 1919, Taubenhaus (42) announced that the causal agent of pink-root was Fusarium mali n. sp. (F. solani (Mart.) App. et Wr.) (49). Taubenhaus and Mally in 1921, (43), affirmed F. mali as the causal agent of pink-root of onions.

In 1924, Sideris (39) working with material collected in California, reported that he had isolated the following 11 species of Fusarium from diseased onion roots: F. oxysporum Schlecht., F. mali Taub. (F. solani (Mart.) App. et Wr.) (49), F. redolens Wr., F. lutulatum Sherb. (F. vasinfectum Atk. v. lutulatum (Sherb. ut sp.) Wr.), F. radicicola Wr. (F. javanicum Koord. v. radicicola Wr.), F. martii App. et Wr. (F. solani v. martii (App. et Wr.) Wr.), F. moniliforme Sheld., F. orthoceras App. et Wr. v. triseptatum Wr. (F. orthoceras App. et Wr.), F. angustum Sherb., F. discolor App.
et Wr. (F. sambucinum Fuck.), and F. culmorum (W. G. Sm.) Sacc. Isolates at this time believed by Sideris to be new species were as follows: F. cromyophthoron, F. rhizochromatistes, F. rhizochromatistes v. microselerotium, F. lonchoras, F. lonchoras v. microsporon, and F. sclerostromaton. All the above "new species" except the last named, are now considered by Wollenweber and Reinking (49) to have been isolates of F. bulbigenum Che. et Mass. Fusarium sclerostromaton is considered to be identical with F. angustum Sherb.

On the etiology of pink-root Sideris states,

What is understood by specificity, in the case of certain Fusarium diseases, does not seem to apply in this particular disease. Species which have been reported by previous investigators as being pathogenic on plants such as potato, pepper, pea and tomato have also been found to occur in diseased onion roots, and in the case of F. rhizochromatistes, to produce pink-root symptoms on two or three different plants.

In 1926, Hansen (16), reported that previous workers had been in error in assigning the cause of pink-root to either a single species or to several species of Fusarium. He observed that if isolations were made from turgid pink roots which had been treated previously with a 0.2 percent solution of bichloride of mercury for three minutes and subsequently rinsed in water blanks prior to agar planting, an organism was obtained which belonged to the genus Phoma. This fungus when introduced into previously steamed soil in which onion bulbs were set, was found capable of inducing severe pink-root.

Early in 1929 in his published thesis, Sideris (40) reported that although Fusarium cromyophthoron n. sp. (F.
bulbigenum Cke. et Mass.) was the principal etiological agent of pink-root, some of the species of Fusarium previously reported by him (39), were also capable of producing pink-root symptoms. (The actual work done in this study had been completed prior to 1926, which was the date of Hansen's first publication). Sideris mentioned in this report that he had isolated a species of Phoma, but assigned no role to the organism as a pink-root-producing agent.

Hansen (17) in 1929 announced that pink-root of onions was caused by a new species of Phoma, which he called Phoma terrestris. He reported further that although none of the species of Fusarium obtained from either 'aubenhaus or Sideris were able to induce pink-root, his isolate (Phoma) invariably produced the disease in its most severe form. Further, in regard to Sideris' (40) report pertaining to the isolation of a species of Phoma, Hansen stated, "The writer was unable to give credit in his article for this find, since Phoma is not mentioned in the thesis (Sideris' Doctorate) deposited in the University of California library." In 1930, Hansen (18) reported that he had isolated Phoma terrestris from onion pink-root specimens from Arkansas, California, Colorado, Louisiana, Massachusetts, New York, and Texas.

Matzulevitch (27) in 1932 reported that pink-root in Russia was caused by Fusarium cepae (?), and DuPlessis (11) in 1934, working in South Africa, stated that both F. cepae (?) and Phoma terrestris Hansen were equally capable of inducing the disease.
On the other hand, the work of the Iowa investigators, Melhuus and Henderson (28) in 1932, and Davis and Henderson (8) in 1937, supported Hansen's contention that *Phoma terrestris* is the causal agent of pink-root, and that species of *Fusarium* play a secondary role.
MATERIALS AND METHODS

Technique of Isolation

In obtaining cultures of the organisms involved in this study, primarily those of the pink-root pathogen, isolations were made following two general methods. Agar plantings were made either directly from the roots of diseased field plants, or from the roots of plants grown under controlled conditions (greenhouse), in field soil known to be infested with the causal agent. This latter method consisted of obtaining a soil sample taken to a depth of from six to eight inches from spots in which diseased plants were growing, instead of attempting to isolate from the roots of these plants. Samples were then placed in four-inch pots in the greenhouse which were planted with treated seed or set bulbs. The roots of growing plants were then examined at biweekly intervals, and isolations were made as soon as the faintest pink coloration appeared in the roots. For want of a better name, this last described method will be referred to as the trap plant technique.

In either case, roots used for isolation purposes were washed and rinsed in tapwater, treated for from two to four minutes in a 0.2 percent solution of bichloride of mercury, re-rinsed several times in sterile water blanks, and finally dried
on pieces of previously sterilized paper toweling.

Studies of Infection

Onion seeds and bulbs (sources and varieties).

The standard variety used in this work was the Yellow globe danvers onion. Other types utilized in the study were Silverskin, Red wethersfield, Mountain danvers, Southport white globe, Bermuda, Red globe and Sweet spanish.

Seed was obtained from the Iowa State Seed Laboratory, the Colorado State Seed Laboratory and local (Colorado) firms. Sets and mature bulbs were also obtained from the latter.

Treatment of Seeds and Bulbs

Prior to planting, seeds or bulbs were treated for 15 minutes in a 0.2 percent solution of bichloride of mercury, then thoroughly rinsed in tapwater and sterile water blanks, and finally dried on sterile paper toweling. The efficacy of the seed treatment was tested by transferring treated seed to petri dishes containing sterile nutrient agar.

Treatment of Soil

The standard soil mixture, for studies of infection, was prepared by mixing four parts of sifted black loam to one part of clean sand, to one part of humus. In order to effect
sterilization, soil samples were moistened and either steamed in gallon earthenware jars for two hours at 20 pounds pressure or in four-inch clay pots for 24 hours at from five to seven pounds pressure. In cases where the soil was added to the pots after steaming, these containers had been previously steamed at 15 pounds for 15 minutes.

Infestation of Soil

Soil was infested with the organism to be studied, by adding several grams of a ground four-to six-week-old giant culture to the contents of a four-inch clay pot, and thoroughly mixing the inoculum into the soil of the upper half of the pot.
THE CAUSAL AGENT

Isolation of the Pink-Root Pathogen

At the outset of this study, isolations were made from three types of abnormal roots: (a) roots appearing translucent in spots (believed to be the "dull lead color" reported by Davis and Henderson [8]), (b) roots yellow to amber-brown in color, and (c) roots showing varying degrees of pinkening to reddening.

By the use of the standard technique of isolation, almost all isolations from types a and b yielded either bacterial colonies or species of Fusarium. These results were also obtained for type c, however, occasionally an organism was obtained which appeared to be morphologically identical with a culture of Phoma terrestris obtained from Dr. G. N. Davis.

Since it was suspected that this organism was present in the majority of diseased roots, especially in those showing reddening, a modification of Hansen's technique of isolation (16) was adopted. Roots still turgid but showing faint discolorations were immersed in a 0.2 percent solution of bichloride of mercury for three minutes, rinsed well in sterile water blanks, and pressed between pieces of sterile toweling to remove most of the excess moisture. By the use of this method, the same Phoma-like
fungus was obtained, frequently from pink to red roots, seldom from yellow roots, and only in a few instances from translucent roots.

In order to investigate the possible pathogenicity of the organisms obtained, the predominant Fusarial types and a Phoma-like organism were grown in separate oat giant cultures in quart milk bottles for 40 days. At the end of this time, each fungus was introduced into a steamed soil mixture in steamed four-inch pots. The soils were then planted with treated Yellow globe danvers onion seed and set bulbs. Observations were made 50 days after planting, and it was found, as noted by Hansen (16,17), and Davis and Henderson (8), that only the Phoma-like fungus was capable of discoloring and subsequently destroying the roots. Although two of the isolates of Fusarium were found to be capable of inducing a severe damping-off of onion seedlings, they were incapable of attacking the roots of set bulbs. Re-isolations from pink roots yielded fungal colonies which were morphologically identical with the Phoma-like organisms used in the original inoculation.

Repeated trials showed that apparently there was no correlation between the appearance of the amber-yellow or translucent root symptom, and the presence of any one of the fungi under test. However, pink or red root discolorations, irrespective of how faint, were found to be associated with invasion by Phoma-like isolates.
Identification of the Phoma-like Isolates

Curiously enough, only one sporulating strain of the fungus causing pink-root was isolated within a four-year period. This culture produced small, dark, sparse, subglobose, carbonaceous pycnidia 200-275 µ in diameter, which when crushed released numerous small oblong-ovoid non-septate biguttulate conidia of the type generally attributed to the genus Phoma. The sporulating isolate studied, compared favorably with Hansen's description of *Phoma terrestris* (17), which is given as follows:

Pycnidia subglobose, ostiolar, papillate, dark brown to black, carbonaceous, 170 to 350 µ may occur singly, frequently gregarious. Conidia continuous, hyaline, oblong-ovoid, 4.5-5.5x1.3-2.3 µ biguttulate, sessile in pycnidium, escaping as a gelatinous mass through ruptures, rarely as a gelatinous cirrus through the ostiole.

In addition, all non-sporulating isolates on hand appeared to be morphologically and pathologically identical with a culture of *Phoma terrestris* which had been previously identified by Dr. H. N. Hansen. For this reason, all such pink-root producing fungi were considered as isolates of *Phoma terrestris* Han.

Morphology of the Causal Agent

The pink-root organism on hard potato dextrose agar, Czapek’s or other media sufficiently rich in sugars, grew slowly, producing a dense, compact, somewhat depressed mycelial mat, requiring about 10 days at optimum temperatures to produce a colony five centimeters in diameter. On potato agar, colonies
were almost white, pale rhodonite pink (32), light purple-grey to darker. Few colonies were completely concolorous, and secor-
ing was a commonly observed phenomenon.

When viewed from the under side of the plate the colonies appeared pansy purple, violet carmine, amaranth purple, dusky auricula purple, to almost a heliotrope slate. Pigmentation was almost entirely confined to the mycelium of the fungus.

Growth on cornmeal agar resulted in a markedly appressed colony, which scarcely showed any aerial mycelium. In addition, pigmentation tended toward redder hues than that observed in media richer in dextrose.

Mycelium.

The hyphae of the fungus were septate, hyaline, anastomos-
ing, and frequently occurred in multifilamentous, rope-like rhizomorphic strands. As a rule, the hyphae were fine, averag-
ing approximately 2μ in diameter, although variations of 0.8-
5μ frequently occurred. Occasionally individual hyphal cells became spherical, attaining a diameter of 8μ, as shown in figure 1. Hyphal cells usually were markedly guttulate, freeing several small oil-like globules when ruptured. The contents of these cells usually revealed a pink coloration. Such chromo-
genic hyphae were characteristically submerged below the sub-
stratum.

Pycnidal primordia.

These bodies were first seen by Hansen (17) who described
Figure 1. The vegetative characters of the pink-root pathogen. From three-month-old stock cultures growing on potato dextrose agar.
A. Mycelial filaments. Greatest diameter 2.5μ; least diameter 1.0μ.
B. Pycnial primordium.
C. Size variation in hyphal segments. Largest cell shown, diameter 8.0μ; smallest hyphal diameter 0.8μ.
D. Pycnial primordium, length 38.7μ.
their occurrence in onion root tissues as follows:

In certain cells sometimes close together, sometimes far apart, the mycelium accumulates, swells into various shapes, and turns dark in color. This phenomenon is the beginning of pycnidium formation and, though it may be observed in all parts of the root it is seen more frequently in the epidermal cells.

Hansen also observed these structures developing in plates of cornmeal agar. In the present study these structures were found constantly in the cortical tissues of infected roots, as shown in figure 2. In addition, they appeared almost invariably in 30- to 60-day-old stock cultures growing on potato dextrose agar, and were recognized in test tube culture by the appearance of darkening fringes at the edge of the colony. Their formation, at least in culture, was due not strictly to "mycelium accumulation", but rather to enlargement and division of certain hyphal cells, which were accompanied by a slight though noticeable cell wall thickening, and the formation of a light olive-brown pigment within the guttulate protoplast. The result was a darkened conglomerate mass composed of swollen multiguttulate cells as illustrated in figures 1 and 2. The size of these bodies is quite variable, ranging from 5-70 x 25-200 μ).

Microscopic examinations of pinkened onion roots obtained from naturally and artificially infected plants revealed the invariable presence of pycnidial primordia. Such observations have been verified by isolations. In addition, these bodies have been found in yellow to amber-yellow roots.

In order to obtain some indication as to the ability of any
Figure 2. The pycnidial primordia of *Phoma terrestris* in the cortical cells of a 40-day-old onion root.
given isolate to produce pycnidial primordia in culture as well as in the tissues of the host, 13 stock cultures of Phoma which had been isolated on separate occasions were transferred to liter flasks containing steamed wheat, and were then incubated at 25°C for three weeks. At the same time, the cultures were transferred to fresh tubes containing a two percent potato dextrose agar. The giant cultures were allowed to grow for a period of three weeks, after which time 10 grams of wheat inoculum of each isolate was introduced into a separate four-inch pot containing steamed soil. Treated Yellow globe danvers onion seed was planted in the infested soil. The roots of onion seedlings were examined in four weeks for the presence of primordia, and the tubes were examined at the end of 30- and 60-day periods. The results of this study are shown in table 1.

The data in table 1 show that primordium formation is constant for the fungus irrespective of whether the organism is developing in culture or in the root tissues of the onion. All isolates tested produced these bodies in the host tissue, although there was some variation as to the formation of these structures in culture. Isolate 34A was cultured from the roots of plants grown from non-treated seed, in pots and soil which had been steamed for four hours at 20 pounds pressure. This culture was the only pycnidial-forming isolate obtained during the course of the study.

