PATHOGENIC CAUSES OF PREMATURE DECLINE AND DEATH OF ALSIKE CLOVER, *TRIFOLIUM HYBRIDUM* L., IN CENTRAL OREGON

by

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PATHOGENIC CAUSES OF PREMATURE DECLINE AND DEATH OF ALSIKE CLOVER, TRIFOLIUM HYBRIDUM L., IN CENTRAL OREGON

INTRODUCTION

Oregon has been the leading state in production of alsike clover (Trifolium hybridum L.) seed over the past 18 years (54, p. 306), and 49, p. 7). Three geographic districts within the state have produced the majority of this seed; the Willamette Valley Counties, the Central Oregon Counties of Crook and Deschutes, and Klamath County. Alsike seed production in the Willamette Valley represented approximately half the state's total in the period 1936 to 1939, but today seed producing fields in this region are almost nonexistent. Central Oregon and Klamath County remain as the main producing regions.

The culture of alsike clover is markedly different in these two regions. Alsike is commonly planted during late August or early September in Klamath County. With sufficient irrigation the plants are up three to four inches by early winter. A snow cover usually assures adequate moisture during the winter months, and irrigation is resumed in the spring before lack of moisture becomes critical. Water is withdrawn from the plants in late summer to insure maturity of seed before frost damage. Harvesting usually is completed in September, and the plants are irrigated again to develop a vigorous

plant for over-wintering. Normally a second seed crop is cut the following September. The standard rotation in this region is two years of alsike clover followed by one year of potatoes and one year of a cereal crop, usually barley. Fall planted alsike is drilled into the cereal stubble.

In Central Oregon the same crops are grown in the standard rotation, but the sequence of planting is different. Alsike clover commonly is spring-planted with a cereal. Water is supplied until late summer when the cereal is dried off before harvesting. Unfortunately the clover rarely is watered after the cereal has been harvested. Water is usually unavailable to the farmer in this season because of a general shortage in the area. The first seed crop is cut the following fall from second-year plants. The clover is followed by one year of potatoes, and the following spring the alsike clover cereal mixture is again planted.

Although yields have been maintained at a high level in both regions, sometimes there has been considerable difficulty in getting a second seed crop in Klamath County. The problem has become so acute in Central Oregon that a second seed crop is no longer attempted. Total seed yield for this area is not a valid indication of clover failures, because fields with depleted stands are taken out of production in the spring of the second seed-crop year, or they are not harvested in the fall.

Failure of alsike clover stands in spring of the second seed-crop year was recorded by Hardison in 1949 (20, p. 11). In 1951

Hardison (22, pp. 21-36) demonstrated that the primary cause of plant death was the destruction of the root system by pathogenic fungi. Dying plants were depleted of fine rootlets, and often showed a severe wounding and rotting of the cortical tissue of primary and lateral roots. Insect injury was said to be an important associated factor, but soil fertility was eliminated as a possible direct cause of failures (22, pp. 28-29).

Hardison (24, pp. 22-41) also investigated the fungi associated with rootlet damage. However, several phases of the problem were not studied. An investigation of these unstudied phases forms the basis of this thesis. Observations were taken and experiments were designed to fulfill the following objectives: (1) enlarge the general survey of root diseases, (2) determine the nature and origin of crown tissue deterioration, (3) test new isolates from crown rots for pathogenicity, and (4) determine the causes of stunting and vascular discoloration in the Klamath area.

LITERATURE REVIEW

Alsike clover stand failures have been reported, but there are no specific studies of causes other than the unpublished work of Hardison (22, pp. 21-36) and 24, pp. 22-41). On the other hand there is a voluminous literature dealing with difficulties encountered in growing red clover, Trifolium pratense L. The similarity of problems attending the culture of these two plants necessitates a review of the pertinent literature on red clover failures as a background for the consideration of alsike clover root diseases. Many studies primarily concerned with failures of various other forage legumes have included an investigation of the relation of these failures to alsike clover. These papers will be reviewed when they relate to alsike clover failure in Oregon.

Fergus and Valleau (16, pp. 143-148) have traced reports of clover failures in both Europe and America that occurred during the last 200 years. Many early reports described clover difficulties as "clover failure" or "clover sickness", and often did not mention contributing factors or even the clover species involved. In Europe prior to 1870, clover sickness was generally considered to be due to the lack of some essential mineral or minerals, or to other unfavorable non-pathogenic soil factors (16, p. 145). In the 1870's Sclerotinia trifoliorum Erik. was demonstrated to be a causal agent of clover sickness, and there followed a new era of clover failure investigations in which the role of microorganisms as disease agents

was determined.

The role of fungi in clover failures in America was first indicated in reports from the North Carolina Agricultural Experiment Station in the early 1890's (37, p. 87), and 38, p. 151), and by 1952 there had been over 100 species of fungi reported on clovers (44, pp. 421-424), 6, pp. 10-20), and 53, pp. 658-664). Many clover failures resulting from disease have been attributed to stem and leaf pathogens, but these conditions are of minor importance in alsike clover failures in Central Oregon.

Losses and symptoms associated with root rots vary greatly from locality to locality and year to year. Second year failure of alsike clover has been reported from Kentucky (30, p. 29) where loss of roots during the first summer was said to be the main cause of death in the second year.

Cormack (12, p. 75) has indicated that moderate to severe losses in the alsike clover seed growing regions of Canada were due to winter crown rot caused by an unidentified Basidiomycete. Hardison (21, pp. 12-15) has described a second year alsike clover failure in Oregon that was due primarily to a rootlet rot. Other symptoms associated with clover losses include stunting, wilting, and red or yellow discoloration of the margin of leaflets. Additional below-ground symptoms include vascular discoloration and cortical rots of primary and secondary roots.

Although in most cases the direct cause of plant death was attributed to the action of pathogenic fungi, several predisposing

factors have been shown to exert a varying influence. According to Fergus and Valleau (16, pp. 200-205) the interaction of low soil fertility, unfavorable H-ion concentration, and poor soil aeration may cause inferior root development in both red and alsike clovers. The exact relation between root development and effects of root rot was not determined. Pieters (42, p. 21) has suggested that over-grazing or cutting too late in the fall of the first year were the most common forms of abuse of red clover stands. He recommends that plants be allowed to make at least six inches of growth before winter in areas where winters are snowless and severe. Wheeler (54, p. 305) makes essentially this same recommendation for alsike clover. Feene (15, p. 281) also attributes clover failure in Virginia to the combination of winter injury and fungus attack.

Probably the most universally reported predisposing factor is insect wounding of roots by the clover root borer Hylastinus obscurus Marsh. and various species of Sitona, the clover root curculios. According to unpublished reports of the Department of Entomology, Oregon State College, only Sitona hispidula (F.) has been found associated with alsike clover root damage in Central Oregon and Klamath County. Elliott (14, p. 41) believes the effective control of fusarial root rot of red clover depends on control of insects that feed on the root system. In Pennsylvania (41, p. 16) this same association of insect injury and Fusarium attack is said to have caused widespread loss of red clover stands during July and August, 1947. To be sure, insect wounding may present an excellent avenue

for fungus attack.

Although root rots of clovers are seldom the pathogenic expression of a single fungus, for reasons of clarity and convenience each pathogenic genus will be discussed separately.

Fusarium species.

Fusarium spp. are the most commonly isolated root pathogens of clovers (19, p. 219). In 1943 Chilton, Henson, and Johnson (6, p. 13) listed the following species that had been reported on alsike clover: F. avenaceum (Fr.) Sacc., F. moniliforme Sheldon, F. oxysporum Schlecht., F. scirpi var. acuminatum (Ell. and Ev.) Wr., F. solani (Mart.) Appel and Wr., F. solani var. martii f.3 Snyder, and F. vasinfectum Atk. Hardison (24, p. 39) has isolated F. lateritium, and Gordon (18, p. 588) isolated F. acuminatum Ell. & Ev., F. avenaceum (Fr.) Sacc., F. equiseti (Cda.) Sacc., F. moniliforme Sheld. emend. Snyder & Hansen, F. oxysporum Schlect. emend. Snyder & Hansen, and F. poae (Pk.) Wr. from Canadian seed samples of alsike clover. Several earlier reports (55, pp. 158-159), 56, p. 63), and 27, p. 78) have indicated the presence of Fusarium species among isolates from rotting clover roots and crowns, but only one species determination was made. Young (55, pp. 158-159) has described one isolate as the macroconidial (Fusarium) stage of Gibberella saubinettii.

Fergus and Valleau, (16, pp. 197-199) cultured F. moniliforme, F. solani (Mart.) App. et Wr., and F. oxysporum from small rotting

rootlets of alsike clover. Red clover and alfalfa plants with similar symptoms yielded the same three species of Fusarium. The pathogenicity of these Fusarium species was established with seedlings grown in tubes on nutrient agar.

According to Cormack (10, p. 500) F. avenaceum (Fr.) Sacc. produced light to moderate infection on roots of alsike clover during winter and summer. However, F. culmorum (W. G. Sm.) Sacc. was considered to cause more damage in the summer on roots of Trifolium spp. The complexity of this problem was demonstrated by the fact that seedlings of wheat, barley, and oats were attacked by isolates of Fusarium from legumes and vice versa. Cormack believes that Fusarium spp. live over in Canadian soil on the roots of wild legume hosts.

In 1951 Hardison (22, pp. 29-34) isolated F. roseum, F. oxysporum, F. solani and F. lateritium from black rootlets of alsike clover grown in Oregon. These species represented 27 percent of the fungi cultured from diseased rootlets of Central Oregon alsike, while for Klamath County plants this percentage dropped to 9.3. The most prevalent isolate was an unidentified species of Phoma. However, rots of primary and lateral roots yielded 41.9 percent Fusarium spp. from Central Oregon plants, and 47.7 percent from Klamath County alsike. The frequency of isolation of the individual species was not determined.

Fusarium spp. alone did not produce black rootlet rot symptoms, but stunting, leaf reddening, and occasionally damping off occurred (22, p. 35). Pathogenicity tests were carried out by incorporating

oat cultures of the fungi into sterile soil before planting with surface disinfected seed. Hardison (24, pp. 39-40) was able to reproduce the lesions on primary roots, but the severity generally was rated as mild. A number of legumes and grasses were seeded in the greenhouse in field soil from three locations in Central Oregon. Fusarium spp. were recovered from diseased rootlets and primary roots of Melilotus alba, Pisum arvense (Austrian Winter), Trifolium pratense, and Vicia villosa.

Kreitlow and Hanson (33, p. 16), and Kilpatrick, Hanson and Dickson (32, p. 256) found that Fusarium spp. were most prevalent among isolates from roots of red clover plants grown in Pennsylvania and Wisconsin, respectively. In Pennsylvania severe depletion of second year stands was attributed mainly to the action of F. solani and F. oxysporum. The pathogenicity of Fusarium isolates was demonstrated by placing inoculum in contact with scarified or pruned tap roots of young plants. Increased infection was observed with soil temperatures above 30 degrees C.

In Wisconsin cortical rots and vascular discoloration are commonly observed symptoms associated with losses of first- and second-year red clover plants (32, pp. 254-257). The dominant species isolated, which represented approximately 65 percent of all isolates, were F. oxysporum, F. solani and F. roseum. Fusarium moniliforme occurred in one percent of the cases. The pathogenicity of these fungi was demonstrated only on seedlings grown in test tubes (31, p. 293).

Phoma species.

Phoma spp. are most commonly associated with black-stem disease of legumes. However, some species are root pathogens. Valleau and Fergus (50, pp. 507-509) first described black-stem symptoms on alfalfa, red clover, and sweet clover, and later Johnson and Valleau (28, pp. 69-75) found that the causal agents on these hosts were respectively: *Phoma medicaginis* Malbr. and Roum., *Phoma trifolii* Johnson and Valleau, and *Mycosphaerella lethalis* Stone. Cormack (11, p. 840) isolated unidentified species of *Ascochyta* and *Phoma* from black-stem lesions on alsike and red clovers in Canada, and Kilpatrick, Hanson, and Dickson (32, p. 257) found unidentified species of *Phoma* among their isolates from red clover roots.

Phoma trifolii was first isolated from red clover stems, leaves, petioles, and decaying rootlets near the surface of the soil (28, pp. 72-73). This species caused black-stem symptoms when inoculated on red clover, alfalfa, and sweet clover stems, but root pathogenicity was not determined. Leach and Elliott (34, p. 1042) isolated *P. trifolii* from black-stem lesions on red clover in West Virginia. Hardison (24, pp. 37-38) found that this species is of minor importance in alsike failure in Oregon. *P. trifolii* was isolated from Klamath County only, and represented much less than one percent of all *Phoma* isolates. However, inoculation tests carried out under greenhouse conditions showed this species to be pathogenic on alsike roots. Infected rootlets were light brown,

and 20 to 40 percent were completely killed. Vascular discoloration occurred in approximately 20 percent of the 119 plants inoculated. Artificial wounding of the roots three and one-half months after initiation of the experiment had no effect on the severity of disease.

