FUNGUS ROOT DISEASES OF ALFALFA

by

WILLIAM G. WILLIS

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Approved by:

S. M. Pady

Major Professor

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INTRODUCTION

Alfalfa is man's oldest cultivated forage crop. Wing (104) surmises that this is the "grass" Nebuchadnezzar ate as recorded in Daniel 4: 33. Bolton (7) says it was probably the "principal fodder for the cavalry and chariot horses of the ancient Persians, Greeks and Romans". The Greeks included this crop in their series of husbandry books (7) and Greek and Roman writers at the beginning of the Christian era mentioned it as an important forage. Alfalfa culture nearly died out in Europe during the dark ages but was re-introduced about the 15th century. Harte (43) in 1770 extolled its virtues for English farmers in a flowery essay. He quotes an old Spanish poem - -

> Alfalfa, whole, luxuriant herbage feeds
> The lab'ring ox, mild sheep and fiery steeds:
> Which ev'ry summer, every thirtieth morn
> Is six times reproduc'd and six times shorn!

Medicago, the genus to which cultivated alfalfa belongs, includes many species. Bolton includes descriptions of 61 species (7). The most common in the United States is *Medicago sativa* L. *M. falcata*, a yellow flowered winter hardy species has been used in breeding hardy varieties in this country and results of these crosses are referred to as variegated and classed as *M. media* by some authorities. Since its cultivation predates historical records its origin is obscure. But wild plants growing in the high dry region of south and central Asia resemble cultivated varieties and this is considered to be the first place it was cultivated. Alfalfa comes from the Spanish name which in turn may be from an Arabic word meaning "best forage" (104). Lucerne is the more common name in Europe and probably comes from Lucerne, Switzerland (7). Greeks called it medicai and Romans medica from the Greek meaning "feed from Media".

Spanish invaders introduced alfalfa into the new world in Mexico, Chile, and Peru (17). Coburn (17) quotes a report from Jacob Downing that he had seen "near Mexico City fields of alfalfa 300 years old constantly cropped and never reseeded". From Central and South America alfalfa was brought to California in 1854 and reached Kansas in 1869 (7). East coast introductions were made from Europe in the 18th century.

J. L. Bolton (7) in a recent book published in England in 1962 "Alfalfa - Botany, Cultivation and Utilization" reviews this crop from a world-wide viewpoint.

Alfalfa acreage in Kansas (57) climbed from 34,384 acres in 1891 to a million acres in 1912 and a peak of 1,538,000 acres in 1955. Since then the acreage has declined to 1,018,000 acres in 1960 when Kansas ranked 2nd in the nation in dehydrated alfalfa production, 3rd in alfalfa seed production with 10,625,000 pounds and 10th in alfalfa hay with 2,647,000 tons (58).

EARLY REPORTS OF DISEASES

Early writers made little mention of diseases of alfalfa. Harte in 1770 (43) warned that "few things hurt more than cold marshy grounds, wet clays, and stagnating waters". He recommended growing plants in a nursery and transplanting to the field. This could have been a preventative to damping-off. He also suggested cutting with sickle to avoid crown injury with the scythe "for the scythe... will frequently slice off such parts of the bulb as stand above ground: in consequence thereoff, the root will weep and air and rain will cause it to perish".

A Kansas State University graduating thesis on alfalfa in Kansas by Syms (93) in 1901 mentions no specific diseases. And even as late as 1929 Melchers (71) lists only violet root rot and an undetermined crown rot. However Freeman (31) in 1908 reported red (violet) root rot rapidly spreading in the state and anthracnose and Texas root rot in Arizona and Texas.

GENERAL STUDIES TO DETERMINE CAUSES OF FUNGUS ROOT DISEASES

Several investigators have surveyed causes of seedling blights and root rots of alfalfa and related legumes with rather inconsistent results. McDonald (68) in one of the most comprehensive surveys found in Manitoba a wide variety of organisms involved. These varied considerably with soil type, time of year, age of plant, and part of root involved.

Table 1. Fungi associated with root and crown rotting of alfalfa in Manitoba showing percent of roots infected under various conditions. Data from McDonald (68).