Microscopic examinations showed that the primordia in
Table 1. Formation of pycnidial primordia in tube culture and in the tissue of the host.

<table>
<thead>
<tr>
<th>Culture number</th>
<th>Source of isolate</th>
<th>Presence of Tube culture primordia in cultures showing root cells of ing primordia</th>
<th>Plants grown in infested soil-28 days</th>
<th>Plants grown in infested soil-60 days</th>
<th>Plants grown in infested soil-90 days</th>
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<tr>
<td>1</td>
<td>Clear Lake, Iowa, 1935</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>&quot;</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>&quot;</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
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<tr>
<td>9</td>
<td>&quot;</td>
<td>+</td>
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<td>&quot;</td>
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</tr>
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<td>16</td>
<td>&quot;</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>California (Hansen)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>27A</td>
<td>Rocky Ford, Colo. 1936</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>29A</td>
<td>Greeley, Colo. 1936</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>50A</td>
<td>Littleton, Colo. 1936</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>54A</td>
<td>Seed borne 1936 (?) (Colorado seed)</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>53A</td>
<td>Brighton, Colo. 1937</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>58A</td>
<td>Littleton, Colo. 1937</td>
<td>+</td>
<td>-</td>
<td>+</td>
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</tbody>
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culture were identical morphologically, even to cell pigmentation and guttulation, with those found in the tissues of the onion root. No other fungus to the best of the present knowledge, produces such bodies in the tissues of the onion root. The presence of primordia in the tissues, then, appears to be a definite indication of Phoma invasion.

**Attempts to induce sporulation.**

As soon as isolates of Phoma were recognized, they were transferred to petri dishes containing either one or two percent cornmeal agars. This is the method recommended by Hansen (17) to induce pyenidial formation. In all cases except one, such attempts resulted in failure. In the instance named, a few pyenidia formed in the colonies in the original dishes prior to transfer. This was the only case in which sporulation occurred, the medium used in this instance being the standard hard potato dextrose agar used for isolation purposes throughout the course of the work. One mycelial transfer was all that was necessary to bring about complete loss of the pyenidial-forming-capacity of the isolate.

In attempting to induce cultures to sporulate, a number of methods were used. Cultures were grown for two to four month periods on sterile onion leaves and roots, hard potato dextrose agar, either 1 or 2 percent yellow and white, strained and unstrained cornmeal agars; oatmeal agar, sugar-beet agar, peptone-beef broth, water agar (non-nutrient), prune juice
extract, Czapek's agar, and beanmeal agar. In addition, cultures were grown on 1, 2, or 5 percent cornmeal agars for eight months. Further, the pathogen was allowed to grow for months on the outer scales of onion bulbs and on decaying roots. Isolates of the fungus obtained from different localities were grown opposite one another in petri dishes containing cornmeal or hard potato dextrose agars. In addition, cultures growing on cornmeal or potato dextrose agars were dried and wetted alternately for several weeks. Isolates were exposed to bright sunlight, diffused light, and darkness for varying periods. The fungus was grown on 1 or 2 percent cornmeal, or 2 percent potato dextrose agars held at 10, 20 and 30°C. for intervals of one to four months. Finally, isolates were introduced into steamed soil in which onions were growing, recovered from the ensuing infected root tissues, grown in culture, and then reintroduced into the soil. This process was repeated in order to constitute from two to four passages through the tissues of the host.

Since all of the above outlined methods gave negative results in regard to sporulation, it is considered that there are non-pycnidal-forming strains of Phoma terrestris, and that consequently, the so-called pycnidal primordia which appear as constant features of these isolates, might be considered as vestigial structures.
Physiology of the Causal Agent

The pigment of the pathogen.

Early in the study of the pink-root disease, a phenomenon was observed which had escaped the notice of the earlier investigators. It was found that onion roots growing in soils infested with Phoma, occasionally failed to reveal the pigmentation characteristic of pink-root, except in some portions of certain turgid and apparently healthy roots. In these last named instances, the roots revealed a faint rhodonite pink (32) coloration which was detected only after a minute examination. Dead and dying roots instead of appearing rhodonite pink to spinel red as expected, were yellow to yellow-brown. A microscopic examination of such tissues revealed the presence of pyenidial primordia, which as has been pointed out previously, are characteristically formed in tissues invaded by Phoma. Isolations from such tissues yielded colonies of the fungus. Diseased roots showing yellow discolorations were more frequently found in infested field soils of Iowa than in soils of Colorado which harbored the pink-root pathogen. Further, where the red-pink pigmentation was present, the color of the diseased roots in the latter was deeper and noticeably more pronounced that in cases of the former. Since certain lots of roots infected with Phoma terrestris did not show a pink to red coloration, it is evident that the absence of this pigmentation may not be a de-
pendable negative criterion.

It was observed early in the investigation that tempera-
ture and moisture variation had little to do with the nature of
the pigment expression. In addition, *P. terrestris* was grown
for several months at a time on all types of standard media
(31), (33), which in some instances were supplied with varying
amounts of sugars and salts, to ascertain whether such nutrient
variation would affect pigmentation. Although the intensity
of the typical red-purple cultural coloration fluctuated some-
what with the medium used, it was soon evident that a study
of the role of the nutrient factors involved, would not solve
the question. In this particular study, however, it was ob-
served that a standard acid medium such as prune agar, effect-
ively prevented the manifestation of a red-purple color. Since
this observation appeared to parallel the phenomenon previously
observed in diseased roots, a study was conducted into the nature
of the influence of the hydrogen-ion concentration on the mani-
festation of color by the pink-root fungus.

The relation of hydrogen-ion concentration to the expres-
sion of characteristic pink-root pigmentation. It has been
known for a considerable length of time that fungi produce a
great variety of pigments, some of which can be used as indi-
cators of hydrogen-ion concentration, or of oxidation-reduction
equilibria (25). Color changes in fungal pigments especially
in species of Fusarium, induced by the change of pH in the cul-
"The phenomenon in the root showed a more rapid response than that in the hypophyseal bed. Changes in other organs were observed. At pH 4-5 the color changed from yellow-brown to brown.

A color change was observed at pH 0.1. 分子 weight drops of 0.1 ml. of distilled water, each containing 50 ml. of water, were placed in each of three 200 ml. beakers. Several agar slabs and dried roots were placed in each of the beakers and dried roots were placed in each of the beakers to prepare a surface where the fungus had been growing. Several plates of potato starch were prepared. The experiment was performed to determine whether the reaction of the hydrogen ion was unaffected.

In order to determine whether the reaction of the hydrogen ion was unaffected, a series of preliminary titrations was conducted on the samples prepared for the experiment with the buffer solution (as a source of hydrogen ions). The concentration of the solution was determined by the pH measurement.

The only treatment in the literature to date on this phrase is that of Kurtz and Mollenweider and Matthew's (25) work. The medium was mentioned by Lockett and Moyer (26)."
Neutralizing the solutions and bringing them into the alkaline range by the addition of 0.1 N NaOH restored the original color hues at approximately pH 8.5.

The extraction of the pigment and further studies on the influence of the H-ion concentration on manifestation of color. In the belief that a more accurate study of the influence of the H-ion concentration on the manifestation of color would be possible if the pigment were to be obtained in solution, steps were taken to extract the coloring substance. In this study, mats of the fungus which had been growing on potato dextrose agar for several weeks were used for extraction purposes. Negative results were obtained using the following solvents and solutions at room temperatures and heated to boiling: acetone, ethyl alcohol (95%), ether, chloroform, KNO₃ (1%), NaNO₃ (1%), KNO₂ (1%), NH₄Cl (1%), HCl (1%), NaCl (1%), 0.02 N HCl, 0.02 N NaOH, 0.1 N NaOH, 0.1 N HCl and water. These trials were next repeated using infected roots as a source of material. As before, the results obtained were negative. In this connection, it is interesting to note that Sideris (40) found that by merely wetting the pinkened root tissue he was able to extract the pigment. This is further evidence that Sideris was not dealing with the pigment produced by *Phoma terrestris*.

Successful pigment extractions were obtained only by treating the fungal mats with 0.1 N HCl, allowing the material to remain for 15 minutes to several hours in the acid, then decanting
this solution and adding 0.1 N NaOH. With the addition of the acid solution, the pigmented material underwent the characteristic color shift of pansy purple to chestnut, and with the subsequent removal of the acid and the addition of the alkaline solution, the reverse color shift took place, with the immediate indications of pigment extraction. Allowing the material to remain in the solution for several hours gave the solution a deep pansy purple coloration. These trials were repeated several times with the same result.

Portions of the colored extract were next treated with various substances in an attempt to remove or precipitate the pigment from solution. Success was attained only by the use of a saturated Na₂SO₄ solution, which when added to a small amount of the dissolved pigment, flocculated the coloring material, separating it from its solution after an interval of from 15 minutes to an hour. The flocculate was next removed by filtration, and was washed thoroughly with distilled water. All the solvents which were originally used in the first attempt to extract the pigment, were reemployed in an effort to redissolve the flocculated pigment. All such attempts resulted in failure.

In order to obtain a more accurate concept of the color shift of the pigment as affected by pH, 50 ml. portions of the original deeply colored alkaline extract were set aside in 250 ml. beakers. Holding several samples as controls, the contents of the four beakers were treated with drops of a 0.1 N HCl solution until a definite color change was observed (toward the
yellow-brown or chestnut shades). The point at which the color shift took place was relatively sharp. Two of the beakers were then put aside and the contents of the other two were treated with drops of a 0.1 N NaOH solution, until the original pansy purple color gave indication of returning. The pH's of the solutions in the beakers were then obtained by use of the Beckman Acidimeter (glass electrode) with the following results:

<table>
<thead>
<tr>
<th>Sample</th>
<th>Color Changing Information</th>
<th>pH Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample 1</td>
<td>Color changing to yellow-brown</td>
<td>7.00</td>
</tr>
<tr>
<td>Sample 2</td>
<td>Original pansy purple color returning</td>
<td>7.05</td>
</tr>
<tr>
<td>Sample 3</td>
<td>Original pansy purple color returning</td>
<td>7.86</td>
</tr>
<tr>
<td>Sample 4</td>
<td>Original pansy purple color returning</td>
<td>7.70</td>
</tr>
</tbody>
</table>

From these results it is apparent that a more sensitive test is obtained when the pigment is in an extracted solution rather than in the hyphae of the fungus. Subsequent trials indicated, that as the hydrogen-ion concentration dropped below pH 7, the color of the solutions approached a light amber-yellow. Raising the pH to 8 and above, gave a complete return of the original pansy purple coloration identical with the color of the controls.

The chemical nature of the pigment. Using the washed, flocculated pigment, numerous tests were made to ascertain the chemical nature of the coloring material.

In a series of qualitative tests conducted, i.e., the biuret reaction, the Millon reaction, Liebermann's reaction, the Adamkiewics reaction, the Acree-Rosenheim reaction, and the Molisch test, only the latter gave positive results. All of
We have noted that some phenomena in this paper (20) reported variation in the reaction of methionine and

appearance to be a common observed phenomenon. Characteristics and

variation in pathogenesis of trophozoites of a given parasite

The variation in pathogenesis of trophozoites of the protozoa

read out to determine the effect of the color of the enzyme
 complexes in the nature of a chemical test. It was

interpreted to indicate that the best part of the pigment
 release of the pigment by the enzyme emulsion. Such evidence may

emulsion. This was considered to be fairly evidence of hydro-

emulsion was found in the control solutions, which contained only the

solution and contained the recognized pigment. In such evidence

presence of reduced sugars in small amounts in the solutions

reduced was back in solution. Pending the test indicated that

after an interval of 30 minutes at the end of 1 hour at

reaction would be observed. The emulsion solution

after some study it was found that if the dried pigment

consideration.

possible existence of such enzymes cannot be excluded from

of the emulsion tested are available. For this reason, the

ages cannot be related upon, especially where only small amounts

it is recognized that quantitative color tests for protein link-

whereas the colorimetric test is specific for carboxylating enzymes (14).

these reactions except that last are specific for proteins.
lates of species of Rhizoctonia. Hansen (18) working with a pycnidium-forming isolate of *Phoma terrestris*, reported that cultures grown from single spores, varied in pathogenicity on seedlings. In addition, further changes in the pathogenic nature of an organism after having been retained in culture for a period of time has been reported frequently (4) (13) (22).

In the course of the work, studies on the pathogenicity of *Phoma terrestris* to onion plants revealed that isolates from certain localities appeared to differ in virulence when tested under uniform conditions just subsequent to their isolation. Further, trials conducted at varying intervals over a period of time, indicated that no one isolate was stable in its virulence. All isolates reported in this study were retained in tubes of two percent potato dextrose agar, were transferred at the same time to tubes of nutrient agar prepared from the same lot of media, and kept under the same conditions. In no case was any culture subjected to passage through its host's tissues.

In each separate trial, the plants were grown in a steamed, uniform soil-mixture in four-inch clay pots. Infestation was effected by the addition of eight grams of a four-week-old wheat giant culture of Phoma to the soil in each pot. These studies were made under comparable conditions, using the same variety of onion (*Yellow globe danvers*). Observations were made 50 to 70 days after planting. The data are presented in table 2.
Table 2. Differences in pathogenicity between isolates of Phoma terrestris.