Phoma medicaginis has been most widely reported as an alfalfa pathogen. Due to the fairly frequent occurrence of uniseptate spores this organism has been named Ascochyta imperfecta Peck. Toovey, Waterston, and Brooks (48, pp. 709-711) also considered the two synonymous but preferred the Ascochyta designation, even though they observed a great preponderance of unicellular spores from pycnidia produced on artificial media.

Melchers (39, p. 183) reported A. imperfecta from clover in 1924 and indicated that this fungus was the cause of a leaf spot of red clover. Sprague (45, pp. 924-925), while studying A. imperfecta cultures from alfalfa, demonstrated that these isolates were also pathogenic on alsike and red clovers. Root inoculations were not made. Dr. Hardison, in conversations with the author, has reported the rare appearance of this species among alsike clover rootlet isolates from Central Oregon and Klamath County.

The most important fungus species associated with rootlet rot of alsike clover in Oregon according to Hardison (22, pp. 21-36), 24, pp. 22-41), and 23, p. 514), is an unidentified species of Phoma designated as Phoma X (24, p. 33).

Phoma X constituted 40.5 percent of the isolates from black

rootlets of alsike clover from Central Oregon, and 53.3 percent from Klamath County plants. From Central Oregon isolations from rots of primary and lateral roots yielded 29.4 percent Phoma X, and 20 percent of the isolates from Klamath County plants were Phoma spp. (22, pp. 29-33). Phoma X has also been isolated from rootlets of the following plants grown in Central Oregon field soil in the greenhouse: Melilotus alba, Vicia villosa, Lotus corniculatus, Medicago sativa, Trifolium fragiferum, T. hybridum, T. pratense, T. repens and T. repens var. ladino (24, pp. 23-33).

After incorporating oat cultures of Phoma X into steam sterilized field soil, black rootlet rot symptoms were noted, and Phoma X was reisolated from Trifolium fragiferum and T. hybridum. Failure to reisolate Phoma X from discolored rootlets of the grasses and cereals grown in these tests prompted Hardison (24, p. 36) to conclude that these Gramineae are not attacked by Phoma X and, therefore, might be useful in reducing root rot if used more and for longer periods in the crop rotations of Central Oregon.

Similar greenhouse tests have shown the over-all importance of Phoma X as the major cause of severe killing of small roots of alsike clover (22, p. 34). Further significance of this species is demonstrated by its role in causing vascular discoloration (24, pp. 36-37). Phoma X was the only pathogen that was uniformly recovered from artificially wounded and unwounded plants.

Stagonospora species.

Stagonospora spp. have long been considered as leaf pathogens of legumes, but in 1938, Jones and Weimer (29) found that species of this genus are root rot pathogens of forage legumes. S. meliloti (Larsch) Petr. was recovered from reddish brown lesions of the tap root and crown branches of alfalfa in Wisconsin and California. Greenhouse inoculation studies showed that these symptoms could be reproduced in three months by placing bits of fungus mycelia from agar cultures in artifical wounds on the tap root (29, p. 796). Jones and Weimer (29, p. 798) made no attempt to inoculate alsike clover, but cultures of S. meliloti from alsike were proved to be root pathogens of Melilotus alba.

Conners (7, p. 16) has reported that S. meliloti also produces a leaf spot on alsike clover. Hardison (24, p. 40), however, has never isolated this species from root tissue in several thousand plantings, although abundant perithecia of Leptosphaeria pratensis found on overwintered alsike straw in one field in Central Oregon produced the pycnidial stage (S. meliloti) when ascospores were cultured.

Rhizoctonia species.

Cherewick (5, p. 674) found that Rhizoctonia solani Kühn caused seedling damping off as well as death of older sweet clover plants in Canada. Isolates from this host were pathogenic on alsike clover in artificial inoculation studies. Rhizoctonia solani was considered

to cause the early phases of sweet clover rot in the field, while Fusarium spp. were associated with later stages.

According to Benedict (2, p. 217), R. solani Kühn can cause seedling stunting of alsike as well as other clovers. This symptom was attributed to cankers produced by the fungus in the cortical tissues.

Ducomet (13, p. 371) made the interesting observation that R. violacea would not attack strong, well developed alfalfa plants in inoculation trials but was definitely harmful to weak plants. The relation of this observation to failure of crops on shallow soils is indicated. Species of Rhizoctonia are commonly isolated from soil the world over and have been associated with root rots of most crops. Bruehl (4, pp. 375-377) has studied this genus and its relation to cereal root rot. The association of Rhizoctonia with potato "Black Scurf" has been known for many years.

Cylindrocarpon species.

In 1937 Cormack (9, pp. 404-410) reported a root rot of alfalfa caused by Cylindrocarpon spp. The results of fungus attack were most evident at the end of the dormant period, and infection was found to occur only rarely during the growing season. Root lesions first appeared as water-soaked areas that soon turned light brown and finally dark brown. In severe cases entire roots were rotted in one to two weeks. The major cause of these symptoms was C. Ehrenbergi Wr., although C. obtusisporum (Cke. & Hark.) Wr., and C. radicicola

Wr. were demonstrated to be mildly pathogenic. By artificial inoculation studies C. Ehrenbergi, and C. obtusisporum displayed mild pathogenicity on alsike clover roots.

Miscellaneous species.

Various species of Colletotrichum, Gleosporium, and Kabatiella have been reported on alsike clover associated with the well known anthracnose symptoms of stem and leaf blights. Kilpatrick, Hanson, and Dickson (32, p. 257) have isolated C. destructivum from diseased red clover roots in Wisconsin.

Sanford (43, pp. 337-348) found that Plenodomus meliloti Dearn. & Sanf. was the cause of a root rot of sweet clover and alfalfa that appears in the field during early spring. Conners (8, p. 30) isolated this fungus from alsike clover in Canada where it was associated with a trace of root rot of field plants.

The following fungi have been demonstrated as pathogens of alsike clover roots, although they were originally isolated from other host plants: Leptodiscus terrestris Gerdemann (17, pp. 548-549), Thielavia basicola Zoph. (36, p. 39), and Streptomyces scabies (Thaxt.) Waksman & Henrici (26, p. 452).

Hardison (22, p. 34) occasionally found species of Verticillium, Sporotrichum, Chaetomium, Xylaria, and Gliocladium among isolates from rootlets of field-grown alsike clover in Oregon.

Kilpatrick, Hanson, and Dickson (32, p. 257) recorded the isolation of the following fungi from red clover root rot in amounts

ranging from less than 0.5 percent of the total isolates to 4 percent: Gliocladium roseum, Alternaria spp., Mucor spp., Penicillium spp., Aspergillus spp., Chaetomium spp., Pythium spp., Trichoderma spp., Nigrospora, Helminthosporium, Epicoccum, Coniothyrium, Stemphylium, Curvularia, Rhizopus, Hormodendrum, Sphaeropsis, Chaetomella, Geotrichum, Thielavia, Totteria, Monilia, and Zythia. A fungus that appeared to be Verticillium was also isolated.

GENERAL METHODS AND MATERIALS

Several methods and materials that were used repeatedly will be described in this section. Special techniques used only in particular experiments will be included later along with the respective experiment.

Field sampling.

At least ten plants were dug from each of four or more sites selected at random within the field. Loose soil was shaken from the roots, and the plants were placed in paper sacks. An attempt was made to keep all plant material cool and moist in transit from field to laboratory. The plants were then thoroughly washed under running water, and symptom ratings and selection of plant parts for isolation were made as soon as possible. If delays were necessary the plants were stored in moistened, clean paper sacks in a refrigerator.

Culture media.

Potato-dextrose-agar, "PDA", was prepared from the following formula:

Infusion from 200 grams of peeled potatoes

Dextrose, 20 grams

Agar, 20 grams

Distilled water to make 1 liter.

This medium varied from pH 6.5 to pH 7.0 after autoclaving for final sterilization. A second medium was prepared by the same formula with

sufficient lactic acid to lower the pH range to 3.5 - 4.0 to favor growth of fungi over bacteria. The acid was added after final sterilization. This medium was designated "Acid-PDA".

Isolation techniques.

Root sections were selected for isolations that included the margin between healthy and diseased tissue. Selected tissues were again thoroughly washed under running water. The root sections were stored in a refrigerator in Petri plates containing a few milliliters of tap water, if isolations could not be made immediately.

All plant materials were surface sterilized in a 1:4 solution of commercial sodium hypochlorite in water. The final solution was approximately one percent active ingredient. Root sections, according to their size and the severity of rot, were submerged in the sterilant for one to five minutes. Rinsing in sterile water was not necessary due to lability of the sterilant.

Tissues were then plated in Petri plates on PDA and Acid-PDA. Ten separate bits of tissue were cut from each of the larger surface-sterilized sections. After each cut the scalpel blade was flamed, cooled in 95 percent ethanol, and reflamed to burn off the alcohol. Each bit of tissue was pushed into the surface of the medium so that only the top surface of the tissue was exposed. The result of this tissue selection and plating technique was an Acid-PDA and a PDA plate that each contained five pieces of tissue representing a sample of a particular symptom from one plant.

Isolations from plants showing vascular discoloration were made by first dissecting the cortical tissue from the vascular cylinder. The vascular tissue was then cut into bits approximately two millimeters in length. Rootlets were similarly cut into two millimeter sections, but no attempt was made to separate the cortex from the vascular cylinder. Cortical tissues were plated separately except in cases of severe rotting where the discoloration had penetrated into the vascular tissues.

The Petri plates with root sections were incubated at room temperature. As colonies appeared hyphal tip transfers were made to PDA slants. All fungi were maintained on this medium.

Greenhouse cultural methods.

Clover plants used in the various experiments were grown in a sandy loam, peat moss, and sand mixture in standard greenhouse flats. All components were mixed thoroughly, screened, and autoclaved before planting. A complete commercial fertilizer was added when the plants were transplanted for inoculation. All plants were well nodulated within the first month, and inoculation of the soil with Rhizobium trifolii Frank was unnecessary. The nodulation was probably due to soil contamination occurring in a greenhouse in which legumes have been grown continuously for the past five years.

Oregon grown commercial alsike clover seed was used throughout this investigation. All seeds were either surface-sterilized for 10 minutes in the previously described one percent sodium hypochlorite

solution and allowed to dry, or treated with Arasan before planting.

Terminology

The term "cortex" is commonly misused in describing secondary tissues outside the cambium in older clover roots. Actually the cortex is sloughed off at the time secondary thickening begins. The so-called "cortical tissues" include phloem, pericycle, and cork tissues. Since cortex has been used universally to describe this tissue it will be maintained here with the understanding that other tissues are involved.

"Tap root" and "primary root" are synonymous. All roots arising from the primary root will be termed "secondary" or "lateral roots", and those originating from secondary roots have been termed "rootlets" or "tertiary roots".

The age of field plants will be given with reference to the planting date. Plants collected on the same date from both areas will be of different ages, because alsike clover is fall-planted in Klamath County and spring-planted in Central Oregon. Spring-planted alsike in Central Oregon will be termed "first-year clover" until the following spring, when it will be termed "second-year clover". The first seed crop is cut from "second-year clover". A second seed crop if attempted would be cut from "third-year clover". In Klamath County fall-planted alsike is harvested the next fall. Thus the first seed crop is harvested from "first-year clover", and a second seed crop is harvested from "second-year clover".

Pathological histology.

Root tissues were killed and fixed in formalin-aceto-alcohol, dehydrated with tertiary butyl alcohol, and embedded in paraffin. The embedded tissues were softened prior to sectioning by paring away the wax to expose the tissue and soaking the paraffin blocks overnight in a mixture of 10 milliliters of glycerol, 1 gram of Dreft, and 90 milliliters of water (1, pp. 55-56). The embedded tissue was sectioned at a thickness of eight to twelve microns with a rotary microtome. The best staining results were obtained with a regressive iron hematoxylin-safranin schedule.

FIELD OBSERVATIONS AND DISEASE SYMPTOMS

Since initiation of this investigation in 1952, no clover fields that were carried over into a second seed-crop year could be located in Central Oregon, and no failures were observed in second seed-crop clover stands in Klamath County. Although the actual failures as described by Hardison (22, p. 21) were not observed, ample evidence of their reality was often detected in plants collected the preceding fall.

A system of classification and disease severity rating was established to evaluate the frequency of the several root rot symptoms. Root rot symptoms were classified as rootlet rots, cortical rots, and vascular discoloration. The symptom-severity classes, (none, slight, moderate, and severe), were determined for each symptom after the total variation was observed in a large sample of field plants.

Rootlet rot.

Black rootlet rot was previously described by Hardison (22, pp. 21-22). All degrees of rootlet discoloration from light brown through dark brown to black were observed (Figures 1 and 2). In many cases the most severely rotted rootlets were lost when the plants were dug, but discolored rootlet stubs on secondary roots indicated the fate of the missing rootlets. The following symptom-severity classes were delimited to rate rootlet rot in field plants.