<table>
<thead>
<tr>
<th>Trial</th>
<th>Date</th>
<th>Isolate, Source of culture and date</th>
<th>Plants showing percentage of number</th>
<th>date of isolation</th>
<th>plants showing surviving plants - root</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>8/19/35</td>
<td>Clear Lake, Iowa 1935</td>
<td>-</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>10</td>
<td></td>
<td>&quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>-</td>
<td>55.5</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td></td>
<td>&quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>-</td>
<td>55.8</td>
<td></td>
</tr>
<tr>
<td>12</td>
<td></td>
<td>&quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>-</td>
<td>35.4</td>
<td></td>
</tr>
<tr>
<td>13</td>
<td></td>
<td>&quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>-</td>
<td>4.0</td>
<td></td>
</tr>
<tr>
<td>14</td>
<td></td>
<td>St. Ansgar, Iowa 1934</td>
<td>-</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>15</td>
<td></td>
<td>Clear Lake, Iowa 1935</td>
<td>-</td>
<td>15.9</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>&quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>-</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>17</td>
<td></td>
<td>&quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>-</td>
<td>1.3</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>California, 1932</td>
<td>-</td>
<td>22.0</td>
<td></td>
</tr>
<tr>
<td>19</td>
<td></td>
<td>Clear Lake, Iowa 1935</td>
<td>-</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td>&quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>-</td>
<td>20.9</td>
<td></td>
</tr>
<tr>
<td>21</td>
<td></td>
<td>California, 1932</td>
<td>-</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>22</td>
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<td>&quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>-</td>
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</tr>
<tr>
<td>23</td>
<td></td>
<td>&quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>-</td>
<td>65.0</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>1/30/37</td>
<td>Clear Lake, Iowa 1935</td>
<td>38.0</td>
<td>12.2</td>
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<td>30.6</td>
<td>22.8</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td></td>
<td>California, 1932</td>
<td>24.3</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>27A</td>
<td></td>
<td>Rocky Ford, Colo. 1936</td>
<td>33.6</td>
<td>45.5</td>
<td></td>
</tr>
<tr>
<td>29A</td>
<td></td>
<td>Greeley, Colo. 1936</td>
<td>4.6</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>30A</td>
<td></td>
<td>Littleton, Colo. 1936</td>
<td>51.3</td>
<td>34.0</td>
<td></td>
</tr>
<tr>
<td>Control</td>
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<td></td>
<td></td>
<td>48.6</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>10/6/37</td>
<td>Clear Lake, Iowa 1935</td>
<td>39.0</td>
<td>18.0</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td></td>
<td>&quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>45.0</td>
<td>86.0</td>
<td></td>
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<tr>
<td>17</td>
<td></td>
<td>&quot; &quot; &quot; &quot; &quot; &quot;</td>
<td>52.0</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td>27A</td>
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<td>Rocky Ford, Colo. 1936</td>
<td>34.0</td>
<td>0.9</td>
<td></td>
</tr>
<tr>
<td>30A</td>
<td></td>
<td>Littleton, Colo. 1936</td>
<td>24.0</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td>55.6</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>10/7/37</td>
<td>Greeley, Colo. 1936</td>
<td>17.0</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>53A</td>
<td></td>
<td>Brighton, Colo. 1937</td>
<td>20.0</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>59A</td>
<td></td>
<td>Littleton, Colo. 1937</td>
<td>24.5</td>
<td>27.0</td>
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</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td>33.5</td>
<td></td>
</tr>
</tbody>
</table>
Table 2. Concluded.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Source of culture and year</th>
<th>10/27/37 Date of isolation</th>
<th>Percentage of surviving plants showing pink-root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clear Lake, Iowa 1935</td>
<td>18.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>22.0</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>18.5</td>
<td>11.1</td>
<td></td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>47.0</td>
<td>46.8</td>
<td></td>
</tr>
<tr>
<td>&quot; &quot;</td>
<td>41.5</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>California, 1932</td>
<td>45.0</td>
<td>2.5</td>
<td></td>
</tr>
<tr>
<td>Littleton, Colo. 1936</td>
<td>42.0</td>
<td>4.7</td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>41.2</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

*Calculated as a percentage of the number of seed planted.

A study of table 2 reveals that isolates varied from a complete lack of virulence to a condition where all of the plants were severely infected. The results obtained in trial "B", as illustrated with representative sample containers, are shown in figure 3.

In order to obtain information pertaining to the variability of any one isolate, a compilation of the data from the trials shown in table 2 and certain other comparable studies, are given in table 3.

Table 3 indicates that there is no constancy in the degree of virulence shown. Pathogenicity appears to vary from interval to interval, and curiously enough, the expected phenomenon of loss of pathogenicity, as illustrated by cultures 14 and 27A, does not appear to be a general occurrence. Instead, fluctuations such as those illustrated by cultures 1 and 10, and increases in virulence, as shown by isolates 15 and 16,
Figure 3. The differences in pathogenicity between certain isolates of *Phoma terrestris*. No. 1, control; No. 2, isolate 30A (Colorado); No. 3, isolate 10 (Iowa); No. 4, isolate 18 (Iowa); No. 5, isolate 27A (Colorado); No. 6, isolate 1 (Iowa); and No. 7, isolate 29A (Colorado). Note the extreme thinning effect due to *Phoma* No. 29A (isolated 11/11/36 from Greeley, Colorado material), as contrasted with *Phoma* No. 30A (isolated 11/12/36 from Littleton, Colorado material).
<table>
<thead>
<tr>
<th>No.</th>
<th>Date of isolation</th>
<th>Experiment</th>
<th>Type of soil used</th>
<th>Percentage of diseased plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Iowa 7/15/35</td>
<td>Iowa peat</td>
<td>Iowa peat</td>
<td>0.0</td>
</tr>
<tr>
<td>10</td>
<td>Iowa 5/3/35</td>
<td>Iowa peat</td>
<td>Iowa mixture</td>
<td>0.0</td>
</tr>
<tr>
<td>15</td>
<td>Iowa 6/10/35</td>
<td>Iowa peat</td>
<td>Iowa mixture</td>
<td>0.0</td>
</tr>
<tr>
<td>14</td>
<td>Iowa 1934</td>
<td>Iowa peat</td>
<td>Iowa mixture</td>
<td>0.0</td>
</tr>
<tr>
<td>15</td>
<td>Iowa 7/15/35</td>
<td>Iowa peat</td>
<td>Iowa mixture</td>
<td>0.0</td>
</tr>
<tr>
<td>16</td>
<td>Iowa 6/10/35</td>
<td>Iowa peat</td>
<td>Iowa mixture</td>
<td>0.0</td>
</tr>
<tr>
<td>18</td>
<td>Calif. 1932</td>
<td>Iowa peat</td>
<td>Iowa mixture</td>
<td>0.0</td>
</tr>
<tr>
<td>27A</td>
<td>Colo. 8/28/36</td>
<td>Iowa peat</td>
<td>Iowa mixture</td>
<td>0.0</td>
</tr>
<tr>
<td>29A</td>
<td>Colo. 11/11/36</td>
<td>Iowa peat</td>
<td>Iowa mixture</td>
<td>0.0</td>
</tr>
<tr>
<td>30A</td>
<td>Colo. 11/11/36</td>
<td>Iowa peat</td>
<td>Iowa mixture</td>
<td>0.0</td>
</tr>
<tr>
<td>27A</td>
<td>Colo. 8/28/36</td>
<td>Iowa peat</td>
<td>Iowa mixture</td>
<td>0.0</td>
</tr>
<tr>
<td>29A</td>
<td>Colo. 11/11/36</td>
<td>Iowa peat</td>
<td>Iowa mixture</td>
<td>0.0</td>
</tr>
<tr>
<td>30A</td>
<td>Colo. 11/11/36</td>
<td>Iowa peat</td>
<td>Iowa mixture</td>
<td>0.0</td>
</tr>
</tbody>
</table>
present a situation difficult to explain.

According to Hansen (18), Phoma terrestris produces numerous saltations in culture. Such phenomena were frequently observed in this study. However, Hansen reported that segregation (?) apparently occurred during the formation of conidia, since mycelial transfers gave cultures of constant character. He reported further, that variants produced from conidia differed pathologically as well as morphologically. Since the cultures used in this study were typically non-pycnidal forming, only mycelial transfers were possible. It appears, therefore, that Hansen may be incorrect in his assumption that cultures obtained from mycelial transfers are constant in character.
SYMPTOMS

As Expressed on Roots and Aerial Leaves

Plants suffering root attack by *Phoma terrestris* showed a stunting of the aerial portions, and an abnormal amount of withering and dying of the leaf tips. In addition to these symptoms, leaf flaccidness and general yellowing were common. (See figures 4 and 5).

According to Davis and Henderson (8), the first symptom on the root was the change from the normal whiteness to a dull lead color; the reddening of the roots not occurring until they lost turgidity and shriveled. This is not in accord with the results obtained in the present study. Roots just invaded by *P. terrestris* remained turgid, exhibiting a faint and barely discernible pale rhodonite pink, which later became spinel red, and occasionally exhibited almost as dark a hue as an amaranth purple (32). Invaded roots soon became flaccid and dried, remaining in the soil as reddened and shriveled structures, as illustrated in figure 5.

The so-called "nipped" stem symptom as stressed by Taubenhaus and Mally (43), and later questioned by Hansen (17), did not appear to be a constant feature of the disease. As a rule the bulbs of diseased plants were small and "bottle necked", as shown in figure 5.
Figure 4. Symptoms of the pink-root disease. The two plants on the left were grown in steamed soil infested with *Phoma terrestris*. The plants on the right were grown in steamed soil in which the pathogen had not been introduced. Note the darkened shriveled roots and the withered leaves of the stunted plants.
Figure 5. Pink-root symptoms on the Yellow Spanish onion. These specimens were taken at harvest from a field in which the crop was commercially valueless. Note the failure to form normal bulbs, the flaccid aerial leaves, the discolored and shriveled roots, and the absence of the so-called "nipped" stem.
As Expressed on Bulbs

According to Hansen (17), and Davis and Henderson (8), the pink-root organism does not advance from the roots into the interior of the onion bulb. Although *Phoma terrestris* was neither isolated from the interior of the bulb nor the stem of naturally infected plants, it was possible to obtain the organism from the outer surface of the stem of artificially infected plants. In addition, the fungus frequently has been isolated from the outermost scales of both artificially and naturally infected onion bulbs. As a rule, isolations from the interior of the stem or the onion bulb, yielded only colonies of *Fusarium* spp. In addition, microscopic examinations of such tissues failed to reveal any mycelium characteristic of *Phoma*.

**Invasion of the outer bulb scales**

In growing almost any white set or mature onion bulbs, such as the Silverskin or Southport white globe varieties, in soil previously infested with the pink-root fungus, it was observed that such bulbs characteristically showed pink to purple-red outer scale blotchings seven to 21 days following planting, as shown in figure 6. Bulbs grown in non-infested soils did not show this condition. Microscopic examinations of portions of such discolored tissue revealed the presence of fine intricately branching hyphae, as shown in figure 7, and isolations from these
Figure 6. Southport white globe onion bulbs. The two on the right were grown in soil infested with the pink-root pathogen, while the bulb on the left was grown in non-infested soil. Scale blotch induced by Phoma is apparent on the two bulbs shown at the right.

Figure 7. The invaded outer scale tissue of a Southport white globe onion bulb grown in artificially infested soil. The profuse mycelium of the pink-root organism which has been stained with cotton blue, may be seen in the cells.
spots yielded the pink-root fungus in the majority of instances. Since any commercial lot of white set or mature onion bulbs will almost invariably show a certain percentage of bulbs revealing such scale discolorations, further investigations were made into the nature of this phenomenon.

The pink-root fungus has been isolated in a few instances from the discolored scales of white onion bulbs taken directly from the field. In addition, a painstaking examination of such affected tissues usually revealed the presence of pycnidial primordia. No invasion from the dead outer scales into the living turgid leaf tissue of the bulb was ever observed, and for this reason, it may be inferred that the fungus is acting in a purely saprophytic role.

Fungi mentioned in the literature as being capable of attacking the scales of onion bulbs producing slightly blemishes, are *Macrosporium porri* Ell., *M. parasiticum* Thüm. (44), *Colletotrichum circinans* (Berk.) Voglino (45), and *Phoma alliiicola* Thüm. (34, 45). The last named fungus according to Walker (45), "Produces small black pycnidia which are often difficult to distinguish macroscopically from the stromata of the smudge fungus (*Colletotrichum circinans*)." There is apparently no evidence to the effect that *P. alliiicola* will invade onion roots. In addition, no true pycnidia were found during the present study on scale tissues attacked by *Phoma terrestris*. 
PREVALENCE AND DESTRUCTIVENESS OF PINK-ROOT

The pink-root disease of onions was first observed in Texas in 1917 (41, 42, 43). Since this time, the malady has been found in 20 states. At present, according to the Plant Disease Reporter (1919 to 1937 inclusive), the disease has occurred in California, Oregon, Washington, Utah, Colorado, New Mexico, North Dakota, Wisconsin, Indiana, Iowa, Ohio, Texas, Arkansas, Missouri, Louisiana, Mississippi, North Carolina, New York, Massachusetts, and Connecticut. The losses in these states have been reported as a trace, to crop reductions as high as 50 percent (California).

The disease has been recorded also as occurring outside of the United States. Dickson (9) found pink-root in western Quebec in 1922, and Howitt (21) observed the malady about the same time in the Leamington district of Ontario. In regard to its destructiveness in this area, Howitt stated,

At least 50 acres of onions were affected by the disease this year. Many fields contained large patches of diseased plants, and the growers estimated their losses at not less than 10 per cent of the crop.

Outside of North America, the trouble was observed in Russia by Matsulevitch (27), in 1932, and Samuel (35) noted its occurrence in South Australia in 1930. DuPlessis (11) in 1934, attributed a 30 percent crop loss to pink-root and bulb-rot in South Africa. In addition, Walker (47) recorded the pre-
sence of the disease in 1924 in the Canary Islands.

In the two states, Iowa and Colorado, which are concerned in the present study, the pink-root malady has caused considerable damage in some of the principal onion growing areas. In Iowa, according to Davis and Henderson (8), heavy losses occurred in the Saint Ansgar and Clear Lake districts in 1930, which brought about the abandonment of about 200 acres of valuable onion land. In Colorado, the disease was first observed near Littleton in 1928 by LeClarg (12, 23), and a serious outbreak of the trouble occurred during the following years in the onion fields of the Arkansas River Valley district. During the summer of 1936, at least 50 percent of the onion fields examined in Colorado showed the presence of diseased plants. The most serious outbreak of the malady at this time was observed in the Greeley (Weld County) onion growing district.