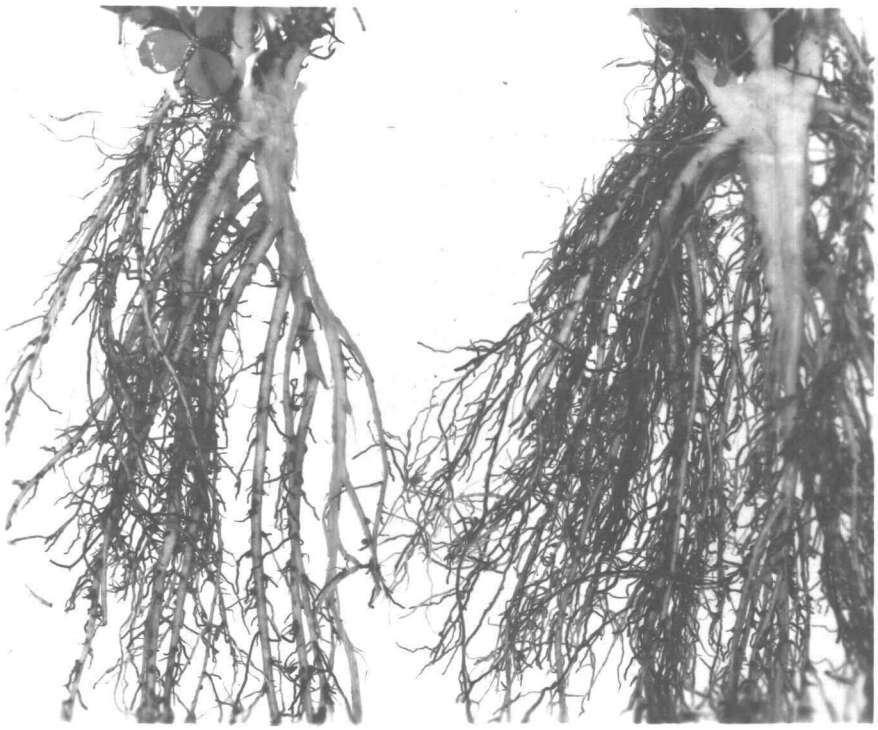

Figure 1. Portions of two alsike clover plants with severe rootlet rot. X1.

Figure 2. Secondary root of an alsike clover plant with dead rootlets. X5.

None — No discolored rootlets or rootlet stubs on secondary roots.

Slight — One-tenth to 10 percent discolored rootlets and/or loss of rootlets as judged by discolored rootlet stubs.

Moderate — Eleven to 30 percent discolored rootlets and/or loss of rootlets as judged by discolored rootlet stubs.

Severe — Over 30 percent discolored rootlets and/or loss of rootlets as judged by discolored rootlet stubs.

Cortical rot.

Early stages of cortical rotting appeared as light brown, water-soaked areas on the exterior of the primary and lateral roots. The lesions increased in size, and turned dark brown or black as the tissues became necrotic. Several lesions coalesced to girdle the root and to extend several centimeters vertically (Figure 3). In later stages the discoloration which was initially limited to the cortical tissues often penetrated the secondary xylem.

Discoloration of the xylem tissue was limited to parenchyma cells, and was found first in the xylem rays. Discoloration of xylem parenchyma was extended vertically several centimeters in cases of severe rotting and was connected with pith discoloration of the lower stem. Extreme severity of this condition frequently

Figure 3. Portions of two alsike clover plants with severe cortical rot. X1.

resulted in a severing of the primary root just below the soil level, in which case death of the plant was delayed only by adventitious roots arising from stem tissue. Such plants were too weak to produce a seed crop of any value, although with abundant soil moisture they often stayed alive.

The development of cortical rots was further studied from permanent slides prepared from diseased plant materials collected at different locations and different times of year in Klamath County and Central Oregon. Two types of margins between healthy and diseased tissues were found. One type had a definite cork cambium walling off the diseased tissues (Figures 5 and 6). The second type was distinguished by the lack of a cork cambium (Figures 4 and 7).

The tissues usually were attacked more severely in roots in which a cork cambium was initiated. When extreme disorganization of the diseased tissues occurred, fungus mycelia and nematodes were frequently present several cells outside the cork cambium (Figures 5 and 6). The fact that no dead cells or fungus mycelia were found inside the cork cambium suggests that the initiation of a cork cambium arrested the advance of parasitic fungi in the clover roots. In several roots the cork cambium was only three or four cells outside the vascular cambium.

Tissues in which a cork cambium had not formed were characterized by the lack of disorganization of the dead tissues (Figures 4 and 7). Much less fungus mycelia was found away from the disease margin, and no nematodes were discovered. This suggests rapid

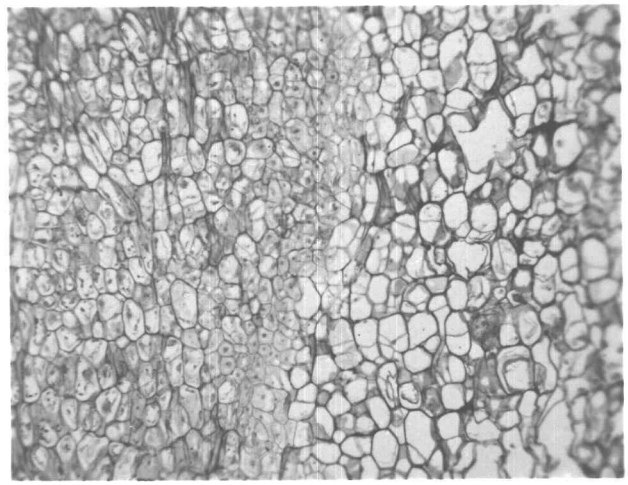

Figure 4. Portion of a longitudinal section of the primary root of a two year old alsike clover plant with the boundary between healthy and diseased cortical tissues. The diseased tissues are on the right. X100.

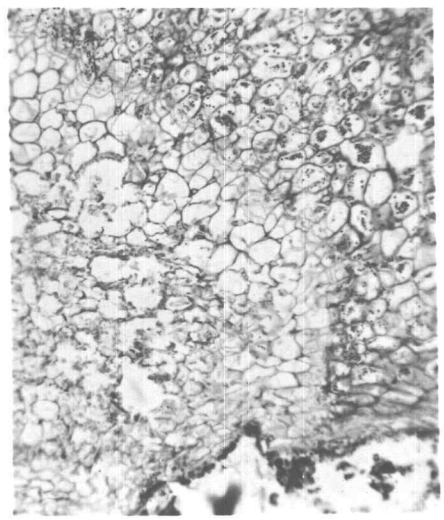

Figure 5. Portion of a cross section of the primary root of a two year old alsike clover plant with the boundary between healthy and diseased cortical tissues. The diseased tissues are on the left. X100.

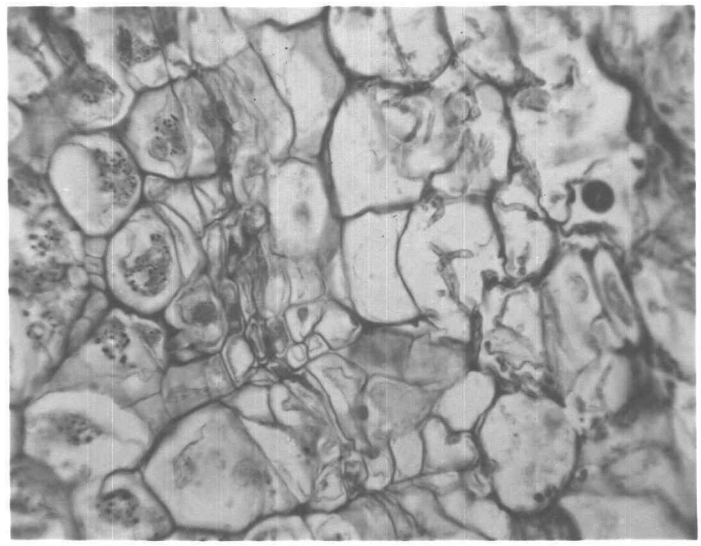

Figure 6. Portion of a cross section of the primary root of a two year old alsike clover plant with a cork cambium between healthy tissues (on the left) and diseased tissues (on the right). Note the fungus mycelia and the cross section of a nematode in the diseased tissues. X400.

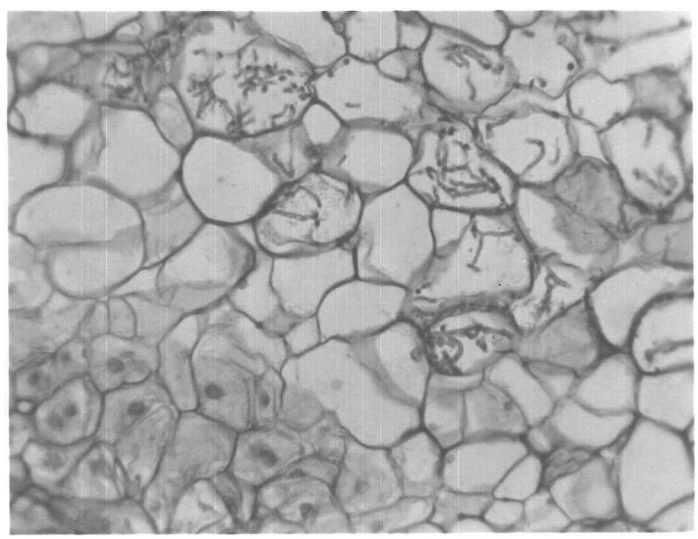

Figure 7. Portion of a cross section of the primary root of a two year old alsike clover plant with the boundary between healthy tissues (lower left) and diseased tissues. Note the extensive development of fungus mycelia in the diseased tissue and the lack of a cork cambium. X400.

penetration of the root tissues by the primary parasites without accompaniment of secondary organisms, and before wound cork can be laid down. The possibility exists that diseased tissues lacking secondary organisms and a cork cambium were merely early stages of cortical rot in which a cork cambium would have been initiated later. Some roots with no cork cambium had rotted a short distance into the phloem parenchyma and the possibility remained that wound cork could have been initiated before the disease reached the secondary xylem. However, in other roots with no cork cambium, the rot had progressed to the vascular cambium. Insufficient diseased plant material was collected at various times of year to discover the relation between environmental conditions and cork formation by the clover plants. In roots that showed no cork cambium possibly fungus attack occurred at soil temperatures that were unfavorable for wound cork formation.

The same symptom-severity classes used to express rootlet rotting were maintained to rate cortical rots, but the class definitions were suited to cortical symptoms. Cortical symptoms of secondary roots were included with those of primary roots because of the similarity of disease development and causal agents.

None — No cortical discoloration of primary or secondary roots. Insect wounds were allowed in this class if the wounds were healed over by cork tissue.

Slight — Up to three small discolored lesions: not coalesced or girdling the root. Penetration limited to the cortical tissues.

Moderate — Exterior of the primary and/or secondary roots with four or more lesions, but not more than 30 percent of the surface area of the roots covered by coalesced lesions. Penetration limited to the cortical tissues.

Severe — More than 30 percent of the surface area of the root covered by lesions, and frequent discoloration of the secondary xylem parenchyma.

Vascular discoloration.

Vascular discoloration results from the deposition of resinous materials in the vascular elements of the xylem (Figures 8 and 9). This symptom, although commonly associated with fungus attack, may be caused by various physiological disturbances (35, pp. 15-17). Isolations from diseased tissues are always necessary to establish the relationship of parasitic fungi with the vascular symptoms. Vascular discoloration is distinguished from the discoloration in the secondary xylem associated with cortical rot in that the former is the result of plugged vessels, while the latter is caused by infected parenchyma cells. Vascular discoloration may or may not be accompanied by cortical symptoms, while discoloration of the secondary xylem parenchyma is never found without an associated cortical rot.

All degrees of severity from a few plugged vessels to almost 100 percent plugged vessels have been found, but there was always

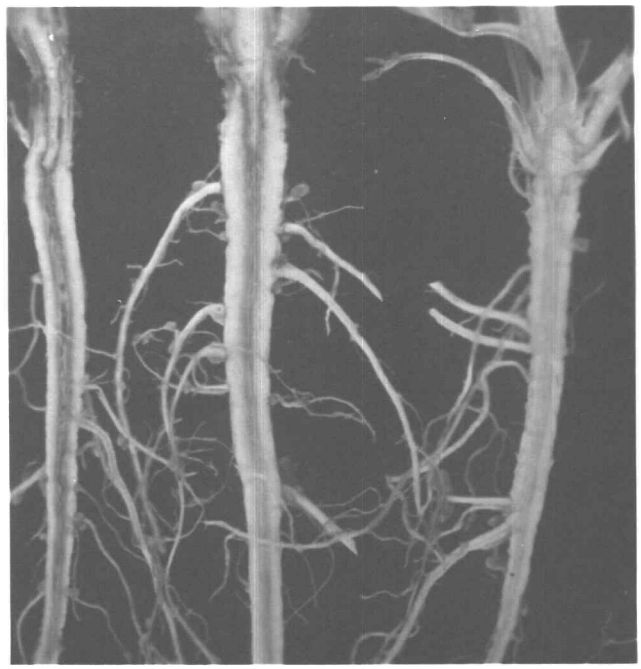

Figure 8. Portion of the primary roots of alsike clover plants with vascular discoloration. The plant on the right is healthy. X1.

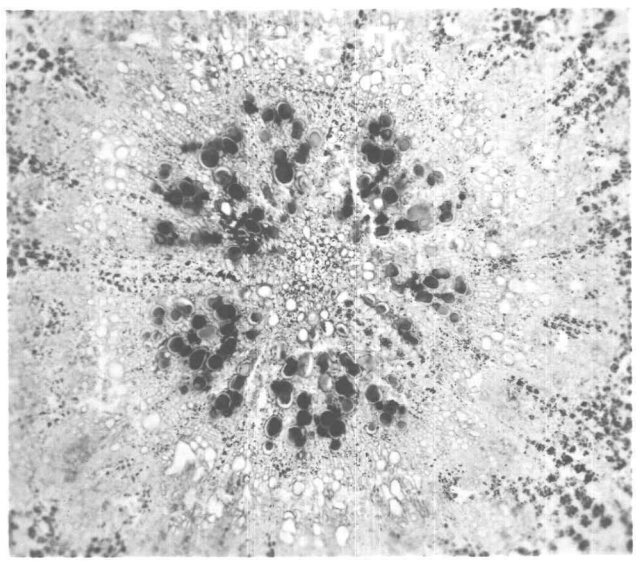

Figure 9. Portion of a cross section of a secondary root of a one year old alsike clover plant with vascular plugging. Note the unaffected vessels on the outer circumference of the secondary xylem. X150.

a ring of unaffected vessels around the outside of the secondary xylem (Figure 9). Evidently the clover plant may be maintained by few functional xylem vessels which would explain why infected plants with severe vascular discoloration seldom wilt in the field as would be expected from vascular attack.

Early stages of vascular attack were distinguished by fungus mycelia in unplugged vessels (on the left of Figures 12 and 13), but the fungus mycelia was frequently found in the resinous material within the vessels (Figures 10 and 13). However, some fungi associated with vascular plugging were seldom found in the plugged vessels and were difficult to find because of their small hyphal dimensions.

In advanced stages of vascular disease there was considerable distortion of the secondary xylem due to proliferation of the xylem parenchyma around infected vessels (Figure 14). In healthy secondary xylem there was an orderly arrangement of vessels and parenchyma in radial rows (Figure 15).

Symptom-severity classes used to evaluate the importance of vascular discoloration were as follows:

None	No discolored (plugged) vascular elements in the secondary xylem of primary or secondary roots.
Slight	Discoloration localized in one to five vessel elements of the primary or secondary roots.
Moderate	Discoloration present in one-third of the vascular elements, but not covering the

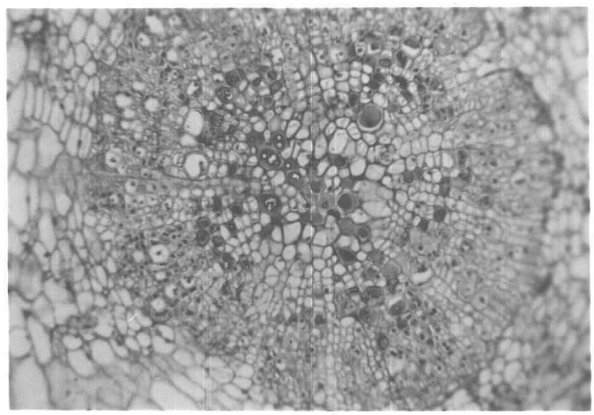

Figure 10. Portion of a cross section of a secondary root of a one year old alsike clover plant with plugged vascular elements and associated fungus mycelia. X150.

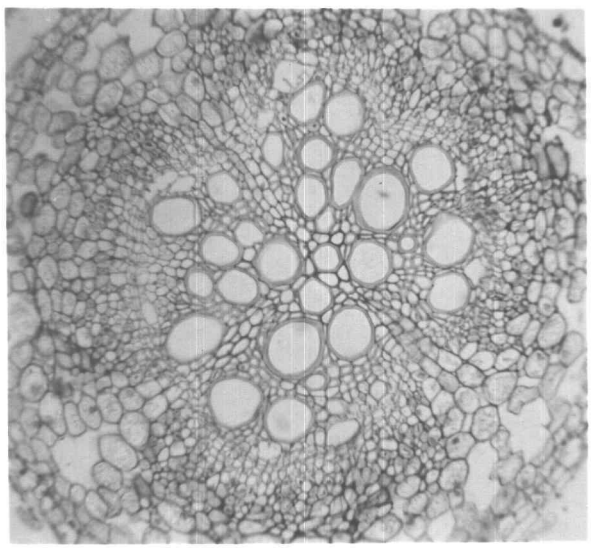

Figure 11. Portion of a cross section of a secondary root of a one year old healthy alsike clover plant. X150.

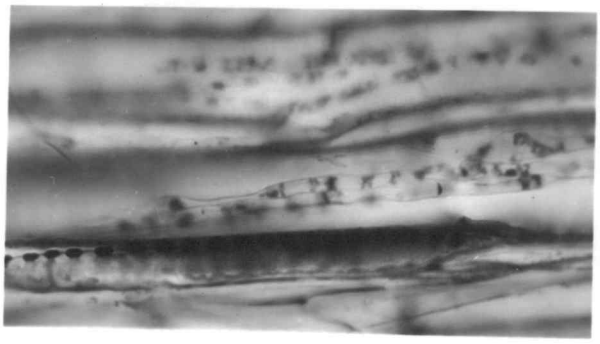

Figure 12. Portion of a longitudinal section of the primary root of a two year old alsike clover plant with fungus mycelia in the vascular elements. X700.

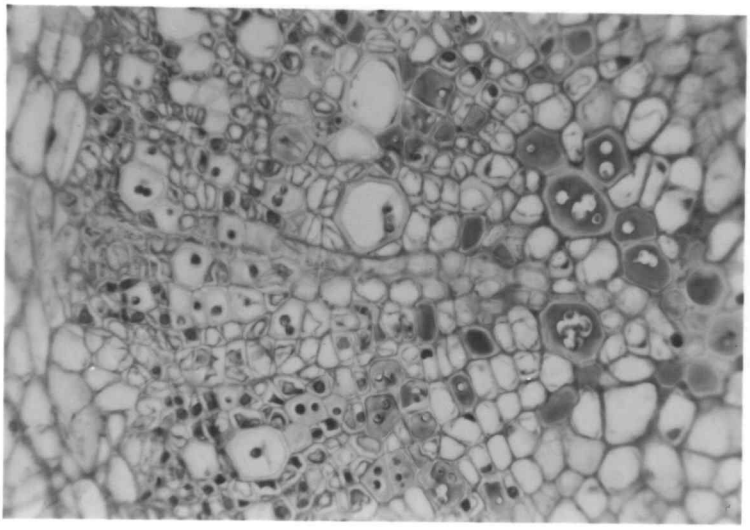

Figure 13. Portion of a cross section of a secondary root of a one year old alsike clover plant with fungus mycelia in plugged and unplugged vascular elements. X300.

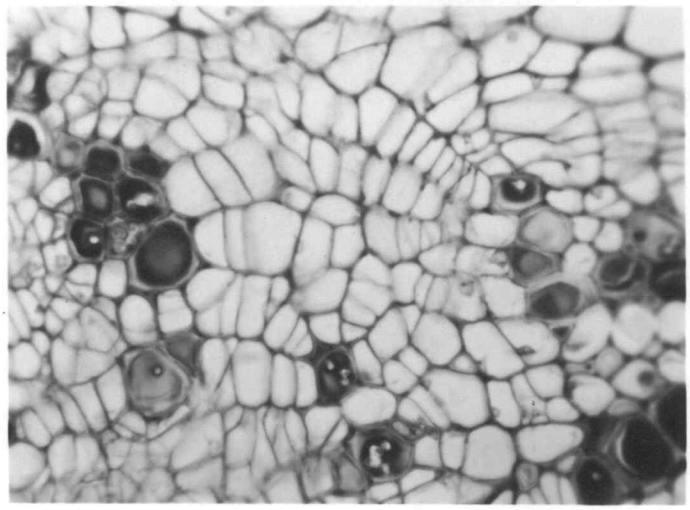

Figure 14. Portion of a cross section of the primary root of a two year old alsike clover plant with proliferation of the secondary xylem parenchyma around plugged vascular elements. X400.

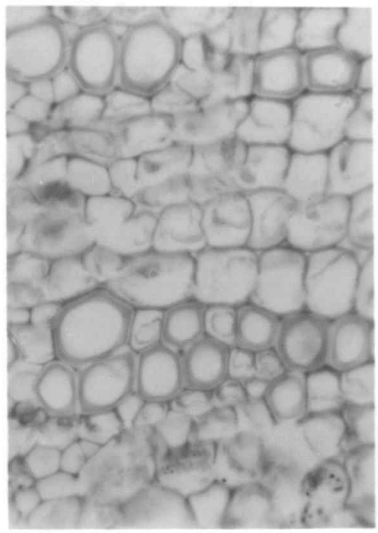

Figure 15. Portion of a cross section of the primary root of a two year old healthy alsike clover plant in the region of the secondary xylem. X400.

complete circumference of the secondary xylem or spread throughout the primary and secondary roots.

Severe — Over one-third of the vascular elements discolored. Distributed throughout the primary and secondary roots, and/or present in the entire circumference of the secondary xylem.

Miscellaneous disease symptoms.

Stem and petiole lesions were observed so infrequently that their severity and frequency were not recorded. "Leaf spot" due mainly to the conidial stage, Polythrincium trifolii Kunze & Schmidt, of Cymadothea trifolii (Pers.) Wolf is present in Klamath County and Central Oregon. This fungus has never been identified among isolates from root rots.

Typical winter injury symptoms seldom were encountered in Central Oregon or Klamath County. This condition when present was distinguished by a light brown discoloration of all root and stem tissues at the soil surface level.

Symptom frequency.

Table 1 summarizes the frequency of symptoms and symptom-severity classes in first-, second-, and third-year alsike clover plants from Central Oregon. The observations were taken from 192 first-year plants collected from five fields, 323 second-year plants from six fields, and 63 plants from two third-year fields. The two

third-year fields were not maintained for seed production. One was pastured. The other was a strip remaining from a previous seed-crop stand that received water by drainage from a nearby potato planting.

Table 2 summarizes the frequency of symptoms and symptom-severity classes in first-, and second-year plants from Klamath County. The observations are based on 172 first-year plants from five fields and 192 second-year plants from five fields.

The plants were collected in 1952, 1953, and 1954. Data taken at various times of year over the three-year period were combined under the age of the plants. The majority of plants were collected during the growing season, and the results in Tables 1 and 2 are from comparable random samples. Additional plants were examined, but because the samples were selected for specific symptoms or from special locations, they could not be included in the summary ratings. None of the fields analyzed deviated radically from the results given in Tables 1 and 2.

Several trends are evident by summing the percentages of plants with disease symptoms from Central Oregon and Klamath County. However, plants from the two regions are not exactly comparable, due to the difference in planting dates.

Rootlet rot was the most important disease of first-year plants from both regions. Second- and third-year plants from Central Oregon and second-year plants from Klamath County were 100 percent affected, but plants from Central Oregon were significantly more severely attacked.

Table 1. The frequency of symptom-severity classes in first-, second-, and third-year alsike clover plants from Central Oregon in 1952, 1953, and 1954.

Symptoms	Age of plants in years*	Percentage of plants in each symptom-severity class			
		None	Slight	Moderate	Severe
Cortical rot	1	79.2	9.2	11.5	0.0
	2	22.0	25.7	36.5	15.8
	3	0.0	6.3	9.5	84.1
Vascular discoloration	1	94.3	3.6	2.1	0.0
	2	87.9	7.7	4.3	0.0
	3	49.2	3.2	41.3	6.3
Rootlet rot	1	51.6	19.3	29.2	0.0
	2	0.0	12.4	70.3	17.3
	3	0.0	0.0	14.3	85.7

* Observations based on 192 first-year plants from five fields, 323 second-year plants from six fields, and 63 third-year plants from two fields.

Table 2. The frequency of symptom-severity classes in first- and second-year alsike clover plants from Klamath County, Oregon in 1952, 1953, and 1954.

Symptoms	Age of plants in years*	Percentage of plants in each symptom-severity class			
		None	Slight	Moderate	Severe
Cortical rot	1	65.9	31.8	1.7	0.6
	2	13.0	4.2	67.7	15.1
Vascular discoloration	1	84.4	5.8	9.8	0.0
	2	56.8	7.3	13.5	22.4
Rootlet rot	1	42.2	43.3	14.4	0.0
	2	0.0	12.5	82.3	5.2

* Observations based on 172 first-year plants from five fields, and 192 second-year plants from five fields.

The incidence of cortical rots, although much lower than rootlet rot in first-year plants, increased rapidly in the second year in both areas. In Central Oregon 20.9 percent of the plants showed cortical rot symptoms the first year, while 78.0 percent were infected the second year. Klamath County plants showed approximately the same increase of cortical rot. The Central Oregon plants were more severely attacked, and third-year stands were so heavily damaged that hardly any root parenchyma tissue was unaffected in 84 percent of the plants.

Vascular discoloration was significant in third-year, Central Oregon plants. Plants of this age were so severely rotted in all tissues that penetration of vascular elements was not considered important. However, in Klamath County vascular discoloration was of much greater importance, because second-year plants were 43.2 percent affected.