The Incidence of Phoma terrestris in Certain Representative Colorado Onion Field Soils

Early in the study of the pink-root disease (during the summers of 1936 and 1937), surveys were made of representative onion fields in Colorado suspected of harboring Phoma terrestris. These surveys covered portions of both the Eastern and Western Slope onion growing regions. In a given field, the roots of numerous plants from random portions of
the field were examined, and where the roots of the plants were obviously diseased, whether they showed the characteristic pink-root coloration or not, representative soil samples were taken. In a few cases samples were obtained from fallowed fields or fields on which a crop other than onions was growing. In such instances information obtained previously from the grower indicated that P. terrestris was present in these soils. All samples were then tested for the presence of Phoma by the previously described trap plant technique.

Table 4 presents a survey which was made during the summer of 1937. This study covered representative areas in the state in which onions were grown. The survey included localities on both the Eastern and Western slopes of the Continental Divide. In the study, all soils tested were placed in four-inch clay pots and planted with treated onion seed (variety Yellow globe danvers). Observations were made 142 days after planting.

Littleton, Rocky Ford, and Greeley are representative Eastern Slope localities, whereas Delta, Montrose and Grand Junction, are on the Western Slope of Colorado. In figure 8 several pots are shown which give a characteristic picture of the results obtained in this study. The differences in degrees of pink-root severity as indicated both by the preceding table and figure 8, may be due in part to differences in degree of soil infestation by Phoma and to differences in degree of pathogenicity.
Table 4. The incidence of *Phoma terrestris* in representative onion fields in Colorado.

<table>
<thead>
<tr>
<th>Locality</th>
<th>Condition of plants</th>
<th>Condition of soil was obtained</th>
<th>No. of pots Isolated from which plants (onions) in field at time samples were taken in previous year or years</th>
<th>Incidence of root rot</th>
<th>Incidence of pink rot of root</th>
<th>Disease of root</th>
<th>Disease of Phoma disease of root</th>
<th>Disease of <em>Phoma terrestris</em> disease of root</th>
<th>Disease of <em>Phoma terrestris</em> disease of root</th>
</tr>
</thead>
<tbody>
<tr>
<td>Littleton Field 1</td>
<td>Pink-root; no bulb rot</td>
<td>Pink-root; no</td>
<td>15</td>
<td>15</td>
<td>+</td>
<td>Moderate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Littleton Field 2</td>
<td>&quot;</td>
<td>&quot;</td>
<td>15</td>
<td>15</td>
<td>+</td>
<td>&quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RockyFord Field 1</td>
<td>Trace of pink-root &amp; bulb rot present</td>
<td>No pink-root; traces of bulb rot</td>
<td>15</td>
<td>3</td>
<td>+</td>
<td>&quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RockyFord Field 2</td>
<td>Fallow</td>
<td>Pink-root; high incidence bulb rot</td>
<td>15</td>
<td>15</td>
<td>+</td>
<td>&quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greeley Field 1</td>
<td>Field planted to field corn</td>
<td>Onion crop a total failure</td>
<td>15</td>
<td>15</td>
<td>+</td>
<td>Severe</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Greeley Field 2</td>
<td>Fallow</td>
<td>Poor crop</td>
<td>15</td>
<td>8</td>
<td>+</td>
<td>Moderate</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delta</td>
<td>No pink-root; some bulb rot</td>
<td>Good crop</td>
<td>15</td>
<td>0</td>
<td>-</td>
<td>None</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Montrose</td>
<td>&quot;</td>
<td>Fair crop; some bulb rot</td>
<td>15</td>
<td>0</td>
<td>-</td>
<td>&quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grant Jct. Field 1</td>
<td>No pink-root; no bulb rot</td>
<td>&quot;</td>
<td>15</td>
<td>0</td>
<td>-</td>
<td>&quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grant Jct. Field 2</td>
<td>&quot;</td>
<td>Plants lacked vigor</td>
<td>15</td>
<td>0</td>
<td>-</td>
<td>&quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Grand Jct. Field 3</td>
<td>No pink-root; some bulb rot</td>
<td>&quot;</td>
<td>15</td>
<td>0</td>
<td>-</td>
<td>&quot;</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Figure 8. The results of planting Yellow globe danvers onion seed in representative Colorado Eastern slope soils suspected of harboring Phoma. No. 1, soil from Littleton field 2; Nos. 2 and 3, field 1, Greeley, and No. 4, field 2, Rocky Ford. Note dwarfing of plants in the Littleton soil as contrasted with the thin stand effect obtained in the Greeley soil, and the relatively normal appearance of plants in the Rocky Ford soil. The roots of all plants shown were infected with Phoma terrestris.
In table 4 it will be seen that Phoma was obtained from all Eastern Slope soils used in the test, whereas all isolations for Phoma from plants which grew in Western Slope soils proved to be negative. In this regard it must be mentioned that pink-root was observed for the first time in Western Colorado during the summer of 1938 in the Olathe area. In addition to the Eastern Slope localities already mentioned, pink-root has been found in the Pueblo, La Junta, Brighton, Denver, Longmont, and Fort Collins areas. All epiphytotics of the pink-root disease have occurred on the Eastern Slope to date, principally in the Greeley (Weld County), Rocky Ford (Otero County), and Littleton (Arapahoe County) onion growing districts.
HOST RELATIONSHIPS

The Internal Pathology of Affected Onion Roots

Naturally infected roots showed primary invasion at any point. The hyphae of Phoma were intra- and inter-cellular and as a rule were confined to the cortex and epidermis, rarely entering the vascular tissues. Whether the roots collapsed soon after invasion depended primarily upon the degree of soil infestation and virulence of Phoma, as well as environmental conditions conducive to attack and consequent rapid tissue disintegration. Secondary invaders, such as species of Fusarium, played a leading part in the collapse of the affected root.

In order to investigate the histological pathology of the pink-root disease, treated yellow globe danvers onion seed was transferred to petri dishes containing potato dextrose agar, incubated at 20°C, and allowed to germinate. At the end of from six to eight days, one lot of seedlings on the agar surfaces showing neither fungal nor bacterial growth, was removed and introduced into petri dishes containing 14-day-old colonies of Phoma terrestris. Care was exercised to place the seedlings on the edge of the colonies. The cultures were then incubated at 25°C; the seedlings removed at
12-hour intervals, cut into small pieces, killed and fixed. A second lot of sterile seedlings were removed and planted in steamed soil in which the pathogen had been introduced. These seedlings were removed at seven-day intervals, cut into small pieces, and killed and fixed. In most instances, the root material was killed and hardened in either of the two following fluids: (a) 1 percent chromic acid, 20 cc.; 1 percent acetic acid, 75 cc., and formalin (40 percent), 5 cc.; and (b) 95 percent ethyl alcohol, 50 cc.; glacial acetic acid, 5 cc.; formalin (40 percent), 7 cc., and distilled water, 40 cc.

The root material was then gradually dehydrated with increasing concentrations of acetone, cleared with xylene, and finally embedded in paraffin. Sections were stained successfully with the following standard stain combinations: fast green and safranin, Mayer’s haemalum and safranin, Delafield’s haematoxylin and safranin, or Delafield’s haematoxylin and eosin (5, 31, 33).

Frequently, examinations of fresh roots were made using a hot cotton blue-lacto-phenol solution (31) to stain the hyphae of the pathogen.

The mechanism of invasion.

Onion roots grown in artificially infested soil and those placed adjacent to the edges of colonies of the pathogen, showed the same general type of invasion. The fungus first
appeared on the surface of the young root as a small irregular colony. Such colonies were seen readily when the roots were cleaned in water, stained in hot cotton blue-lacto-phenol solution, mounted and examined microscopically in the uncrushed condition. The colony took a deep blue stain leaving the surface of the root relatively free from color, as shown in figure 9A. Primary invasion-hyphae were observed as a result of the deep stain taken by the external hyphal segment, as contrasted with the lack of stain in that portion of the same segment within the epidermal cells. (See figure 9B). In the examination of prepared root sections, other cases of hyphal constriction were observed. (See figure 11). In these instances, the constriction occurred in the walls of the cortical cells.

After entering the young root, the fungal hyphae proceeded in all directions from the point of primary invasion, ramifying throughout the cortex, intra- and intercellularly, as illustrated in figures 10 and 11. Pigmentation was usually confined to the hyphae of the pathogen, although observations frequently revealed that there had been some diffusion of the coloring substance into invaded cells. The pigment was not manifested in cells which were not invaded. The extensive diffusion of pigment mentioned by Hansen (17) was not observed. Two types of pigmentation have been observed in the invaded cells: (a) a pink coloration of the entire cellular content as in cells containing anthocyanin, and pink granular
Figure 9. Initial stages in the invasion of the onion root by *P. terrestris*. A. Small colony of the fungus growing on the surface of a young root (stained with cotton blue). B. Primary invasion-hypha of the pathogen, showing the stained external portion (left) as contrasted with the unstained segment (right) within the tissues.
Figure 10. Invasion of the onion root by *Phoma terrestris*. The transverse section of a normal root just back of the promeristem (left); transverse section of a root in approximately the same stage of development, showing invasion (right).
Figure 11. Invasion of the onion root by *Phoma terrestris*. The penetration of a cortical cell wall by hyphal constriction (left); invasion through the cortex (right).
masses within the cells, but not staining the cytoplasm. Of the two types, the first was the more frequently observed.

As a rule, in cases of artificial inoculation, the fungus appeared to sweep across the cortex in a hyphal mass, as illustrated by figures 11 and 12. Under such conditions, the pycnidial primordia failed to form, as the root soon collapsed. (Figure 12).

Invasion of onion root-tips.

Since abnormal protoplasmic responses in the cells of the cortex could not be ascertained with any degree of certainty, a study of the reaction of the meristematic and root-cap cells to invasion was undertaken. Microscopic examinations of prepared root-tip sections revealed a condition such as that shown in figures 13 and 14. In the latter, the invaded root-cap tissues (A) soon collapsed, showing only the vestiges of distorted cell walls. Cells in which fewer hyphal segments were manifest (B and C), revealed marked plasmolysis of their contents and nuclear distortion. This latter condition was frequently accompanied by a failure of the nucleus to either take or retain a safranin stain. Cells adjacent to those invaded, but apparently not parasitized, showed slight plasmolysis. In no case did the promeristematic region reveal any extensive invasion. (Figure 13).
Figure 12. Invasion of the onion root by *Phoma terrestris*. Longitudinal section showing the parasitized cortical cells (left); transverse section illustrating the final stage, showing the completely invaded and necrotic tissues. (right).
Figure 13. Invasion of the root-cap by Phoma (left). Later stage (right); the darker areas are filled with the hyphae of the pathogen. The proseristem shows no invasion.
Figure 14. The invasion of cells adjoining the promeristematic region by Phoma terrestris. At A the cell structure has been destroyed, leaving only the cell wall vestiges. Profuse mycelial accumulation is apparent. At B nuclear distortion with a failure to take the stain, as well as general plasmolysis, are evident. Zone C shows plasmolysis and nuclear distortion. At D the cells appear to be normal with the exception of those adjoining the invaded area.
Host Plants Other Than Allium cepa

In studying the resistance of some of the cultivated species of Allium to Phoma terrestris, Porter and Jones (30) found that although garlic, Allium sativum L., and shallot, A. ascalonicum L., were susceptible, the Nebuka onion, A. fistulosum L., leek, A. porrum L., and chives, A. schoenoprasum L., were extremely resistant.

In 1929 Hansen (17) reported that he had isolated Phoma terrestris from lesions on the roots of cow pea, Vigna satiarg Walp., lima bean, Phaseolus lunatus L., and from potato tubers, Solanum tuberosum L.

In order to investigate the probability of other common crop plants being attacked by Phoma terrestris, a study was undertaken which was confined to the types of standard field crops which might be expected to precede or to follow onion plantings. A steamed soil mixture (one part humus, one part sand, and four parts loam, steamed for three hours at 17 pounds pressure) was placed in 10 well washed flats and allowed to cool. Into the soil of each flat, 40 grams of ground wheat inoculum (a 45-day-old culture of Phoma terrestris No. 29A) was introduced. The inoculum was mixed thoroughly into the soil by hand, and was then allowed to stand for a few days before planting. At the end of this time, seeds of various crop plants were planted in the flats
in rows. Between each of these rows, a row of Mountain danvers, Red weathersfield, or Riverside sweet Spanish onion was planted as a control. Observations and isolations were made 30 days following planting. This trial was repeated employing the same methods and varieties of seeds as used in the first test. The results of these studies are given in Table 5.

A study of Table 5 shows that *Phoma terrestris* attacked the roots of seedlings of 19 different crop plants. Pink root was severe or moderately severe in the cases of onion (Red weathersfield), *Allium cepa* L., cane, *Sorghum vulgare* var. *saccharatum* (L.) Boerl., millet, *Panicum miliaceum* L., cucumber, *Cucumis sativus* L., carrot, *Daucus carota* L., and spinach, *Spinacia oleracea* L.