Clover stands must be carried into a third year of plant growth to harvest a second seed crop in Central Oregon. However, by the end of the second year 100 percent of the plants showed rootlet rot symptoms, and 78 percent had cortical lesions. The resulting loss of absorptive tissues, and the great reduction of stored food materials in the cortical region appear to be important factors limiting plant growth in the third-year stands. Second-year plants are equally diseased in Klamath County. As the second seed crop is harvested from second-year plants the disease problem

is not a limiting factor since third-year stands are not attempted in this area.

FUNGI ISOLATED FROM DISEASED PLANTS

Relation of the age of plant to fungi isolated.

The frequency of fungi isolated from all diseased tissues of first-, second-, and third-year alsike clover plants from Central Oregon was determined (Table 3). The observations were taken from 665 tissue isolations from first-year plants, 1006 isolations from second-year plants, and 501 isolations from third-year plants. Five first-year fields, six second-year fields, and two third-year fields are represented. The plants were collected in 1952, 1953, and 1954.

Table 4 gives the corresponding data for isolations from all diseased tissues of first- and second-year alsike clover plants from Klamath County. The observations were taken from 548 tissue isolations from first-year plants, and 552 isolations from second-year plants. The plants were collected from five first- and five second-year fields in 1952, 1953, and 1954. All tissues selected for isolations were taken from plants collected at random from the various fields.

Phoma spp. were the most commonly isolated fungi from first- and second-year plants in both Central Oregon and Klamath County (Tables 3 and 4). However, from third-year Central Oregon plants Fusarium spp. were the most frequently isolated fungi. Fusarium roseum (Lk.) emend. Snyder & Hansen was the most frequent species followed by F. oxysporum Schlecht. emend Snyder & Hansen and F. solani App. & Wr. emend. Snyder & Hansen. Rhizoctonia spp. were

Table 3. The frequency of fungi isolated from first-, second-, and third-year alsike clover plants grown in Central Oregon in 1952, 1953, and 1954.

Fungi isolated	1st year plants		2nd year plants		3rd year plants	
	Frequency*	Percent**	Frequency	Percent	Frequency	Percent
Phoma spp.	88	51.2	217	48.2	118	39.6
Fusarium spp.	38	22.1	125	27.8	128	42.9
Fusarium roseum	22	12.8	49	10.9	64	21.5
Fusarium oxysporum	11	6.4	46	10.2	57	19.1
Fusarium solani	5	2.9	30	6.7	7	2.3
Stagonospora meliloti	9	5.2	21	4.7	3	1.0
Rhizoctonia spp.	1	0.6	9	2.0	9	3.0
Cylindrocarpon radicicola	2	1.2	12	2.7	0	0.0
Gleosporium bolleyi	2	1.2	3	0.7	12	4.0
Pythium spp.	2	1.2	5	1.1	4	1.3
Penicillium spp.	10	5.8	32	7.1	10	3.3
Stemphylium spp.	10	5.8	2	0.4	6	2.0
Mucor spp.	0	0.0	1	0.2	3	1.0
Botrytis spp.	0	0.0	1	0.2	1	0.3
Epicoccum spp.	1	0.6	3	0.7	0	0.0
Trichoderma spp.	3	1.7	1	0.2	0	0.0
Aspergillus spp.	1	0.6	1	0.2	0	0.0
Cladosporium spp.	5	2.9	4	0.9	0	0.0
Gliocladium spp.	0	0.0	8	1.8	0	0.0
5-44A	0	0.0	5	1.1	4	1.3
Total identified	172		450		298	
Unidentified fungi	8		21		12	
Bacteria	45		121		62	
Sterile	440		414		133	
Total tissue plantings	665		1006		501	

* Number of times fungus appeared in tissue isolations.

** Percent based on total fungi identified.

Table 4. The frequency of fungi isolated from first- and second-year alsike clover plants grown in Klamath County in 1952, 1953, and 1954.

Fungi isolated	1st year plants		2nd year plants	
	Frequency*	Percent**	Frequency	Percent
Phoma spp.	34	28.6	117	56.5
Fusarium spp.	18	15.1	33	15.9
Fusarium roseum	13	10.9	22	10.6
Fusarium oxysporum	5	4.2	7	3.4
Fusarium solani	0	0.0	4	1.9
Stagonospora meliloti	3	2.5	0	0.0
Rhizoctonia spp.	14	11.8	18	8.7
Cylindrocarpon radicicola	2	1.7	4	1.9
Gleosporium bolleyi	7	5.9	2	1.0
Pythium spp.	2	1.7	0	0.0
Penicillium spp.	23	19.3	27	13.0
Stemphylium spp.	1	0.8	4	1.9
Alternaria spp.	1	0.8	0	0.0
Mucor spp.	1	0.8	1	0.5
Botrytis spp.	5	4.2	1	0.5
Epicoccum spp.	1	0.8	0	0.0
Rhizopus spp.	2	1.7	0	0.0
Chaetomium spp.	5	4.2	0	0.0
Total identified	119		207	
Unidentified fungi	1		3	
Bacteria	99		87	
Sterile	181		203	
Total tissue plantings	548		552	

* Number of times fungus appeared in tissue isolations.
** Percent based on total fungi identified.

commonly isolated from Klamath County plants, but were much less common from Central Oregon. Gleosporium bolleyi Sprague was about equally frequent from both areas, but Stagonospora meliloti (Lasch.) Petr. and Cylindrocarpon radicicola Wr. were more common from Central Oregon.

Fungi associated with root rot symptoms.

The data from total isolations were broken down further to determine the frequency of fungi isolated from the various diseased tissues of first-, second-, and third-year plants grown in Central Oregon, and first- and second-year plants from Klamath County (Tables 5, 6, 7, 9, and 10). Only the species proved later in this thesis to be root parasites are listed in the following tables. Other species isolated are included under "Miscellaneous".

Phoma spp. were the most frequently isolated fungi from cortical tissues of first- and second-year plants from Central Oregon (Table 5). However, Fusarium spp. were the most frequent from third-year plants. Stagonospora meliloti and Rhizoctonia spp. were also associated to a lesser degree with rotted cortical tissues.

Two trends were discovered in the isolation results from cortical tissues; (1) Phoma spp. consistently decrease in frequency with increase in the age of the plants, and (2) Fusarium spp. consistently increase in frequency with increase in the age of plants. The frequency of Fusarium spp. seems to be correlated with the degree of symptom severity. The initial high frequency of Phoma spp.

Table 5. The frequency of fungi isolated from cortical tissues of primary and secondary roots of first-, second-, and third-year alsike clover plants grown in Central Oregon in 1952, 1953, and 1954.

Fungi isolated	1st year plants		2nd year plants		3rd year plants	
	Frequency*	Percent**	Frequency	Percent	Frequency	Percent
Phoma spp.	62	56.9	104	40.6	60	31.1
Fusarium spp.	27	25.1	84	32.8	104	53.9
Fusarium roseum	17	16.0	29	11.3	48	24.9
Fusarium oxysporum	7	6.4	42	16.4	49	25.4
Fusarium solani	3	2.7	13	5.1	7	3.6
Stagonospora meliloti	6	5.5	6	2.3	3	1.5
Rhizoctonia spp.	1	1.0	8	3.1	5	2.6
Cylindrocarpon radicicola	0	0.0	8	3.1	0	0.0
Miscellaneous	13	11.9	46	18.0	21	10.9
Total identified	109		256		193	
Unidentified	5		12		7	
Bacteria	13		62		32	
Sterile	212		245		98	
Total tissue plantings	339		575		330	

* Number of times fungus appeared in tissue isolations.
** Percent based on total fungi identified.

Table 6. The frequency of fungi isolated from cortical tissues of primary and secondary roots of first- and second-year alsike clover plants grown in Klamath County in 1952, 1953, and 1954.

Fungi isolated	1st year plants		2nd year plants	
	Frequency*	Percent**	Frequency	Percent
Phoma spp.	8	26.7	53	49.5
Fusarium spp.	6	20.0	21	19.6
Fusarium roseum	3	10.0	12	11.2
Fusarium oxysporum	3	10.0	5	4.7
Fusarium solani	0	0.0	4	3.7
Stagonospora meliloti	3	10.0	0	0.0
Rhizoctonia spp.	8	26.7	6	5.6
Cylindrocarpon radicicola	0	0.0	0	0.0
Miscellaneous	5	16.7	27	25.2
Total identified	30		107	
Unidentified	0		0	
Bacteria	0		49	
Sterile	12		112	
Total tissue plantings	42		268	

* Number of times fungus appeared in tissue isolations.
** Percent based on total fungi identified.

from first-year plants suggests that this genus may be a primary root parasite, while the later build-up of Fusarium spp. suggests a secondary parasitic role for this genus.

Phoma spp. were the most frequently isolated fungi from cortical tissues of first- and second-year plants collected in Klamath County (Table 6). Fusarium spp. were the next most frequent isolates, and Rhizoctonia spp. occurred fairly commonly. Phoma spp. were approximately two and one-half times more frequent than Fusarium spp. from second-year Klamath County plants, while from second-year Central Oregon plants, Phoma spp. were less than one-third as common as Fusarium spp. The failure of Fusarium spp. to build-up in second-year Klamath County plants, which were less severely damaged by cortical rots than plants of exactly the same age from Central Oregon, also suggests the secondary nature of parasitism by Fusarium spp. and the primary nature of parasitism by Phoma spp.

Phoma spp. were the most frequently isolated fungi from vascular tissues of plants of all ages from Central Oregon (Table 7). Fusarium spp. were the next most common isolates. However, the pathogenicity of these species in vascular tissues must be weak as indicated by the low frequency of vascular discoloration in Central Oregon.

The frequency of fungi isolated from diseased vascular tissues of first- and second-year alsike clover from one field in Central Oregon was determined (Table 8). The diseased plants were found only in low areas of the field, and in one corner where water

Table 7. The frequency of fungi isolated from vascular tissues of primary and secondary roots of first-, second-, and third-year alsike clover plants grown in Central Oregon in 1952, 1953, and 1954.

Fungi isolated	1st year plants		2nd year plants		3rd year plants	
	Frequency*	Percent**	Frequency	Percent	Frequency	Percent
Phoma spp.	9	45.0	55	51.4	18	75.0
Fusarium spp.	5	25.0	31	29.2	5	20.8
Fusarium roseum	2	10.0	14	13.1	5	20.8
Fusarium oxysporum	1	5.0	3	3.0	0	0.0
Fusarium solani	2	10.0	14	13.1	0	0.0
Stagonospora meliloti	0	0.0	5	4.7	0	0.0
Rhizoctonia spp.	0	0.0	1	1.0	0	0.0
Cylindrocarpon radicicola	0	0.0	0	0.0	0	0.0
Miscellaneous	6	30.0	15	14.0	1	4.2
Total identified	20		107		24	
Unidentified	3		5		1	
Bacteria	15		18		21	
Sterile	131		133		4	
Total tissue plantings	169		263		50	

* Number of times fungus appeared in tissue isolations.
** Percent based on total fungi identified.

Table 8. The frequency of fungi isolated from diseased vascular tissues of first- and second-year alsike clover plants collected from one field in Central Oregon.

Fungi isolated	1st year plants		2nd year plants	
	Frequency*	Percent**	Frequency	Percent
Phoma spp.	1	3.3	38	71.7
Fusarium roseum	0	0.0	2	3.8
Unknown Pythiaceae	29	96.7	6	11.3
Miscellaneous	0	0.0	7	13.3
Total identified	30		53	
Unidentified	0		1	
Bacteria	10		10	
Sterile	34		32	
Total tissue plantings	74		96	

* Number of times fungus appeared in tissue isolations.
** Percent based on total fungi isolated.

frequently stood several days after irrigation, 90 percent of the plants were affected. Isolations from diseased tissues of first-year plants yielded an unidentified species of the Pythiaceae in 96.7 percent of the tissue plantings. However, from second-year plants Phoma spp. were the most frequent isolates. The unidentified species of the Pythiaceae cannot be considered at present an important cause of alsike clover failures in Central Oregon because of the limited distribution, but it does present a potential disease problem.

The frequency of fungi isolated from diseased vascular tissues of alsike clover plants grown in Klamath County was determined (Table 9). The 573 tissue isolations were made from first- and second-year plants selected for vascular discoloration symptoms from three fields. Verticillium albo-atrum R. & B. was the most frequently isolated fungus from first- and second-year plants. The high frequency of Verticillium albo-atrum among isolates from Klamath County plants, and the fact that this fungus was not isolated from Central Oregon seems to be correlated with the higher percentage of plants with vascular discoloration collected in Klamath County. Phoma spp., although relatively infrequent from first-year plants, increased considerably from second-year plants from Klamath County.

The frequency of fungi isolated from rootlets of plants grown in Central Oregon and Klamath County were determined (Tables 10 and 11). Relatively few isolations were made from rootlets because of the adequate coverage by Dr. Hardison of this phase of alsike clover failures. Phoma spp. were, however, by far the most frequently

Table 9. The frequency of fungi isolated from vascular tissues of primary and secondary roots of first-, second-, and third-year alsike clover plants grown in Klamath County in 1952, 1953, and 1954.

Fungi isolated	1st year plants		2nd year plants	
	Frequency*	Percent**	Frequency	Percent
Phoma spp.	13	6.9	28	32.2
Fusarium spp.	4	2.1	5	5.6
Fusarium roseum	3	1.6	3	3.4
Fusarium oxysporum	1	0.5	1	1.1
Fusarium solani	0	0.0	1	1.1
Verticillium albo-atrum	126	67.4	38	43.7
Rhizoctonia spp.	4	2.1	7	8.0
Cylindrocarpon radicicola	1	0.5	0	0.0
Miscellaneous	39	20.9	9	10.3
Total identified	187		87	
Unidentified	1		0	
Bacteria	77		34	
Sterile	133		54	
Total tissue plantings	398		175	

* Number of times fungus appeared in tissue isolations.
** Percent based on total fungi identified.

Table 10. The frequency of fungi isolated from rootlets of first-, second-, and third-year alsike clover plants grown in Central Oregon in 1952, 1953, and 1954.

Fungi isolated	1st year plants		2nd year plants		3rd year plants	
	Frequency*	Percent**	Frequency	Percent	Frequency	Percent
Phoma spp.	17	68.0	26	49.1	30	65.2
Fusarium spp.	4	16.0	7	13.1	9	19.6
Fusarium roseum	1	4.0	4	7.6	1	2.2
Fusarium oxysporum	3	12.0	3	5.7	8	17.4
Fusarium solani	0	0.0	0	0.0	0	0.0
Stagonospora meliloti	0	0.0	2	3.8	0	0.0
Rhizoctonia spp.	0	0.0	0	0.0	4	8.7
Cylindrocarpon radicicola	2	8.0	4	7.5	0	0.0
Miscellaneous	2	8.0	14	26.4	3	6.5
Total identified	25		53		46	
Unidentified	0		4		0	
Bacteria	17		2		6	
Sterile	85		44		21	
Total tissue plantings	127		103		73	

* Number of times fungus appeared in tissue isolations.
** Percent based on total fungi identified.

Table 11. The frequency of fungi isolated from rootlets of first-, second-, and third-year alsike clover plants grown in Klamath County in 1952, 1953, and 1954.

	1st year plants		2nd year plants	
Fungi isolated	Frequency*	Percent**	Frequency	Percent
Phoma spp.	13	72.2	32	82.0
Fusarium spp.	3	16.6	0	0.0
Fusarium roseum	2	11.1	0	0.0
Fusarium oxysporum	1	5.5	0	0.0
Fusarium solani	0	0.0	0	0.0
Stagonospora meliloti	0	0.0	0	0.0
Rhizoctonia spp.	1	5.5	5	12.8
Cylindrocarpon radicicola	1	5.5	0	0.0
Miscellaneous	0	0.0	2	5.1
Total identified	18		39	
Unidentified	0		0	
Bacteria	0		4	
Sterile	17		35	
Total tissue plantings	35		78	

* Number of times fungus appeared in tissue isolations.
** Percent based on total fungi identified.

isolated fungi from rootlets of plants of all ages from both areas.

Disease observational nursery.

A disease observational nursery of selected field crops was planted on one farm in Central Oregon (Table 12). The objective of this plot was to discover the value of the various field crops in extending the existing rotations in the area. The disease symptoms caused by alsike clover root rot fungi and the frequency of these fungi from various plants was determined.

The plot consisted of 35 rows 16 feet long and 1.5 feet apart, and was seeded in May, 1952, on land on which alsike clover failures had occurred within the previous two years. The plot was first sampled in early September of 1952. Approximately one-third of the plants in each row were dug and analyzed for root rot symptoms. The severity of symptoms was "slight", and only a few isolations were made from diseased tissues. The plot was sampled again in August of 1953.

All the alfalfa varieties had a "slight" amount of light brown rootlet discoloration the first year. Five Nomad plants out of 29 inspected had "slight" cortical rot symptoms on the primary roots. Fusarium roseum, the only fungus isolated, occurred only once out of 25 tissue plantings.

Black-stem was the most prominent disease symptom on 35 second-year alfalfa plants collected in 1953. Fifty percent of the plant

Table 12. Field crops seeded in a disease observational nursery on the Porter farm in the Tumalo district in Central Oregon in May of 1952.

Row	Scientific Name	Common Name	Variety
1.	Trifolium hybridum L.	Alsike Clover	Commercial
2.	Medicago sativa L.	Alfalfa	Grimm
3.	" "	"	Ladak
4.	" "	"	Ranger
5.	" "	Creeping Alfalfa	Nomad
6.	" "	" "	Rhizoma
7.	Trifolium hybridum L.	Alsike Clover	Commercial
8.	Melilotus alba Desr.	Sweet Clover	Willamette
9.	Pisum sativum L. var. arvense (L.) Poir.	Field Peas	Austrian Winter
10.	Trifolium pratense L.	Red Clover	Kenland
11.	" "	" "	Common
12.	" hybridum L.	Alsike Clover	Commercial
13.	" repens L.	White Clover	Kentish
14.	" repens L. var. Ladino Lagr.-Foss.	Ladino Clover	Commercial
15.	Vicia villosa Roth	Hairy Vetch	Commercial
16.	Trifolium hybridium L.	Alsike Clover	"
17.	Agropyron cristatum (L.) Gaertn.	Crested Wheat Grass	"
18.	" intermedium (Host) Beauv.	Intermediate Wheat Grass	"
19.	" trichophorum (Link) Richt.	Pubescent Wheat Grass	"
20.	Alopecurus pratensis L.	Meadow Foxtail	"
21.	Arrhenatherum elatius (L.) Presl.	Tall Oat Grass	Tualatin
22.	Bromus inermis Leyss.	Smooth Brome Grass	Manchar
23.	Dactylis glomerata L.	Orchard Grass	Commercial
24.	Elymus canadensis L.	Canada Wild Rye	"

Table 12. (cont'd)

Row	Scientific Name	Common Name	Variety
25.	Festuca elatior var. arundinacea (Schreb.) Wimm.	Tall Fescue	Alta
26.	Poa pratensis L.	Kentucky Bluegrass	B-27
27.	Phleum pratense L.	Timothy	Commercial
28.	Trifolium hybridum L.	Alsike Clover	"
29.	Avena sativa L.	Oats	Victory
30.	Hordeum vulgare L.	Barley	Hannchen
31.	" "	"	Trebi
32.	Secale cereale L.	Rye	Prolific
33.	Triticum aestivum L.	Wheat	Federation
34.	Solanum tuberosum L.	Potato	Netted Gem
35.	Trifolium hybridum L.	Alsike Clover	Commercial

stems of all varieties had black lesions in varying amounts. Fifty-five tissue plantings from diseased stems yielded 21 colonies of Phoma spp., nine colonies of F. roseum, nine colonies of Stemphylium spp., and nine miscellaneous fungi. The fungus species were morphologically indistinguishable from similar species isolated from diseased tissues of alsike clover roots. All varieties of alfalfa were equally affected.

Rootlet rot and cortical rot were present on all plants in amounts rated generally as "slight". However, there was an associated discoloration in the interior of the upper part of the primary root of all plants with black-stem lesions. Isolations from the diseased root tissue yielded the same Phoma and Fusarium species in the same proportion as were isolated from the black-stem lesions.

On the basis of these results alfalfa must be considered as a susceptible host for Phoma and Fusarium species associated with alsike clover root rot. Black-stem was an important disease problem on alfalfa, although the root rot symptoms of the type common on second-year alsike clover never became more serious than "slight".

One plant of 15, first-year sweet clover plants examined had a trace of discoloration on the exterior of the primary root. All the other plants were free from root rot symptoms.

One second-year plant of 10 examined had a "trace" of cortical rot, and one plant had "moderate" black-stem lesions. A Phoma sp. similar to one present in alfalfa black-stem lesions and alsike clover root rot tissues was isolated. In general, however, sweet

clover was attacked much less severely by the species of fungi associated with diseased alsike clover roots.

Twenty first-year red clover plants of the two varieties planted were examined in 1952 and had a uniform light brown rootlet discoloration rated as "slight". Three plants had a "slight" amount of cortical rotting, and 15 tissue plantings yielded only six colonies of Fusarium spp. Second-year plants were more severely attacked. Of 20 plants examined, 16 had "moderate" and four "severe" cortical rot, and all had "moderate" rootlet rot symptoms. Twenty tissue plantings from cortical tissues yielded five colonies of Fusarium spp. but no Phoma spp.

Red clover plants one and two years old had the same symptoms and disease severity as alsike clover plants from the same plot. The fact that Phoma spp. were not isolated is not considered important because of the small number of tissue plantings. Phoma spp. were the most frequently isolated fungi from red clover plants from other fields in Central Oregon during the course of this investigation.

All 20 first-year alsike plants examined had "slight" rootlet rot, and only three plants showed a "trace" of cortical discoloration.

Phoma spp. were the most frequently isolated fungi from rootlets, and cortical tissues of second-year plants. All of 50 plants rated for root rot symptoms had "moderate" rootlet rot. The 50 plants had varying degrees of cortical rot, but only three were "severe". One plant had "moderate" vascular discoloration.

The field peas, hairy vetch, white and ladino clovers all had

a trace of rootlet discoloration the first-year, but were otherwise disease free. None of these species were analyzed the second year.

All the grasses and cereals tested had healthy roots the first year except the two varieties of barley. All of the 20 barley plants analyzed had a "moderate" amount of yellow-brown roots with blackened tips. Twenty-five tissue plantings gave six colonies of Fusarium spp., three colonies of Gleosporium bolleyi, three colonies of Cylindrocarpon radicicola, and three colonies of Rhizoctonia spp.

The annual species of Gramineae were not replanted for the second year, but the perennial species were studied.

The species of Agropyron all appeared to be healthy. However, eight out of 20 tall oat grass plants had discolored crown tissue from which Gleosporium bolleyi was the most common isolate. Fusarium roseum was also isolated. Similar symptoms were observed in the crown tissue of meadow foxtail, and tissue plantings yielded the same fungi. One of 10 Canadian wild rye plants had dark brown lesions at the base of two culms, and 10 tissue plantings yielded nine colonies of F. oxysporum. Significantly, out of 95 tissue plantings from root and crown tissue of Gramineae, not one colony of Phoma was obtained.

The roots and tubers of the potato variety analyzed in this experiment were free of any fungus disease symptoms in 1952, but the tubers were affected by root knot nematode.

UNIDENTIFIED PATHOGENIC FUNGI

The unidentified fungi demonstrated later in this thesis to be causal agents of alsike clover root rot will be described in this section. Single spore isolates were used for identification, and the isolates were compared with the original culture to verify their relationship.

Phoma species.

The genus Phoma has long been poorly defined. This genus has been delimited primarily on pathological relationships, and little attention has been given to morphological characters. Spore morphology, which in the past has been the principal character employed in species determination, offers little promise since the range of variability within the genus is comparatively limited, and many species have similar spore morphology.

Identification of the isolated species of Phoma on the basis of host plant relationships and limited morphological characters has been found unsatisfactory. This difficulty in identification is indicated in the current pathological literature where these forms are repeatedly listed merely as "Phoma spp.". Species descriptions are inadequate in that they contain little information on root pathogenicity, and the problem is further complicated by variation in morphological characters between isolates of the same species.

Five distinct types of Phoma were isolated during this investigation. With one exception, the types could not be identified

with any known species. These five types were not segregated in the isolation results, because their frequency was known only for the last two of the three years for which diseased tissue planting results were obtained. The types will be described in their order of decreasing isolation frequency.

The most frequently isolated Phoma type is Phoma X, the species previously reported by Hardison (24, p. 33). Phoma X is distinguished mainly on spore dimensions. The spores obtained from cultures growing on steamed clover leaves are 1.75-2.0 x 4.0-5.0 microns, and the globose pycnidia average 90 microns in diameter. Pycnidia and spores are only occasionally formed on nutrient agar media. On PDA Phoma X forms slow growing, light-gray colonies with a white margin.

A second type was designated as Phoma A. This type is distinguished by broadly elliptical spores. The spores from cultures on sweet clover stems are variable in size (2.5-3.0 x 4.0-5.0 microns), and pycnidia from the same medium are globose to subglobose and vary from 100 to 300 microns in diameter. On PDA Phoma A is slow growing and has light-gray mycelia. From the bottom side of a Petri plate culture the colony has a yellowish coloration. In older PDA cultures numerous pycnidia are produced at the outer margin of the colony.

A third type has been designated as Phoma B. The spores from cultures of this type on sweet clover stems are variable in size (2.0-3.0 x 4.0-6.5 microns). Globose pycnidia from the same medium are 200 to 400 microns in diameter. The spores are similar to the

spores of Phoma medicaginis, but they are smaller and less variable in size. The young cultures of both species produce a white mycelia on PDA. However, the mycelia of P. medicaginis later becomes black and leathery while that of Phoma B stays light-colored and does not become leathery. Numerous pycnidia are produced in older PDA cultures at the margin of the colony.

A fourth Phoma type was identified as Phoma medicaginis Malbr. et Roum.

The fifth type, Phoma C, is classified with the Phoma types only on the basis of the mycelial characters. On PDA Phoma C is light-gray and relatively slow growing. Microscopically the mycelia are indistinguishable from that of Phoma X. Thus Phoma C may be a non-sporulating form of Phoma X.