In addition, light attack was observed for the Sweet Spanish variety of onion. This variety has been reported by Porter and Jones (30) as being less susceptible to attack by *Phoma terrestris* than other commercial varieties of onion. Those plants showing no symptoms of pink-root were leek, *Allium*
<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>Variety</th>
<th>Degree of Isolation of Pink Root Attack by Phoma terrestria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onion</td>
<td>Allium cepa L.</td>
<td>Riverside sweet spanish + light +</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Red Wethersfield mountain + severe + moderate +</td>
<td></td>
</tr>
<tr>
<td>Leek</td>
<td>Allium porrum L.</td>
<td>Giant masselberg -</td>
<td></td>
</tr>
<tr>
<td>Soybean</td>
<td>Glycine hispida Maxim.</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Pea</td>
<td>Pisum sativum L.</td>
<td>Edwards + light +</td>
<td></td>
</tr>
<tr>
<td>Bean</td>
<td>Phaseolus vulgaris L.</td>
<td>Pinto -</td>
<td></td>
</tr>
<tr>
<td>Red clover</td>
<td>Trifolium pratense L.</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Sweet clover</td>
<td>Melilotus alba Desv.</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Alfalfa</td>
<td>Medicago sativa L.</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Cane</td>
<td>Sorghum vulgare var. saccharatum (L.) Boerl.</td>
<td>Black amber + severe +</td>
<td></td>
</tr>
<tr>
<td>Millet</td>
<td>Panicum miliaceum L.</td>
<td>Early fortune + severe +</td>
<td></td>
</tr>
<tr>
<td>Oats</td>
<td>Avena sativa L.</td>
<td>Brunker + light +</td>
<td></td>
</tr>
<tr>
<td>Barley</td>
<td>Hordeum vulgare L.</td>
<td>Trebi + light +</td>
<td></td>
</tr>
<tr>
<td>Wheat</td>
<td>Triticum aestivum L.</td>
<td>Komar + light +</td>
<td></td>
</tr>
<tr>
<td>Corn</td>
<td>Zea mays L.</td>
<td>Imp. golden bantam + light +</td>
<td></td>
</tr>
<tr>
<td>Squash</td>
<td>Cucurbita maxima Duc.</td>
<td>Hubbard + light +</td>
<td></td>
</tr>
<tr>
<td>Cucumber</td>
<td>Cucumis sativus L.</td>
<td>Davis perfect + severe +</td>
<td></td>
</tr>
<tr>
<td>Cantaloupe</td>
<td>Cucumis melo L.</td>
<td>Golden honeymoon + light +</td>
<td></td>
</tr>
<tr>
<td>Muskemelon</td>
<td>L.</td>
<td>Extra early + light +</td>
<td></td>
</tr>
<tr>
<td>Tomato</td>
<td>Lycopersicium esculentum L.</td>
<td>Earliana -</td>
<td></td>
</tr>
<tr>
<td>Pepper</td>
<td>Capsicum annuum L.</td>
<td>Oshkosh -</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>World beater -</td>
<td></td>
</tr>
</tbody>
</table>
Table 5. Concluded.

<table>
<thead>
<tr>
<th>Common name</th>
<th>Scientific name</th>
<th>Variety</th>
<th>Degree</th>
<th>Isolation of root</th>
<th>attack</th>
<th>Phoma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggplant</td>
<td>Solanum melongena L.</td>
<td>New York improved</td>
<td>✓</td>
<td>light</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>Brassica oleracea L.</td>
<td>Super snowball</td>
<td>✓</td>
<td>light</td>
<td></td>
<td>-</td>
</tr>
<tr>
<td>Cabbage</td>
<td></td>
<td>Stem holland</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Carrot</td>
<td>Daucus carota L.</td>
<td>Oxheart</td>
<td>✓</td>
<td>moderate</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>Parsnip</td>
<td>Pastinaca sativa L.</td>
<td>Hollow crown</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Celery</td>
<td>Apium graveolens L.</td>
<td>Early blanching</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Sugar beet</td>
<td>Beta vulgaris L.</td>
<td>Pioneer</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Spinach</td>
<td>Spinacia oleracea L.</td>
<td></td>
<td>✓</td>
<td>severe</td>
<td>✓</td>
<td>-</td>
</tr>
<tr>
<td>Lettuce</td>
<td>Lactuca sativa L.</td>
<td>Black seeded simpson</td>
<td></td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
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**Potorum** L., soybean, *Glycine hispida* Maxim., pinto bean, 
*Phaseolus vulgaris* L., red clover, *Trifolium pratense* L., 
sweet clover, *Melilotus alba* Desv., alfalfa, *Medicago sativa* 
L., tomato (Earliana), *Lycopersicum esculentum*, pepper, *Caps-

*sicium annuum* L., cabbage, *Brassica oleracea* L., parsnip, 
*Pastinaca sativa* L., celery, *Apium graveolens* L., sugar beet, 
*Beta vulgaris* L., and lettuce, *Lactuca sativa* L.

Although showing no characteristic pink-root symptoms, 
isolations from the water-soaked roots of soybean and pepper, 
yielded colonies of *Phoma*. The pink-root organism was iso-
lated from the roots of onion, millet, oats, tomato, pepper, 
soybean, carrot and spinach. The fungus was also obtained 
from the pericarps of germinating caryopses of barley, wheat 
and corn. As indicated in table 5, *Phoma* was isolated from 
pinkened roots in the majority of instances, although some 
failures were encountered.

From this study, it may be inferred that the host range 
of *Phoma terrestris* is of a wide and diverse nature. Such a 
pathogen should not be eliminated easily by crop rotation, 
since the organism shows no marked degree of specificity in 
its parasitism.

The Relation of **Fusarium**-Rot to Pink-Root

Basal bulb-rot of onion caused by *Fusarium* was first
described in 1910 by Selby in Ohio (36). A similar disease was reported from Japan by Hanzawa in 1914 (20), and from Connecticut by Clinton in 1915 (7). Hanzawa first described one of the principal etiological agents as *Fusarium cepae*, n. sp. (*F. oxysporum* f. 7)*. The latter, in 1924, according to Walker and Tims (46), was considered to be the cause of a bulb-rot of onions widespread in the vicinity of Chicago.

Two years later, Link and Bailey (24) observed the malady to be more widespread than earlier supposed, and named *F. zonatum* (Sherb.) *Wf. forma 2* (*F. vasinfestum* v. *zonatum* f. 2), *F. cepae* (Hanzawa) Walker and Tims**, and *F. zonatum* (Sherb.) *Wf. forma 1* (*F. vasinfestum* v. *zonatum* f. 1), as the chief bulb-rotting agents involved. They also reported that *F. moniliforme* Sheld. was capable of inducing the disease. They further stated in regard to the identity of the organism mentioned by Walker and Tims, "At the Fusarium conference it was decided that the organism described by Walker and Tims as *F. cepae* emend. Walker et Tims is *F. zonatum forma 1* (*F. vasinfestum* v. *zonatum* f. 1)." In regard to the pathogenicity of these species of *Fusarium*, Walker and Tims, and Link and Bailey agreed that the organisms were strictly wound parasites which seldom if ever attacked the uninjured bulb.

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*All binomials in brackets are according to Wollenweber and Reinking, 1935 (49).**

**This organism is considered by Wollenweber and Reinking to have been identical with *F. vasinfestum* Atk. v. *zonatum* (Sherb.) *forma 1* (Link et Bailey.) *Wf.*
A possible relationship between the pink-root disease and the basal bulb-rot of onions was first suggested in 1929 by Hansen (17), who found that *F. mali* (*F. solani*), *F. cromyoph-thoron* (*F. bulbigenum*), or *F. rhizochromatistes* (*F. bulbigenum*) plus *Phoma terrestris* killed onion plants in 90 days, whereas plants growing in soil infested only with *Phoma* were still alive at the end of this period. Plants grown in soil in which only the above species of Fusarium had been introduced, showed no attack at the end of this period. Hansen concluded that, "Where the host has already been invaded by a parasite, species of Fusarium are able to enter and materially aggra-vate the diseased condition." More recently, Davis and Henderson (8) in studying the relationship between a basal bulb-rot of onions caused by *Fusarium zonatum forma 1* (*F. vasinf ectum v. zonatum f. l*.) and pink-root, reported that *Fusarium zonatum forma 1*, caused semi-dry rot of onion bulbs in the field and in storage. It did not, however, attack the roots or bulbs, except following injury or the initial invasion by another pathogen, such as *Phoma terrestris*. They agreed with Hansen that *Phoma* would not enter the fleshy portion of the bulb, and considered that *F. vasinf ectum v. zonatum forma 1*, following root injury by *Phoma* will attack the roots and subsequently enter the bulb probably by way of the vascular system.
Experiments with seedlings.

In order to study the effect of the principal basal bulb-rotting fungus and the pink-root organism on the incidence of disease in seedlings, 40-day-old wheat giant cultures of Phoma 27A, and Fusarium vasinfectum zonatum forma 1 2A (Colorado isolates), were introduced into four-inch pots of steamed soil. In the cases where each organism was introduced into the soil alone, eight grams of inoculum were used. When both fungi were added to the soil, four grams of each culture were used which made a total of eight grams. The soil in each pot was planted with 100 treated onion seed of the variety Yellow globe danvers. Observations were made at the end of 80 days. The average greenhouse temperatures during the course of the experiment approximated 70°F. Details of the experiment and subsequent results are given in table 6.

Phoma was isolated with ease from the roots of plants growing in soil infested with the pink-root organism alone, but only with difficulty from the roots of plants taken from soil previously infested with both organisms. Early collapse of the seedlings somewhat resembling a damping-off effect, was characteristic of the infestation in series "D". Typical results of the study are shown in figure 15. In general these data coincide with those obtained by Hansen (17), and Davis and Henderson (8), although none of the above workers
Figure 15. The results of infesting steamed soils with *Phoma terrestris* (27A), and *Fusarium vasinfectum* var. *sponsum* f. l. (2A) (basal-rot organism). No. 1, control; No. 2, 2A infestation; No. 3, 27A infestation and No. 4, 27A plus 2A infestation. Note the failure of the Fusarium to cause noticeable damage alone, but the severe seedling injury brought about by introducing both organisms into the soil.
reported the effect of growing seedlings in soil infested with the pink-root and bulb-rotting pathogens.

Table 6. The effect on onion seedlings of infesting steamed soil with *Fusarium vasinfectum* v. *sonatum* forma 1 and *Phoma terrestris*.

<table>
<thead>
<tr>
<th>No.</th>
<th>Fungi</th>
<th>Percent of seedling survival</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>None</td>
<td>70</td>
<td>A few abnormal roots.</td>
</tr>
<tr>
<td></td>
<td>Fusarium 2A</td>
<td>52</td>
<td>Light invasion.</td>
</tr>
<tr>
<td>B</td>
<td>Phoma 27A</td>
<td>24</td>
<td>Severe pink-root</td>
</tr>
<tr>
<td>C</td>
<td>Phoma 27A plus Fusarium 2A</td>
<td>16</td>
<td>Severe pink-root. Surviving plants badly stunted.</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Experiments with bulbs.

At the outset of this work several trials were conducted to verify the reports of Davis and Henderson (8) and Hansen (17) that *Phoma* would not attack the bulb proper. Treated yellow globe danvers onion bulbs were inoculated by removing a small triangular wedge-like portion from the base of the bulb under sterile conditions, inserting the organism under test into the cavity, and then replacing the tissue. The inoculated bulbs were placed in large clean moist chambers and incubated at 20°C for 10 days. The results of the study are given in table 7.
Table 7. The effect of inoculation of onion bulbs with Phoma terrestris Nos. 17 and 18 (Iowa isolates) and Fusarium vasinfectum v. zonatum forma l. No. 40 (Iowa isolate).

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>No. of bulbs in invasion</th>
<th>No. of bulbs showing</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phoma No. 17</td>
<td>18</td>
<td>0</td>
<td>No indication of attack</td>
</tr>
<tr>
<td>Phoma No. 18</td>
<td>18</td>
<td>0</td>
<td>&quot;</td>
</tr>
<tr>
<td>Fusarium No. 40</td>
<td>18</td>
<td>18</td>
<td>Severe bulb-rot in all cases.</td>
</tr>
</tbody>
</table>

The data in table 7 appear to confirm Hansen's and Davis and Henderson's contention that Phoma does not invade the inner tissue of the onion bulb, and that Fusarium vasinfectum zonatum f. l. will readily attack injured and inoculated bulbs.

To obtain some knowledge pertaining to the influence of bulb injury on the possible pathogenicity of several isolates of Fusarium which had been obtained from rotting bulbs and roots and Phoma terrestris, a second and more extensive study was made. Southport white globe onion bulbs were exposed to infection by three methods: (a) removing a small wedge of tissue of the stem, introducing the agar inoculum and re-plugging; (b) stabbing a very small portion of inoculum into the stem by means of a sterile needle, and (c) pressing the inoculum firmly against the stem, taking precaution not to injure the bulb. All bulbs were then planted immediately in pots containing moist steamed soil. Observations were made after the elapse of a 35-day period.
Table 8. The effect of inoculating onion bulbs with representative species of Fusarium and Phoma terrestris.

<table>
<thead>
<tr>
<th>Inoculum (culture number)</th>
<th>Method of exposure to infection</th>
<th>Number of bulbs used</th>
<th>Number of bulbs showing rot in 35 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>F. vasinfectum v. zonatum 2A</td>
<td>plug</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>no injury</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>&quot; 23A</td>
<td>plug</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>needle</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>&quot; 40</td>
<td>plug</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>needle</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>Fusarium sp. 96A</td>
<td>plug</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>no injury</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>&quot;48A</td>
<td>plug</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>needle</td>
<td>10</td>
<td>1</td>
</tr>
<tr>
<td>&quot;36A</td>
<td>plug</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>no injury</td>
<td>10</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>needle</td>
<td>10</td>
<td>7</td>
</tr>
<tr>
<td>Phoma terrestris 53A</td>
<td>plug</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>needle</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>no injury</td>
<td>10</td>
<td>0</td>
</tr>
</tbody>
</table>

As shown by table 8, isolates of Fusarium vasinfectum v. zonatum were pathogenic only when the bulbs were injured. The uninjured bulbs showed no invasion. Fusarium isolates 48A and 36A were slightly pathogenic to injured bulbs, while isolate 96A was non-pathogenic. With all inoculations using species of Fusarium, the uninjured bulbs showed no invasion. In addition, Phoma terrestris failed to invade the inner bulb tissue.
In order to investigate the possible relationship be-
tween Phoma and the species of Fusarium in inducing root-
and bulb-rot of mature plants, another study was conducted.
Clean pint milk bottles were filled with soil (one part
humus, one part sand, and four parts greenhouse loam) which
had previously been steamed in gallon crocks for 15 hours
at five to seven pounds pressure. Five grams of inoculum
(three-month-old wheat giant culture) were then added to the
soil at the neck of each bottle, and one treated Yellow globe
danvers onion bulb was next inserted in the mouth of each
bottle in the manner described by Hansen (17). Water was added
to each bottle only sparingly and readings were made 65 days
later. Extreme difficulty was encountered keeping the bulbs
embedded in the soil in the neck of the bottle, since the
developing roots repeatedly pushed the bulbs away from the
mouths of the bottles. Consequently it was necessary to
press the bulbs back into the soil, which may have caused
some root and stem injury uniformly throughout the experiment.
Since Hansen used the technique described above and mentioned
no means of fastening the bulbs down to the mouth of the
bottle, it is felt that this investigator must have encountered
the same difficulty, consequently although unintentionally,
injuring the stem and the developing roots. The results of
the trial are given in table 9.
Table 9. The effect of growing mature onion bulbs in soils infested with both *Phoma terrestria* No. 27A and *Fusarium vasinfectum v. zonatum forma 1* No. 40.