Pythiaceae.

The unidentified fungus of the Pythiaceae associated with vascular discoloration of alsike clover plants on the Porter farm in Central Oregon is either a Phytophthora or a Pythium. The two genera differ in the method of zoospore formation, and this has not been determined for this species. However, the structures interpreted as sporangia (Figure 16) do not resemble sporangia produced by Phytophthora but are similar to the filamentous sporangia produced by some species of the genus Pythium. Oogonia and oospores (Figure 17) are not produced on nutrient agar media, but are readily formed on steamed alsike clover tissues. The average diameter of 30 oospores

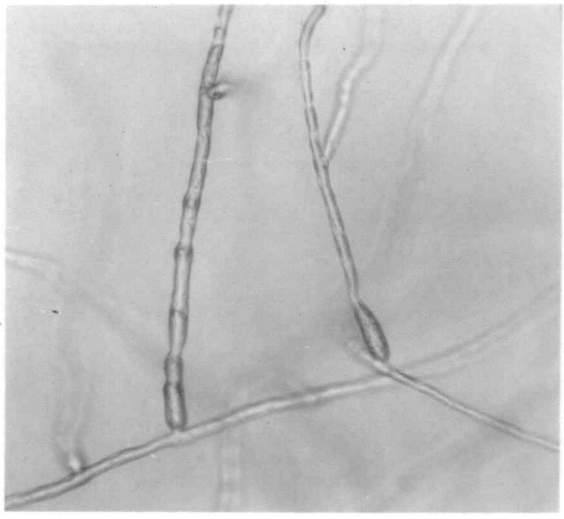

Figure 16. Filamentous sporangia of an unidentified species of the Pythiaceae pathogenic to vascular tissue of alsike clover plants. X200.

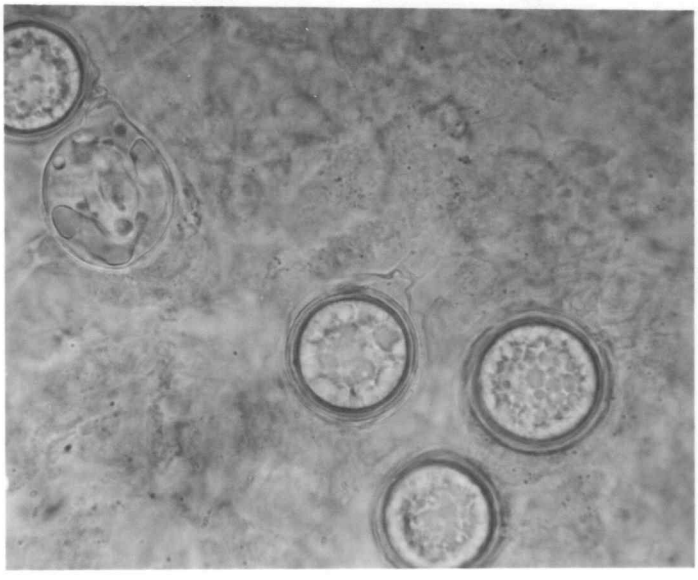

Figure 17. Oospores of an unidentified species of the Pythiaceae pathogenic to vascular tissue of alsike clover plants. X800.

on this substrate was 22 microns. Terminal and intercalary oogonia are equally present, but only one monoclinous antheridium has been found.

The slow growth rate of this fungus is a distinctive characteristic. The rate was determined at five degree intervals from 5° C. to 30° C. on Difco corn meal agar. There was no growth at 5° C. or 30° C., and the maximum was seven millimeters in 24 hours at 25° C. The only Pythium species with a similar growth rate and temperature response, and with similar morphology are two species described by Vanterpool as causing browning root rot of graminicolous hosts, P. tardicrescens Vanterpool (51, pp. 533-534) and P. volutum Vanterpool and Truscott (52, pp. 77-80). Dr. T. C. Vanterpool has examined the author's culture and states that this fungus is not one of the filamentous forms of Pythium which attack cereals, but the final determination has not been made.[1]

Unknown no. 5-44A.

This fungus has been isolated infrequently and proved to be pathogenic to alsike clover roots. The exact taxonomic position has not been determined, but there is a similarity between this organism and Dematophora necatrix Hart. the conidial stage of Rosellinia necatrix (Hart.) Berl. which was reported by Harris (25, p. 407) to be the cause of alfalfa root rot in California. On PDA this fungus

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[1]Privately held letter from T. C. Vanterpool, Professor of Plant Pathology, Department of Biology, University of Saskatchewan, Saskatoon, Canada. April 2, 1955.

is light-gray in mass and produces a faint reddish coloration in the medium. The spores are produced endogenously from the terminal branches of loose synnema. The subglobose spores vary from 2.0 x 3.0 to 2.5 x 3.5 microns.

PATHOGENICITY TESTS

Several inoculation techniques were tested in the greenhouse to determine their value in screening fungi for pathogenicity to alsike clover roots. These techniques were designed primarily to discover the pathogenicity of fungi to cortical and vascular tissues. Rootlet pathogenicity was not specifically tested in the early screening tests because of the coverage given this phase by Hardison (22), and 24).

The method described below proved to be the most satisfactory method for testing large numbers of fungi for cortical and vascular pathogenicity. Clover plants at least eight weeks old were washed, the roots pruned to approximately one and one-half inches, and the primary root wounded by repeated jabs with a dissecting needle. The roots were then rubbed over the surface of a Petri plate culture of the fungus to be tested. A PDA block one centimeter square with the fungus was placed against the wounded area of the primary root at the time of planting. Two treated plants were planted in steam-sterilized greenhouse soil in four inch pots, and three replications were used for each fungus. The controls were treated in the same way except sterile PDA was used. Usually the pots were left on the greenhouse bench for at least six weeks before the results were taken.

By placing the inoculated plants in a controlled temperature chamber at approximately 30° C. the symptoms were more severe, and results could be obtained in three weeks. The use of this higher

temperature was abandoned, however, because of increased infection by common greenhouse soil fungi. The control plants frequently developed disease symptoms, and species of Trichoderma, Chaetomium and Botrytis were isolated.

When the complete range of isolates had been screened by preliminary methods, a set of fungi representing the most pathogenic forms of the various species were selected. More refined greenhouse tests were designed to rate the pathogenicity of these isolates on alsike clover rootlets, and on cortical and vascular tissues. The pathogenicity of these fungi on ladino clover, Kenland red clover, Ranger alfalfa, Federation wheat, and Hannchen barley was also tested in one experiment.

The advantages of higher temperatures for testing root rot fungi for pathogenicity were utilized without the interference of contaminating organisms by conducting an experiment outside the greenhouse in a hot-frame. The inoculum for this experiment was prepared by growing the various fungi on sterile water-soaked oat grain. The fungi were allowed to grow for two weeks before the cultures on infested grain were mixed with sterile greenhouse soil in flats six inches deep with six compartments, each four by ten inches. One pint of oat inoculum was divided among three compartments. Contamination between compartments was not considered important, since Snyder (47, p. 67) has found that appreciable quantities of soil inoculum must be transferred to function in disease production, and diseased tissue plantings were always made

to recover the pathogenic organisms.

Three alsike clover plants at least eight weeks old were washed, root pruned to approximately one and one-half inches, and planted in each compartment. Three replications were used for each fungus. Three controls received sterile oats, and three were planted in greenhouse soil without treatment. The treatments were completely randomized throughout 18 flats. The flats were placed over a heating cable and surrounded by sand (Figure 18). This experiment was carried out during the summer months when, with the aid of the heating cable, the soil temperature at two inches below the soil surface was held in the range 26° C. to 33° C. At the end of six weeks the plants were dug and analyzed for root rot symptoms.

A second method was developed to be used inside the greenhouse to rate the pathogenicity of the various fungi. Contamination effects by common greenhouse fungi were reduced by decreasing the amount of organic matter added to the soil. This was accomplished effectively by preparing the inoculum in a liquid medium. A potato broth made by the same formula as PDA but without the agar, was found to be the most satisfactory medium. Eight week old, or older, plants were washed, root pruned to approximately one and one-half inches and dipped in ten day old cultures that had been macerated in a Waring blender. Three inoculated plants were planted in six inch clay pots, and the culture homogenate was poured around the plants at the rate of 50 milliliters per plant. Three replications were used, and the pots were completely randomized on the greenhouse

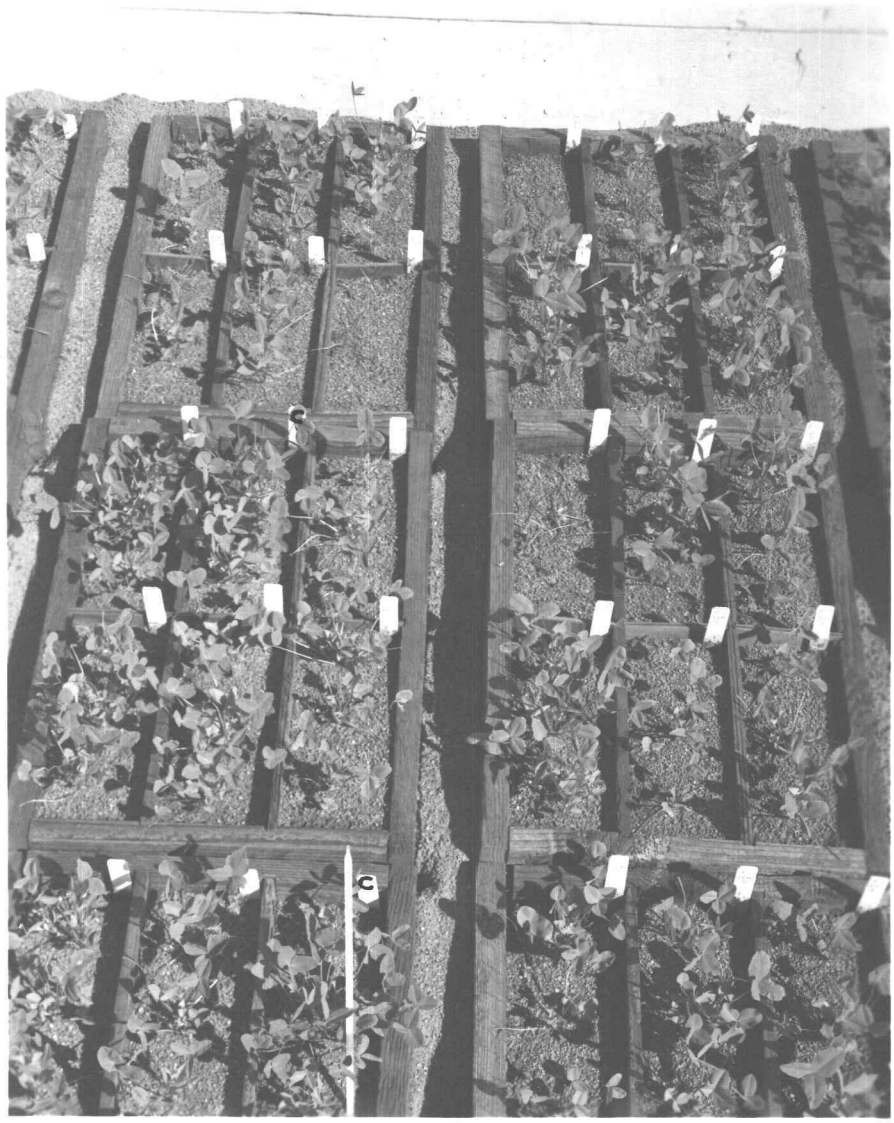

Figure 18. Alsike clover plants growing in soil artificially infested with various root rot fungi. Sections marked with "C" contain the control plants.

bench where the temperature ranged from 18° C. to 23° C. Check plants inoculated with sterile potato broth and uninoculated plants were grown in all experiments.

In September, 1953, a plot was set up on the Porter farm in Central Oregon to test the various alsike clover isolates and thereby determine the validity of the greenhouse tests in predicting pathogenicity in the field. Ten five month old plants were inoculated with each fungus by injecting one milliliter of a spore and/or mycelial suspension into the primary root with a syringe and no. 20 hypodermic needle. Verticillium albo-atrum was the only pathogenic species not tested and all the cultures used were isolated from plants previously collected on the Porter farm.

The plants were dug, and the root rot symptoms were analyzed the following spring. All inoculated plants had a general rotting of the primary root and lower stem typical of winter injury. Evidently by disturbing the soil around the plants they were rendered less resistant to winter injury. Symptoms that might have been produced by pathogenic fungi could not be distinguished, but isolations from tissues away from the wounds in many cases yielded the test fungus in such high percentages that a pathogenic relationship was suspected.

In August, 1954, a similar plot was set up on the Porter farm to be analyzed the same year and thus avoid winter injury. Healthy plants approximately four months old were lifted from an adjacent field, washed, root pruned to one and one-half inches, wounded on

the primary root, and dipped in a culture homogenate. Two rows four feet long were planted with 10 plants each. A PDA block with the fungus to be tested was placed against the wounded area of the primary root when the plants were set in the ground. A treated and an untreated control were planted. During the time this experiment was run the temperature one inch below the soil surface ranged between 32.5° F. and 87.0° F. Nine weeks after inoculation one row of each treatment was dug, washed, and rated for root rot symptoms.