<table>
<thead>
<tr>
<th>Ser.-</th>
<th>Fungi added</th>
<th>bulbs</th>
<th>showing</th>
<th>bulb-rot</th>
<th>pink-</th>
<th>root</th>
<th>plants</th>
<th>centage:plants</th>
<th>age</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Phoma 27A</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>Fusarium 40</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>Phoma 27A plus Fusarium 40</td>
<td>20</td>
<td>10</td>
<td>50</td>
<td>17</td>
<td>85</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>None</td>
<td>20</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The preceding data in general coincide with those obtained by both Hansen (17) and Davis and Henderson (8).

Bulb-rot was obtained only in series "C", where both organisms were combined. Root attack was lighter in series "C" than in series "A". Other than pink-root, no appreciable root necrosis was observed in any case.

Uncertain as to the possible role of injury in the preceding study, a second trial was conducted using four-inch clay pots as containers instead of milk bottles. Large Southport globe onion set-bulbs were selected for both size and appearance, and cleaned and treated as described elsewhere. From this material, 270 bulbs were selected and individually injured by thrusting a sterile needle through the stem. Two other lots of 270 selected bulbs were kept free from injury. Bulbs were planted deeply, three to a pot in steamed soil, into which eight grams of a giant culture had been introduced previously. Cultures of *Fusarium 23A, 2A, 40* (isolated from
enable these species of Prunus to invade the cotton plants
the soil together, root attack by Prunus peresserata did not
prove caused internal seedling losses when introduced into
the seedling at the pink-root and bulb-propagation turned em-
was not controlled. Instead, the foreoeroot data seem to indi-
period. In this study, the work of Parks and Hendeson (6)
test, observations were made at the end of the four-month
attributes from these data given in Table 10. In this latter
ment such as the one just described, these no indications de-
initial root invasion by Prunus peresserata. A second expert-
intersection a sometimes gains entrance into the plant root-
addition, there is no evidence to indicate that Prunus per-
rooting species of Prunus invaded only invaded plants. In
the data in Table 10, tend to indicate again that path-
end of a 70-day period.
largest types (25a, 4a, and 26a) observations were made at the
placed in some containers in the representative series
in which all bulks remained free from phytop and were
in some instances or in the proceeding series; and series "n"
in which all bulks remained free from phytop and were planted
read with different inoculates of Prunus species "n" in
"b" in which all bulks were invaded and planted in soils in-
this study was divided into three experiment. series; series
jester 26a was also used. As may be seen in Table 10, this
Table 10. The incidence of bulb-rot and pink-root as influenced by mixed soil infestation and bulb injury.

<table>
<thead>
<tr>
<th>Seri-</th>
<th>Fungi used</th>
<th>Stab No.</th>
<th>Fer- No.</th>
<th>Percent of bulb showing pink-root</th>
<th>Percent of stem showing bulb-rot</th>
<th>Percent of bulbs at injury plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>F. vas. zon. 23A</td>
<td>30</td>
<td>4</td>
<td>15</td>
<td>43.3</td>
<td>0.0</td>
</tr>
<tr>
<td>F.</td>
<td>&quot; 2A</td>
<td>&quot;</td>
<td>4</td>
<td>15</td>
<td>43.3</td>
<td>0.0</td>
</tr>
<tr>
<td>F.</td>
<td>&quot; 4O</td>
<td>&quot;</td>
<td>4</td>
<td>5</td>
<td>16.6</td>
<td>0.0</td>
</tr>
<tr>
<td>F.</td>
<td>&quot; 48A</td>
<td>&quot;</td>
<td>4</td>
<td>1</td>
<td>3.3</td>
<td>0.0</td>
</tr>
<tr>
<td>F.</td>
<td>&quot; 46A</td>
<td>&quot;</td>
<td>4</td>
<td>2</td>
<td>6.6</td>
<td>0.0</td>
</tr>
<tr>
<td>F.</td>
<td>&quot; 36A</td>
<td>&quot;</td>
<td>4</td>
<td>12</td>
<td>40.0</td>
<td>0.0</td>
</tr>
<tr>
<td>F.</td>
<td>&quot; 6A</td>
<td>&quot;</td>
<td>4</td>
<td>5</td>
<td>16.6</td>
<td>0.0</td>
</tr>
<tr>
<td>F.</td>
<td>&quot; 36</td>
<td>&quot;</td>
<td>4</td>
<td>12</td>
<td>40.0</td>
<td>0.0</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>&quot;</td>
<td>4</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>B</td>
<td>F. vas. zon. 23A</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>F.</td>
<td>&quot; 2A</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>F.</td>
<td>&quot; 4O</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>F.</td>
<td>&quot; 48A</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>F.</td>
<td>&quot; 46A</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>F.</td>
<td>&quot; 36A</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>F.</td>
<td>&quot; 6A</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>F.</td>
<td>&quot; 36</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>C</td>
<td>Phoma 58A</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
<td>0.0</td>
<td>30</td>
</tr>
<tr>
<td>Phoma</td>
<td>&quot; 58A F. vas. zon. 23A</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
<td>0.0</td>
<td>30</td>
</tr>
<tr>
<td>Phoma</td>
<td>&quot; 58A F. 36A</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
<td>0.0</td>
<td>30</td>
</tr>
<tr>
<td>Phoma</td>
<td>&quot; 58A F. vas. zon. 23A F. 48A</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
<td>0.0</td>
<td>30</td>
</tr>
<tr>
<td>F.</td>
<td>&quot; 58A F. 48A</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
<td>0.0</td>
<td>30</td>
</tr>
<tr>
<td>Phoma</td>
<td>&quot; 58A F. 48A</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
<td>0.0</td>
<td>30</td>
</tr>
<tr>
<td>F.</td>
<td>&quot; 58A F. vas. zon. 23A F. 48A</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
<td>0.0</td>
<td>30</td>
</tr>
<tr>
<td>F.</td>
<td>&quot; 58A F. 48A</td>
<td>&quot;</td>
<td>&quot;</td>
<td>0</td>
<td>0.0</td>
<td>30</td>
</tr>
</tbody>
</table>
sterile tissues. An equal part of any one extract was added to a sterile filter paper into which a sterile sealer filter into sterile frames portion was filtered under pressure through a sterile paper portion. Portion was filtered under pressure through three filters. Portion thus filtered through sterile frames the juice was then dried into three. After extraction by ethanol, the juice from 80 grams of green Allium sativum. To determine whether such substances might play a part in the resistance of the suspension public sector to attack by Pelarium, supplementary experiment on the water of a sterile comparative particulate filteration experiment showed no apparent effect. In studying the effect of one of the higher boiling fractions in food, a number of tests were made of the same extract, and a compound was determined to semicircle (10) the vortega of the flame containing 4% according to Figure 10)

and showing no apparent relationship.

are two distinct diseases manifesting different symptoms. It appears that at least in Colorado, both are and pink-root. The data of these investigators and those reported here in reference to the above account for the discrepancies between these studies and those used by Davis and Henderson could easily account for the discrepancies between the direct data. It is recognized that possible differences between these two types...
Figure 16. Differences between the basal bulb-rot disease (Fusarium vasinfectum v. zonatum), and the pink-root malady (Phoma terrestris). Note the soft disintegrating bulb and the relatively normal root system of the plant on the left (invasion by Fusarium), as contrasted with the diseased roots and firm uninvaded bulb of the plant on the right (attacked by Phoma). Variety, Yellow Spanish.
to an equivalent portion (by volume) of cooling liquid four percent agar just prior to the pouring of petri dishes. Into these dishes mycelial transfers of a pink-root fungous isolate were made, and they were then kept at room temperature for 14 days. The results of this study are given in table 11.

Table 11. The results of transferring Phoma terrestris (isolate 53A) to media containing the variously treated juice extract of the Silverskin onion bulb.

<table>
<thead>
<tr>
<th>Med-; Treatment of</th>
<th>No. of petri dishes</th>
<th>Results at the end of 7 days</th>
<th>Results at the end of 14 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>Furfural extract</td>
<td>15</td>
<td>No growth in 3 dishes; all others showed fair colony growth</td>
<td>No growth in 3 dishes; agar surfaces of all others overgrown growth</td>
</tr>
<tr>
<td>Seitz filters</td>
<td>15</td>
<td>All dishes showed good colony growth</td>
<td>Agar surfaces overgrown</td>
</tr>
<tr>
<td>Juice steamed at 10 lbs. for 30 minutes</td>
<td>15</td>
<td>No growth in 4 dishes; all others showed slow colony growth</td>
<td>No growth in 4 dishes; agar surfaces of all others overgrown growth</td>
</tr>
</tbody>
</table>

Except for seven dishes of media "A" and "C", there was growth in all the dishes used. Colony growth was slowest in medium "C" and most rapid in medium "B". Surprisingly enough, no contamination was observed in any of the dishes containing the unfiltered and unsteamed juice. Although growth appeared to be inhibited in a few cases where the unsteamed juice was
employed, the majority of dishes in these series showed moderately good growth. Although these data justify no conclusions, they do indicate that it would be unsafe to assume that the failure of Phoma terrestris to invade the succulent portion of the onion bulb was due to inhibitory substances present in extracted onion juice.

The Influence of Temperature on the Growth and Pathogenicity of Phoma terrestris

Hansen (17), and Davis and Henderson (8) have investigated the influence of temperature on the growth of Phoma terrestris. The former worker found that the optimum temperature for growth was near 26°C, showing a decline at 28°C, and exhibiting a sharp drop at 30°C. Davis and Henderson, on the other hand, found the optimum growth of their isolate to be at 28°C, their results coinciding with those of Hansen in that growth exhibited the same sharp decline at 30°C.

Growth

A brief study was made of the growth of P. terrestris on artificial media as influenced by temperature. This work was conducted to determine whether Colorado isolates differed in their temperature relations from those reported by the above mentioned investigators, and to establish a foundation for the next phase of the work; namely, the study of the affect of
-78-

varying temperatures on the pathogenicity of *P. terrestris* to seedlings and mature plants.

*Phoma* (isolate 58A) was selected for use in the study, since it was morphologically and pathologically typical of the isolates obtained in Colorado. Agar pieces, approximately four millimeters in diameter containing the mycelium of the fungus, were transferred to 25 petri dishes each containing 15 ml. of hard potato dextrose agar, five dishes of which were then placed at each of the following temperatures: 15, 20, 25, 30 and 35°C. Average colony diameter measurements were recorded at 48-hour intervals for each culture, and at the end of 288 hours, the cultures were removed and colony diameter measurements for each culture series at a given temperature were averaged. The results of this trial are given in table 12.

Table 12. Growth of *Phoma* (isolate 58A) on hard potato dextrose agar at five constant temperatures.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>48 hrs</th>
<th>96 hrs</th>
<th>144 hrs</th>
<th>192 hrs</th>
<th>240 hrs</th>
<th>288 hrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>7.0</td>
<td>8.5</td>
<td>13.3</td>
<td>18.6</td>
<td>20.6</td>
<td>24.6</td>
</tr>
<tr>
<td>20</td>
<td>10.6</td>
<td>20.0</td>
<td>23.6</td>
<td>35.6</td>
<td>45.6</td>
<td>52.3</td>
</tr>
<tr>
<td>25</td>
<td>11.0</td>
<td>23.3</td>
<td>33.5</td>
<td>44.0</td>
<td>49.3</td>
<td>56.3</td>
</tr>
<tr>
<td>30</td>
<td>10.0</td>
<td>18.0</td>
<td>20.0</td>
<td>24.5</td>
<td>27.5</td>
<td>32.0</td>
</tr>
<tr>
<td>35</td>
<td>4.0</td>
<td>6.0</td>
<td>7.5</td>
<td>10.5</td>
<td>15.5</td>
<td>16.0</td>
</tr>
</tbody>
</table>
The data in table 12 indicate that 25°C. or slightly above, was optimum for the organism. However, it was apparent that an intermediate temperature between 25 and 30°C. was necessary for a reasonably accurate determination of this point. For this reason, another trial was conducted using a modified Czapek's agar, following the same technique as described for the last experiment, with the exception that six petri dishes instead of five were placed in each incubator. The same time intervals and method for recording data as used previously, were employed in this study.

Table 13. Growth of Phoma (isolate 53A) on Czapek's modified agar at six constant temperatures.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>48 hrs.</th>
<th>96 hrs.</th>
<th>144 hrs.</th>
<th>192 hrs.</th>
<th>240 hrs.</th>
<th>288 hrs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>5.8</td>
<td>8.3</td>
<td>13.5</td>
<td>18.5</td>
<td>22.5</td>
<td>27.3</td>
</tr>
<tr>
<td>20</td>
<td>6.8</td>
<td>12.1</td>
<td>20.3</td>
<td>29.1</td>
<td>38.3</td>
<td>48.0</td>
</tr>
<tr>
<td>25</td>
<td>7.3</td>
<td>16.0</td>
<td>32.0</td>
<td>47.5</td>
<td>56.0</td>
<td>66.0</td>
</tr>
<tr>
<td>27</td>
<td>7.7</td>
<td>18.5</td>
<td>32.5</td>
<td>47.0</td>
<td>58.5</td>
<td>73.2</td>
</tr>
<tr>
<td>30</td>
<td>8.0</td>
<td>14.1</td>
<td>19.6</td>
<td>25.0</td>
<td>27.0</td>
<td>31.5</td>
</tr>
<tr>
<td>35</td>
<td>5.8</td>
<td>7.1</td>
<td>7.6</td>
<td>8.8</td>
<td>12.6</td>
<td>15.6</td>
</tr>
</tbody>
</table>

The data in table 13 agree in general with those given in table 12, with the exception that on Czapek's agar, 25°C. gave a greater growth increment at the optimum temperatures than was obtained using hard potato-dextrose agar. Twenty-seven degrees Centigrade appeared to be closer to the optimum temperature for the growth of the isolate used, although the differences in growth increment between 25 and 27 degrees
were only slight. Figure 17 represents a growth comparison between cultures showing the average growth for each five-degree interval.