The severity of symptoms varied slightly from experiment to experiment depending mainly on the soil temperature and the length of time the tests were run. However, the plants inoculated in the field and greenhouse by the methods just described were rated by the same standard. Rootlet rot and vascular discoloration were merely recorded for their presence. The experiments were of such comparatively short duration that moderate and severe vascular symptoms did not have time to develop, and not all the experiments were designed to favor rootlet rotting. Cortical rots, however, were rated by a system similar to the one applied to field plants.

None	No cortical rot of the primary or secondary roots. All wounds were healed over by cork tissue.
Slight	Trace of rot only around wounding.
Moderate	Rot associated with wounding only, but tissues discolored up to five millimeters away from the wounds.

Severe. Rot spread more than five millimeters away from wounding and initiated on roots without apparent wounds.

The pathogenicity of the varous fungi was determined on seedlings grown in laboratory glassware on suitable media. Although in Oregon there has been no difficulty in obtaining first-year alsike clover stands in the field, pathogenicity tests using seedlings were employed with the hope that the results would accurately predict the results of greenhouse and field inoculation studies with older plants. The main advantage of using seedlings is that results can be obtained in two weeks with relatively simple preparations. Two types of tests were used.

In the first method 10 or more surface-sterilized alsike clover seeds were germinated in Petri plates on PDA. Each plate was inoculated by pouring four milliliters of a spore and/or mycelial suspension over the PDA on which the clover was growing. A second method was used in which five or more surface-sterilized clover seeds were germinated in eight inch test tubes on cotton mats saturated with tap water and placed at the bottom of the test tubes. Before seeding the tubes with water soaked mats were autoclaved for 15 minutes at 15 pounds pressure. Each tube was inoculated by placing a 5 millimeter disc of PDA bearing the inoculum on the cotton between the seedlings. In both tests the inoculation of sterile seedlings was made when the plants were four days old. The Petri plates were incubated in the laboratory, and the test tubes were kept in the

greenhouse. There was no difference in the results of the two methods.

The results of all the pathogenicity tests have been grouped, and will be presented under the species of fungus involved. The variation of disease severity between replicates and plants within replications never exceeded that of the symptom-severity class in which the plants were classified. In general the results obtained in the field were correlated with the greenhouse results. The control plants in all tests remained symptomless except that a trace of discoloration occurred in a few plants from the field plot. Diseased tissue plantings were always made to isolate the causal agent. Pathogenicity results were considered valid only in cases in which the test fungus was reisolated.

The seedling tests were not considered valuable in predicting what might be expected by inoculating older plants in the greenhouse and field. All the fungi that were pathogenic in the field and greenhouse tests were also pathogenic on seedlings. However, more species were pathogenic on seedlings than older plants, and relatively few could be eliminated by this method.

Phoma species.

In the tests carried on inside and outside the greenhouse in Corvallis, Phoma X always caused severe cortical rot of alsike clover roots (Figure 20). The rootlets were usually severely discolored, but only a trace of vascular discoloration was observed. Similar

Figure 19. Alsike clover plants inoculated with Unknown no. 5-44A and grown in the greenhouse (on the left) and in the field (center). The plant on the right is a control plant from an inoculation test in the greenhouse. X1.

Figure 20. Two alsike clover plants (on the left) inoculated with Phoma X and grown in the greenhouse. The plant on the right is a control plant. X1.

Figure 19.

Figure 20.

symptoms were produced on red clover and alfalfa roots. On red clover and alfalfa stems, Phoma X caused a black-stem symptom different from the typical symptom associated with attack by P. medicaginis. Phoma X black-stem symptoms were long, broad, black lesions as contrasted with the narrow black lesions of the typical black-stem. Alsike plants from the pathogenicity trials in the field were severely damaged in the cortical tissues of the primary and secondary roots, and in seedling tests this fungus killed the plants in eight days.

The pathogenicity of Phoma A was more variable and usually less severe than that of Phoma X. In one greenhouse test severe rot was produced in alsike clover cortical tissues. The rootlets were discolored, but no vascular discoloration was observed. In two other tests in the greenhouse only moderate cortical rot was produced. Phoma A also caused moderate cortical rot on red clover. In the field test this species caused only a slight amount of cortical rot on alsike clover. On seedlings a distinct reddish discoloration was found on the primary roots, and the plants were so severely attacked they died within ten days of inoculation.

Phoma B was considerably less pathogenic than Phoma X or Phoma A. In the greenhouse tests this species caused only a trace of cortical discoloration on alsike and red clover roots. Cortical tissues of inoculated field plants were only slightly damaged, but the seedlings were moderately discolored on the primary root.

Phoma medicaginis was only moderately pathogenic to alsike

clover roots in the various greenhouse pathogenicity tests. A trace of rootlet discoloration occurred, but no vascular discoloration was observed. Typical black-stem symptoms were produced on red clover and alfalfa. In the field this species was only slightly pathogenic to cortical tissues, and no rootlet or vascular discoloration was observed. Seedlings were severely attacked.

Phoma C was the least pathogenic of all the Phoma species tested. In the greenhouse tests this species caused a moderate amount of cortical rot, but on field plants and seedlings only a slight discoloration of the cortical tissues was observed. The same symptoms were observed on red clover in the greenhouse tests.

Fusarium species.

In greenhouse tests Fusarium roseum caused severe cortical rot, and in the tests with oat inoculum the plants inoculated with one isolate all died one month after inoculation. However, this could not be repeated in a later test. Fusarium roseum caused similar symptoms in red clover cortical tissues. In the field tests this species caused severe cortical rot, but no rootlet rot or vascular discoloration was observed. Seedlings were killed in 10 days by isolates of this species.

In the greenhouse tests Fusarium oxysporum caused cortical rot which was rated as moderate. In the field test this fungus produced moderate cortical rot, and a slight amount of vascular discoloration. Of all fungi tested on field plants this species was the only one

consistently causing vascular discoloration. On seedlings this species caused only a trace of primary root discoloration.

Fusarium solani caused moderate cortical symptoms on alsike and red clover roots in the greenhouse tests. In the field this species produced a slight cortical rot, and seedlings were also only slightly attacked.

Stagonospora meliloti.

Stagonospora meliloti, although infrequently isolated from field plants, was nevertheless strongly pathogenic to alsike clover roots. Severe cortical symptoms were produced by this fungus on greenhouse and field grown plants. The tissue discoloration associated with attack by this fungus was easily distinguished by the light color. Even in advanced stages of attack the tissues remained light brown and often a reddish discoloration occurred at the margin of the diseased tissues. Alfalfa and red clover were similarly attacked, but seedlings were unaffected by this fungus.

Cylindrocarpon radicicola.

Cylindrocarpon radicicola produced slight cortical rot in greenhouse and field tests. On red clover similar symptoms were produced. However, seedlings were severely attacked, and in two weeks 50 percent of the inoculated seedlings were killed.

Rhizoctonia species.

Several Rhizoctonia isolates tested were similar to C.

radicicola in that they were only weakly pathogenic on older clover roots, but inoculated seedlings developed severe discoloration of the primary root and finally died.

Unknown no. 5-44A.

Severe cortical discoloration was caused by this fungus in greenhouse tests in which elevated temperatures were used (Figure 19). At lower temperatures only a trace of cortical discoloration occurred. Similar symptoms were produced on red clover, ladino clover, and alfalfa. In the field 5-44A was the most pathogenic species tested. Of the 20 plants inoculated 11 died within the first month and the rest were severely rotted (Figure 19). The primary roots of seedlings were only slightly discolored by this fungus.

Pythiaceae.

The unidentified species of the Pythiaceae isolated solely from diseased vascular tissues was not tested in all the experiments previously described. This fungus would not grow on the oat grain medium used to build up inoculum, but it was included in the tests in which liquid media inoculum was used. This fungus did not cause disease symptoms on any of the plants tested in the greenhouse or field in the regular experiment, and seedlings were unaffected. However, plants that had been inoculated with liquid media and held over for three months showed moderate vascular discoloration symptoms, and the fungus was reisolated from the vascular tissue of the stem

as much as four centimeters above the wounds on the primary root.

Vascular discoloration was found in greenhouse grown alsike plants six months after transplanting into infested field soil. The unknown Pythiaceae was the only fungus isolated from the diseased tissues.

Verticillium albo-atrum.

Böning (3) found that Verticillium dahliae will attack clovers, and Kilpatrick, Hanson and Dickson (32, p. 257) have found a species of Verticillium among isolates from red clover roots in Wisconsin. Hardison (22, p. 34) only rarely isolated a species of Verticillium from alsike clover in Oregon. None of these reports of Verticillium spp. on clovers have described the specific symptoms of the disease or reproduction of the disease experimentally. On the other hand typical Verticillium wilt symptoms of plugged and discolored vascular elements and wilted plants in the field have been reported on alfalfa from England (40).

The symptoms of Verticillium albo-atrum on alsike clover in Klamath County, Oregon, are similar to those on other plants except that wilted plants have never been found. The failure of plants to wilt in the field was thought due to the abundance of moisture during the growing season. To test this hypothesis diseased field plants were brought into the greenhouse and planted in field soil in six inch pots. The pots were set in soil and heat applied from below with a heating cable to maintain the temperature at 2 inches below

the soil surface in the range of 25° C. to 32° C. The soil was allowed to dry out until the healthy control plants as well as the plants with severe vascular plugging, wilted. When the plants were watered again both controls and diseased plants revived. This procedure was repeated several times, but no difference could be detected in the wilting and recovery of the diseased and healthy plants.

In the greenhouse inoculation studies vascular discoloration was the only disease symptom associated with attacks by V. albo-atrum. Symptoms did not appear until two months after inoculation, and the fungus was isolated from tissues as far as two inches up the stem of diseased plants. Verticillium albo-atrum was not tested in the field, and it was not pathogenic to the alsike clover seedlings.

SUMMARY AND CONCLUSIONS

Failures of alsike clover stands in Central Oregon appear for the most part to be due to the combined result of the three classes of disease, rootlet rot, cortical rot, and vascular discoloration. The three symptoms have been observed in every field in amounts that varied mainly with the age of the plants. The most prevalent symptom was rootlet rot which occurred frequently the first year and was found on 100 percent of the second- and third-year plants. The importance of this type of root rot as the ultimate cause of plant death is doubtful in view of the fact that clovers can regenerate tertiary roots annually (16, pp. 175-190).

The occurrence and build-up of cortical rots seems highly correlated with the death of the plants in spring of the third year. By the beginning of the third growing season the plants are so depleted of cortical food storage tissue that they are unable to support growth when environmental conditions become favorable. The extra burden of producing new top growth after harvest late in the fall certainly is a contributing factor. Two types of cortical rots have been delimited on the basis of anatomical studies. Infected tissues have been found walled off by a cork cambium, while in other cases the infection had progressed to the vascular cambium without wound cork formation.

Vascular discoloration is not an important problem in the production of second-year seed crops in Central Oregon. The more

frequent vascular discoloration found in Klamath County was demonstrated to be due to attacks by the common vascular parasite Verticillium albo-atrum. An unidentified species of the Pythiaceae not previously reported on clovers has been isolated from severely diseased vascular tissue of alsike clover plants from one field in Central Oregon. The pathogenicity was proved in pure culture inoculation tests in the greenhouse.

Root tissue plantings and pathogenicity studies have proved the over-all importance of Phoma. When the Phoma species are ranked in order of decreasing isolation frequency, the order is the same as that of decreasing pathogenicity. However, this observation cannot be made into a generalization because of the relatively infrequent occurrence and yet strong pathogenic nature of Stagonospora meliloti and Unknown no. 5-44A. Fusarium species were isolated relatively frequently from root rots, particularly from the cortical tissues of older plants. A relationship between the increase of symptom severity and an accompanying increase in Fusarium spp. isolates was pointed out. The association of Phoma spp. with early stages of root rot and the later build-up of Fusarium spp. were cited as evidence for the primary pathogenic nature of Phoma spp. and the secondary role of Fusarium spp. Pure culture inoculation studies, however, demonstrated the pathogenic nature of the Fusarium spp. Fusarium roseum was the most pathogenic Fusarium species tested.

Cylindrocarpon radicicola and Rhizoctonia spp. were isolated relatively infrequently and were only weakly pathogenic.

Snyder and Baker (47, pp. 21-22) have observed that many fungi commonly pathogenic on above-ground plant parts are associated with the roots of irrigated crops in semi-arid climates in California. The relationship of the black-stem Phoma species with root rot in Central Oregon seems to fit this generalization. The Phoma spp., however, will cause black-stem disease on alfalfa and red clover in the field and greenhouse, but species of Phoma were never isolated from roots of grasses and cereals nor were they at all pathogenic on wheat or barley.

Alsike clover failures in Central Oregon have been avoided by not attempting a second seed crop. This practice has been expensive for individual growers because of increased farming costs. Also, root rots must surely reduce the yields of the first seed crop. Now that the pathogenic causes of alsike clover failures are better understood, the relation of predisposing factors such as insect wounding, soil fertility and cultural practices should be studied. An understanding of these relationships is a fundamental prerequisite of any program designed to control the disease.

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