Pathogenicity.

To date Hansen (17) and DuPlessis (11), are the only workers who have contributed any information pertaining to the influence of temperature on the pathogenicity of Phoma. Using Ebenezer onion bulbs for his host plants, Hansen reported that the optimum temperature for infection of onion roots (roots produced from bulbs) was also optimum for the best growth of the pink-root pathogen in culture. Hansen found that 75 percent of the plants at 20°C. were infected, 100 percent at 25°C., and that the disease incidence dropped off sharply at 30°C. (25 percent). DuPlessis also reported that onions growing at 25°C were more vigorously attacked than those growing at 30°C.

The first experiments using seedlings as a source of host material were conducted at Ames, Iowa, in the summer of 1936. In this preliminary work, Iowa field soil obtained from a field on Mr. Sam Kennedy's farm at Clear Lake, Iowa, which had previously been demonstrated by Davis and Henderson (8) to be harboring Phoma terrestris, was used. In addition, trials were also conducted using a steamed greenhouse soil mixture (six parts compost-loam to one part of sand), part of
Figure 17. The influence of temperature on the growth of Phoma terrestris on modified Czapek's agar. These cultures were typical of their respective series at the cessation of a 288-hour interval. A 15, B 20, C 25, D 30, and E 35°C.
which was infested, and a portion of which was not infested.

From large air-dried and well mixed lots of the non-treated field soil, the non-infested greenhouse soil mixture, and the infested greenhouse soil mixture, samples were taken to determine their moisture holding capacities. This determination was made by use of standard methods (10, 26). Following this determination, enough water was added to each of 54 glass jars each containing 650 grams of soil (18 containing field soil, 18 containing infested steamed soil, and 18 containing non-infested steamed soil) to raise the moisture content of the soil in each jar to 60 percent of the moisture holding capacity. The soil was next well mixed to bring about a uniform moisture dispersion throughout, several inches were removed from the surface layer, and 100 well washed but non-treated Yellow globe danvers onion seed were planted. The soil was then replaced immediately and the surface was leveled. Following this, the sides of each jar above the soil level were well cleaned with dry cotton, and oven dried sand (12 hours at 140°C.) was added to within an inch or so of the mouth of the jar. A clean dry cotton plug was next placed in the mouth. (For this method see figure 18). The jars were next weighed and removed to an incubator regulated to 20°C. After the duration of a 48-hour period the containers were removed, and nine jars (three of each soil series) were placed in each of six

*250 grams of ground inoculum (60-day-old oat giant culture Phoma isolate 10) added to every 3,500 grams of air-dry soil.*
Figure 18. Equipment used in the major portion of the temperature work. A. dry cotton plug; B. oven dried sand collar; C. soil layer covering seeds or bulbs; D. soil base. Layers "C" and "D" were of the same uniform composition, made up to the desired moisture percentage, and infested with the pathogen or held as a control as desired.
Since several important crops were destroyed from moderate frosts, it was apparent that the amount of inoculation was too great to avoid. Seedling roots in all tissues except at 10°C. showed 100 percent invasion in all cases except at 20°C. Since the inoculated seedlings showed the highest amount of invasion in the non-sterile soil, the maximum amount of invasion in the non-sterile soil is shown in Table 14, the maximum amount of invasion in the non-sterile soil is shown in Table 14.

Roots of any of the plants or bacteria already present in the growth of any of the plant or bacteria already present in the soil were eaten and consumed in a. Po. A. Han and R. P. Lolling and Wright. All seedling roots were examined for any evidence of invasion. All seedling roots were examined for any evidence of invasion. If the hot cotton blue-treato-phenoxyphene, viability, and morphology, such as chromatography, plantations, or reddening, were

since in many cases, experimental root it exceeded your "scare" since in many cases, test root were some noticeable substances lose per jar, and in no case did we find quite consistent at all except at 20°C and 25°C. Here there were more substrates than that at 15-day interval. The jar weights were removed, to

and 25°C.
Table 14. The influence of six constant temperatures on disease incidence in seedlings as determined microscopically.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Naturally infested</th>
<th>Greenhouse soil mixture</th>
<th>Infested greenhouse soil mixture</th>
<th>Infected field</th>
<th>Infected greenhouse soil mixture</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>54.0</td>
<td>6.4</td>
<td>18.1</td>
<td>95.8</td>
<td>57.5</td>
</tr>
<tr>
<td>15</td>
<td>28.5</td>
<td>12.2</td>
<td>2.2</td>
<td>100.0</td>
<td>56.0</td>
</tr>
<tr>
<td>20</td>
<td>63.6</td>
<td>32.4</td>
<td>3.0</td>
<td>100.0</td>
<td>46.9</td>
</tr>
<tr>
<td>25</td>
<td>49.7</td>
<td>38.1</td>
<td>7.5</td>
<td>100.0</td>
<td>42.4</td>
</tr>
<tr>
<td>30</td>
<td>40.0</td>
<td>48.1</td>
<td>0.0</td>
<td></td>
<td>40.1</td>
</tr>
<tr>
<td>35</td>
<td>3.0</td>
<td>16.6</td>
<td>0.0</td>
<td></td>
<td>0.0</td>
</tr>
</tbody>
</table>

In order to further study the effect of temperature on the pathogenicity of Phoma on seedlings, a second experiment was conducted using steamed soil and a reduced amount of inoculum in order to obtain an observable gradation in degree of severity of attack. A well mixed sample of steamed greenhouse soil (one part sand, four parts loam, and four parts humus) was divided into two equal portions. To one lot, 50 grams of moist, ground inoculum were added per kilogram of soil.

The inoculum consisted of 27-day-old wheat giant culture of Phoma 58A which had been ground to a pulpy mass in an ordinary food chopper. After each sample had been well mixed separately by hand, they were brought to approximately 60 percent of their moisture holding capacities (based on oven dry weights) and approximately 80 grams of moist soil was added to each of 35
was isolated from such root sections.

These roots were obtained from the plants at 15°C, a temperature of 20°C. Observations were made from representative samples of

and was some root necrosis in the controls both at 20°C. However, since it was apparent that there

as shown in Table 10, the disease incidence was again

in almost all instances.

Phenocic roots. These observations yielded cocoons of phenocic

observations were made from a representative assortment of

and as the index of the disease. To establish the root-

was evident throughout the treated soil seres, it was con-

was reduced to one of the following temperature: 10°, 20°, 60°

containers were removed to free the horticulturist, each of which

it was apparent that the optimum of seeds red germination, the

allow the seeds to germinate. At the end of this time, when

raised to 20°C. and allowed to remain there for six days to

and removed to a thermostatically controlled temperature. At

proceeding study (see Figure 18). Each jar was then weighed

for the experiment in the same fashion as was done in the

yellow globe dianthus on seed. Each jar was next prepared

were planted with 60 seeds.

clean oven dried bottles (outside diameter 20 mm, height 120

-96-
Table 15. Pathogenicity of *Phoma* to onion seedlings at five constant temperatures.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Infested Seed Treatment</th>
<th>Soil Planted</th>
<th>Germination (%)</th>
<th>Seedling Root Disease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Infested</td>
<td>250</td>
<td>33.6</td>
<td>42.8</td>
</tr>
<tr>
<td></td>
<td>Non-infested</td>
<td>100</td>
<td>47.0</td>
<td>0.0</td>
</tr>
<tr>
<td>20</td>
<td>Infested</td>
<td>250</td>
<td>24.8</td>
<td>80.6</td>
</tr>
<tr>
<td></td>
<td>Non-infested</td>
<td>100</td>
<td>53.0</td>
<td>0.0</td>
</tr>
<tr>
<td>25</td>
<td>Infested</td>
<td>250</td>
<td>22.8</td>
<td>89.4</td>
</tr>
<tr>
<td></td>
<td>Non-infested</td>
<td>100</td>
<td>46.0</td>
<td>0.0</td>
</tr>
<tr>
<td>30</td>
<td>Infested</td>
<td>250</td>
<td>10.4</td>
<td>100.0</td>
</tr>
<tr>
<td></td>
<td>Non-infested</td>
<td>100</td>
<td>51.0</td>
<td>29.0*</td>
</tr>
<tr>
<td>35</td>
<td>Infested</td>
<td>250</td>
<td>8.0</td>
<td>65.0</td>
</tr>
<tr>
<td></td>
<td>Non-infested</td>
<td>100</td>
<td>26.0</td>
<td>65.3*</td>
</tr>
</tbody>
</table>

*Penicillium* sp. was isolated from the majority of seedling roots thus affected.

The fact that no such injury was observed at 15, 20 or 25°C. in the controls, suggests the possibility of abnormal host predisposition at the higher temperatures.

Large treated Southport white globe onion set-bulbs were next planted in infested and non-infested soils. The general method of preparation was identical with that used in the preceding experiment, with the exception that the containers were placed in the five incubators as soon as preparations were complete. Four bulbs were planted in the soil of each container. Observations were made in 12 days.

Since *Phoma terrestris* grew on the outer scales of the bulbs used in this study, some indication as to the action of *Phoma* on dead tissues at high and low temperatures was obtained. Observations on scale blotch as well as pink-root are given in table 16.
Table 16. Pathogenicity of Phoma to onion set-bulbs at five constant temperatures.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Soil treatment</th>
<th>No. of bulbs showing Phoma</th>
<th>Total of infected bulbs</th>
<th>Percent Phoma</th>
<th>Percentage of bulbs</th>
<th>Percent of roots</th>
<th>Involved in 12 scale</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Infested</td>
<td>16</td>
<td>100.0</td>
<td>25.0*</td>
<td>342</td>
<td>7.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-infested</td>
<td>12</td>
<td>91.6</td>
<td>0.0</td>
<td>220</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>Infested</td>
<td>16</td>
<td>87.5</td>
<td>100.0</td>
<td>286</td>
<td>55.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-infested</td>
<td>12</td>
<td>83.3</td>
<td>0.0</td>
<td>244</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>Infested</td>
<td>16</td>
<td>56.2</td>
<td>100.0**</td>
<td>142</td>
<td>61.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-infested</td>
<td>12</td>
<td>66.6</td>
<td>0.0</td>
<td>159</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>Infested</td>
<td>16</td>
<td>0.0</td>
<td>12.5*</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-infested</td>
<td>12</td>
<td>41.6</td>
<td>0.0</td>
<td>14</td>
<td>0.0</td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>Infested</td>
<td>16</td>
<td>0.0</td>
<td>0.0</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Non-infested</td>
<td>12</td>
<td>16.6</td>
<td>0.0</td>
<td>10</td>
<td>0.0</td>
<td></td>
</tr>
</tbody>
</table>

*Superficial blotch.
**Severe blotch.

Root invasion was highest at 25°C., bulbs in infested soil failing to produce roots at either 30 or 35°C. All bulbs showed blotching of the scales at 20 and 25°C., with a sharp decrease in the number so affected at 15 and 30°C. There was no scale invasion at 35°C. Blotching at 25°C was far more severe than at 20°C., although in either case 100 percent of all the bulbs used were affected.

In order to determine the degree of root invasion above 25°C., a second experiment was conducted using large yellow globe danvers set-bulbs. All the bulbs used in this study were treated as was done previously, with the exception that they were allowed to root out in moist steamed sand before being planted in their respective containers. The same methods as used in the previous experiment were followed. Observations
were made in 12 days.

Table 17. Pathogenicity of Phoma to onion set-bulbs at five constant temperatures.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Soil treatment</th>
<th>Number of set-bulbs</th>
<th>Number of roots</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>Infested</td>
<td>9</td>
<td>203</td>
<td>53.8</td>
</tr>
<tr>
<td></td>
<td>Non-infested</td>
<td>4</td>
<td>101</td>
<td>0.0</td>
</tr>
<tr>
<td>20</td>
<td>Infested</td>
<td>8</td>
<td>155</td>
<td>72.2</td>
</tr>
<tr>
<td></td>
<td>Non-infested</td>
<td>4</td>
<td>92</td>
<td>0.0</td>
</tr>
<tr>
<td>25</td>
<td>Infested</td>
<td>8</td>
<td>154</td>
<td>80.5</td>
</tr>
<tr>
<td></td>
<td>Non-infested</td>
<td>4</td>
<td>73</td>
<td>0.0</td>
</tr>
<tr>
<td>30</td>
<td>Infested</td>
<td>9</td>
<td>145</td>
<td>88.2</td>
</tr>
<tr>
<td></td>
<td>Non-infested</td>
<td>4</td>
<td>52</td>
<td>0.0</td>
</tr>
<tr>
<td>35</td>
<td>Infested</td>
<td>10</td>
<td>152</td>
<td>75.6</td>
</tr>
<tr>
<td></td>
<td>Non-infested</td>
<td>4</td>
<td>108</td>
<td>1.8*</td>
</tr>
</tbody>
</table>

*Penicillium sp. isolated.

Although the number of diseased roots are recorded in percentage in table 17, a true picture of variation in severity is not obtained by studying the data presented. The majority of roots showing disease at 15 and 20°C, appeared to be superficially invaded, as compared with those attacked at higher temperatures. The high incidence of disease at 35°C may have been due to at least in part to extreme host predisposition although the evidence of such a phenomenon is not convincingly shown by the controls. Roots of the control plants were free from invasion throughout the experiment except for a few at 35°C. And again from these roots a species of Penicillium was uniformly isolated. Typical plants for each temperature series used in this study are shown in figure 19.
Figure 19. The effect of incubating previously rooted Yellow globe danvers onion bulbs (see table 17), in steamed non-infested and Phoma-infested soils, at five constant temperatures. The plants in the top row were grown in infested soil; those in the bottom row in non-infested soil. Series reading from left to right; 15, 20, 25, 30, and 35°C.
The pertinent data in tables 14, 15, 16 and 17 are summarized in figure 20. It will be observed that with the exception of "E" and "C", all graphs show maximum invasion at 30°C., exhibiting sharp slopes on either side of this point. Graph "E" has little bearing on this general trend, since no roots were produced from bulbs above 25°C. Graph "C" is interesting since bulb scale invasion closely paralleled the actual growth of *Phoma terrestris* in culture (see table 13), with its peak within the 20 to 25°C. range. However, it should be restated at this point that at 25°C. scale invasion was much more marked than at 20°C., thus the parallelism between the growth of the fungus in culture and growth on dead scale tissue becomes apparent. In spite of the fact that the growth of the fungus in culture proved to be best between 25 and 27°C. and that there was a sharp drop in this process at 30°C. (see tables 12 and 13), the indications are that Hansen (17) was incorrect in his conclusion that optimum growth and highest disease incidence are found at the same temperatures (25 to 26°C.). Instead, this study indicates that the pathogenicity of *Phoma terrestris* on living root tissue is highest at 30°C. This apparent paradoxical situation may be clarified somewhat when it is recalled from the foregoing data that the germination of onion seed was retarded at 30°C. and above (table 14), the roots of onion seedlings in non-infested steamed soil showed invasion by organisms at 30°C. and above which are not parasites at lower temperatures (table 15), and finally, onion
Figure 20. The incidence of disease at different temperatures.

Temperature: Degrees C.

Percentage Infection
bulbs failed to root out at 30°C. and above (table 16). Unfortunately table 17 does not lend itself to this same general trend, since no appreciable retardation or irregular invasion of roots was evident in the controls. It appears that the relatively delicate seedling roots react more readily to unfavorable temperatures than do the fleshy thickened roots produced from bulbs. The bulk of the data presented in this study bring out one important point, namely, that a temperature of 30°C. may predispose onion root tissues to attack more than enough to offset the decline in growth exhibited by the pathogen (Phoma terrestris) at the same temperatures.
Although the pink-root pattern did not invade the scene,

which P. perforatella is described in nature.

that this type of attack may afford an additional means

site was readily found in such affected tissues. It appears

of "methion" on onion plants. Since the symptoms of P. perforata
that such a condition is considered to represent a new type

on onion plants in the field and in artificial stirred affected soils.

Evidence of outer scale attack has been found so consistently

the appearance of water-soaked areas on the outer scales

opposite direction as red-wheat rust, invasion was witnessed by P.

as beady red-speckled patches and streaks on the outer surface

decoration which followed a small P. perforatella absorbed

the southern end of the root-globe. In this variety, the red-purple

pose to invasion and most evident in white onions such as

vision, although occurring on all the varieties of onion ex-

perforata appeared to continue the attack to the lateral roots

any species of Prunus is able to induce this material

Hansen is the central agent of the pink-root disease of onions

The data herein presented show that P. perforatella

DISCUSSION
lent tissues of the onion bulb, the fungus was grown on agar media to which both the filtered and unfiltered extract of onion juice had been added. From this evidence it appears that the known substances in onion juice (37), which Walker et al. (49) reported as having an inhibitory affect on the spore germination and growth of certain onion pathogens, such as *Colletotrichum circinans*, may not be responsible for the resistance of the onion bulb to invasion by *Phoma terrestris*.

Root attack by *P. terrestris* has been considered to afford an entrance into the bulb for the bulb-rotting fungus, *Fusarium vasinfectum* v. *zonatum*. Although Hansen (17), and Davis and Henderson (8) found that their isolates of *Fusarium vasinfectum* v. *zonatum* and *Phoma terrestris* when inoculated together into steamed soil were capable of inducing death of mature plants, such an effect was not obtained in this study. Possible dissimilarity between the strains of fungi used by these investigators and those reported herein, and probable environmental experimental differences may account for the disparity in results. It was found, however, that a greater degree of seedling and young plant injury was obtained when both of the above mentioned organisms were introduced together as against the infestation of soil by either organism singly.

*Fusarium vasinfectum* v. *zonatum* has been shown to be capable of readily invading and rotting injured onion bulbs, although no such attack was ever observed to occur in the
cases of bulbs not previously injured. For this reason, it is considered probable that epiphytotics of bulb-rot of onions could be brought about in soils infested with the bulb-rotting pathogen by either excessive bulb injury in cultivation or by insect injury. The evidence presented in this study shows that bulb-rot induced by Fusarium and pink-root caused by *Phoma terrestris*, at least in Colorado, are two dissimilar and distinct diseases. Pink-root appears to have no measurable influence upon the incidence of bulb-rot.

The indication that *P. terrestris* is an organism which would be difficult to eliminate from soils once infested with the fungus, is borne out by the fact that it attacked the roots of young crop plants such as carrot, spinach, cane, millet and the like. In addition, the indications that the organism has been in the soils of onion fields in Colorado for a considerable period of time, are borne out by the fact that the fungus has been shown to occur in the majority of representative soils of onion fields on the Eastern Slope of Colorado. Isolates of *P. terrestris* which were obtained from the roots of onion plants grown in some of these representative soils, showed considerable differences in virulence at the time of isolation. Variation in pathogenicity between isolates of a given pathogen is a phenomenon which has been observed frequently in the study of parasitic fungi (6, 29). The indications, however, that these isolates varied in virulence without undergoing host passage, even showing in occasional in-

stances actual increases in virulence, was unexpected. The only explanation for such a phenomenon would be to consider as Brierly (1) did, that a fungal colony may not be represented as any one genotype but rather as a heterogeneous collection of many genotypes. In addition, possible heterokaryotic phenomena (15, 19), could still further tend to complicate the picture. With these assumptions, it could be clearly understandable that individual mycelial segments may be genetically dissimilar in potential virulence. Transferring a small segment of a colony, therefore, may not insure one of obtaining a genotypic representation of that colony.

There are data presented in this study which appear to show that the optimum temperature for the incidence of the pink-root malady was not the optimum temperature for the most vigorous growth of the pink-root fungus. Although maximum invasion of outer bulb scales and most vigorous growth in culture was obtained at temperatures of 25 to 27°C., the highest disease incidence (root invasion by Phoma) was observed at 30°C. Curiously enough, growth showed an abrupt drop at this latter temperature. Onion seed failed to germinate and onion bulbs failed to root at a temperature of 30°C. In addition, a species of Penicillium normally non-pathogenic to onion roots, readily invaded roots at this temperature. It appears then, that a temperature of 30°C. was distinctly unfavorable to the host, more so perhaps than to the parasite. It can be seen therefore, that such a temperature could predispose
tissues of the onion root to attack by \textit{P. terrestris}, to an extent great enough to offset the unfavorable effect produced on the organism.

Since colony-like growths of \textit{Phoma terrestris} were observed on the onion root, it was thought that the products of fungal metabolism might eventually bring about the death of the underlying onion cells, thus enabling the organism to invade saprophytically. However, because such a postulated means of entry into the tissues of the host has been questioned by Brown (2), and Brown and Harvey (3), a search was made for penetrating hyphae showing indications of entrance by mechanical means. Such hyphae were observed both on the exterior of the root and within the parasitized cortical cells. These hyphae were characterized by marked constrictions in their diameters at the point where the cell wall of the host was invaded. In studying the attack of the root-tip by \textit{P. terrestris}, it was observed that the hyphae of the pink-root fungus invaded all portions except the promeristematic region. It is possible that the high metabolic activity of this tissue precluded attack. However, cells of the promeristem adjoining those cells invaded showed slight plasmolysis. Such evidence may indicate that the hyphae of the pathogen secreted substances toxic to protoplasm, which diffused in advance of the invading filaments. It would appear then, that the pathogen invaded only dead and dying cells. Invaded cells showed plasmolysis of their contents and nuclear distortion, with a re-
sultant failure of the nuclei to either take or retain a safranin stain. Such evidence may be taken to indicate that the chemical nature of these nuclei had been altered.

Infected roots usually showed the presence of those cellular aggregates in the cortical tissues which Hansen (17) has described as "pycnidial primordia." Although these bodies have been considered to be the precursors of pycnidia, all efforts to induce the formation of the latter in isolates of *Phoma terrestris* resulted in failure. Only one pycnidial-forming isolate of *P. terrestris* was found during the entire course of the work. From the results of a four-year study, it is concluded that there are true non-pycnidial-forming strains of *P. terrestris*. Accordingly, it follows that for such strains, the pycnidial primordia may be considered as vestigial structures.

In the light of the evidence obtained, it is considered that the presence of the pycnidial primordia in the root tissues, is the best diagnostic criterion of the pink-root disease, since, as stated above, they were invariably found in tissues attacked by *P. terrestris*. Since *P. terrestris* was quite difficult to isolate from affected root tissues, diagnosis based on isolations was uncertain at best. Further, the pink to red coloration which has been considered to be the best diagnostic index of the pink-root malady in the past was not always manifested. Diseased roots of affected plants growing in certain soils infested with *P. terrestris*, instead
of appearing rhodonite pink (32) to spinel red as expected, approached a yellow in hue. A microscopic examination of such tissues, usually revealed the presence of pycnidal primordia. In addition, isolations from such tissues yielded colonies of *P. terrestris*. Roots showing the typical hues (pink to red), became yellow-brown when immersed in solutions slightly acidified. In addition, a solution of the pigment of *P. terrestris* showed a color shift within the pH range of 7.05 to 7.86, from yellow-brown on the acid to red-purple on the alkaline side. It is apparent from this study that roots may be invaded by *P. terrestris* even though they do not show the pink-root color symptom. Consequently, too great a reliance should not be placed on the characteristic pink to red coloration in diagnosing the disease produced by *P. terrestris*. 
SUMMARY

By the use of a modification of Hansen's (16) technique, Phoma-like isolates were obtained from diseased onion roots in Iowa and Colorado which showed characteristic pink-root symptoms. In all cases these isolates were found to be capable of causing the disease. Comparisons with previously identified cultures showed that these pink-root producing fungi were isolates of Phoma terrestris Hansen. No isolate of Fusarium obtained from affected roots either produced pink-root or was pathogenic to onion roots.

Only one isolate was obtained during the course of the study which was capable of producing pycnidia. Efforts to induce sporulation in all other isolates resulted in failure. Phoma terrestris invariably formed pycnidial primordia in culture, although in only one instance were they observed to develop into pycnidia.

Examinations frequently revealed the presence of pycnidial primordia in the tissues of onion roots which were yellow to yellow-brown in color instead of the characteristic pink to red hue. In addition, the organism was isolated from roots showing such atypical discolorations. Cultural studies revealed that variation of temperature and nutrients had little influence on pigment manifestation. Increasing the H-ion con-
centration, however, produced a color change which induced a hue suggestive of that observed in nature. Preliminary trials, using diseased roots and fungal mats showed a color shift from the red to red-purple shades at pH 8.5 to a yellow to yellow-brown color at pH 4.5. By extracting the pigment from the fungal hyphae using 0.1 N HCl followed by 0.1 N NaOH, the coloring material was obtained in a soluble state. By use of the pigment in solution, the color shift was observed to take place within the range of pH 7.00 to 7.36. The pigment of *P. terrestris* was obtained from solution by the addition of saturated Na₂SO₄, and was returned to the soluble state only by treatment with 0.1 N HCl followed by 0.1 N NaOH. Since the material apparently was hydrolyzed by emulsion it appeared that at least part of the pigment complex was of the nature of a D-glucoside.

Isolates of *P. terrestris* obtained from various localities differed considerably in virulence. Further, isolates retained in culture and without undergoing passage through host tissue, appeared to show both increases and decreases in virulence.

Onion plants attacked by *P. terrestris* generally lacked vigor and were stunted. Leaf flaccidity, withering, and dying of the tips were common symptoms. In addition, leaf yellowing seemed to be associated with the malady. Roots just invaded, although still turgid, revealed a faint rhodonite pink coloration in the area attacked. Later, these roots
lost their turgidity, became spinel red in color and collapsed, persisting as shriveled and reddened structures.

Although *P. terrestris* did not invade the living leaf tissue of the onion bulb, the fungus invariably attacked the dead outer scale tissue. Such invasion, because of the typical reddish discoloration produced, was much more evident in the case of white onions than in that of the colored varieties.

The pink-root pathogen has been found to occur in the soils of the majority of onion fields on the Eastern Slope of Colorado. Only the soil of one onion field on the Western Slope of the state showed the presence of the organism.

The fungus formed small colony-like growths on onion roots, and invasion was effected by means of hyphae which showed a characteristic constriction at the point of entrance. Having once gained access to the inner tissues, the hyphae ramified throughout the cortex, eventually forming the so-called cellular glomerations known as pycnidial primordia in the cortical cells. Under conditions favorable for the growth of the organism, affected roots then underwent complete necrosis. Invaded cells near the proteristematic region revealed plasmolysis of their contents and nuclear distortion. In addition, the nuclei usually failed to take or retain a safranin stain. Cells showing no invasion but adjoining those containing the hyphae of the pathogen, revealed a slight plasmolysis of their contents.

*Phoma terrestris* attacked the roots of numerous young
observed to occur at the temperature

set difference increase (root inhibition by P. terrestris) was
common but did not produce roots at 20°C. Further, the high-
temperature fluctuations common seed fall to germinate and
these obtained in the reeontration of the medium in culture to
scale these was most marked at 25°C. These results pertained
exhibited a sharp drop at 20°C. The growth of the fungus
temperatures between 25 and 37°C. The growth of the fungus

P. terrestris grew most thermostoically in culture at

butts had been added

which the killed and untreated juice extract from common
tone plants

was not observed to affect the roots of

V. zonata' was not observed to affect the roots of

The latter fungus readily attacked injured plants but could

that root affect by P. terrestris and P. variocinctus

that seedling injury then either origination when introduced

introduced into steamed soil together, induced a greater de-

P. terrestris and P. variocinctus, a common

crushed, coriander, muskmelon, pepper, eggplant,

soybeans, peas, cicer, millet, oats, barley, wheat, corn, sugarcane,

crop plants. Plants shown to be susceptible to attack were:

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LITERATURE CITED


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