Organism	Soil Type			Time of Year			Age of Plant				Part of Root			
	Heavy	Sandy	High Lime	Spring	Summer	Autumn	1 Month	1 Year	2 Year	3 Year	Crown	Vascular	Primary	Non-cambial
Pyrenochaeta terrestris	7	43	4	13	20	24	1	1	17	20	16	9	23	25
Fusarium acuminatum	14	15	15	11	14	7	1	14	17	12	23	24	6	5
F. oxy. var. redolens	11	1	6	1	7	4	18	27	8	4	8	2	6	8
Fusarium solani	11	T	T	0	4	3	8	2	4	4	3	6	5	3
Ascochyta imperfecta	6	8	5	6	4	4	0	2	6	7	4	15	4	3
Cephalosporium sp.	4	T	8	2	5	7	0	8	5	3	8	6	3	0
Cylindrocarpon ehrenbergii	3	3	5	5	4	8	0	1	2	5	2	3	6	3
Stagnospora meliloti	T	3	7	1	1	3	0	0	T	6	1	10	2	0
Rhizoctonia solani	3	1	3	0	4	3	19	0	2	3	6	1	3	1
Plenodomus meliloti	3	1	1	30	2	3	0	0	2	1	T	0	3	3
Pythium debaryanum	0	0	0	0	0	0	17	0	0	0	0	0	0	0
Misc. Identified	14	9	24	6	14	9	15	12	18	14	12	14	15	20
Misc. Unidentified	16	13	20	25	15	20	4	11	14	18	8	7	20	27

Pythium debaryanum was important only in cool wet springs on young seedlings. *Fusarium* species predominated in isolations from first year plants but their pathogenicity was questionable. Rhizoctonia solani, usually regarded as an important incitant of root and crown rots was relatively unimportant. McDonald (68) concluded that no one fungus was the primary cause.

Kilpatrick (60) in a survey of fungi associated with root and crown rot of red clover in Wisconsin found three species of Fusarium to be most prevalent and Pythium only on a small percentage, commonly on young plants.

Kainski (56) in a similar study in New York on birdsfoot trefoil, Lotus corniculatus, found several Fusarium species causing root rots and seedling blights, especially in the fibrous root and pith. He found cortical rots caused by several other common root parasites.

Duffield (25) isolated fungi from alfalfa roots in Kansas over a three year period and ranked them in order of importance - Fusarium sp., Cylindrosporium sp., Alternaria sp., Leptosphaeria pratensis, Rhizoctonia solani, Chaetomium sp., Ascochyta imperfecti, and Phytophthora cryptogea.

Leach (63) collected 1050 alfalfa plants from 59 fields in Oregon and examined them for cortical and vascular discoloration and decay. Cortical discoloration or decay was found in 39% and 59% showed vascular discoloration. Older plants showed markedly more of both effects. Isolations from steles yielded Fusarium oxysporum, F. solani, Phoma herbarum var. medicaginis.

Chilton et al. (16) reported 204 species of fungi in 97 genera on Medicago sativa including 26 species of Fusarium, 11 of Phoma and 8 of Pythium. Many of these would fall into Garret's (34) definition of unspecialized parasites, soil saprophytes and mycorrhizal fungi.

Jones (54) in an interesting study of the decay of transient (non-cambial) roots of alfalfa views this sloughing off as a more or less natural process not to be attributed to the action of outside organisms. Erwin and Kennedy (29) noted a collapse of roots of alfalfa following application of irrigation water and called it "scald". They were able to reproduce the symptoms in the greenhouse with saturation of soil with water at 39°C. Clipping predisposed plants to scald.

It is the purpose of this report to review literature available on fungus diseases of alfalfa roots and crowns. In some cases it was necessary to draw a rather fine line between stem infecting fungi and crown infecting ones. From this review it should be possible to know what fungi might be found infecting the below-ground parts of alfalfa and conditions under which they occur. Techniques useful in study and control are important parts of this report. They will provide a basis for further study.

The main body of the report is arranged with a section on each parasitic genus with the species involved and their effect and symptoms. Control measures have been included when given in the literature. The parasites have been arranged more or less in order of their importance with some grouping of those causing damage under similar conditions.

With the number of fungi involved, it is beyond the scope of this report to include any discussion of their taxonomy or morphology.

SPECIFIC FUNGI INVOLVED IN ROOT AND CROWN ROTS

Rhizoctonia

Rhizoctonia has been studied by many workers as a pathogen in alfalfa roots and crowns. It causes pre-emergence and post-emergence damping-off of young seedlings and root and crown rot and canker of established plants.

Rhizoctonia violaceae Tul. (R. medicaginis DC.) causing violet root rot is one of the earliest reported fungus root diseases of alfalfa. Kühn (62) reported it from Germany in 1859 and is credited with the first description. Tubeuf (97) in England in 1897 stated that it had been known in Europe for some time. Early writers on this subject in the United States mentioned this disease prominently: Melchers (71) in Kansas in 1899; Heald (46) in Nebraska in 1906; Fromme (33) in Virginia in 1915; and Reed and Crabill (79) in Virginia in 1913.

First visible symptoms are yellowing of the entire plant which soon wilts and eventually dies. Dying plants appear in conspicuous spots in the field which gradually increase in size 3 to 20 feet per year. Poorly drained soils encourage this disease. Roots are covered with a mat of reddish-brown or violet colored fungus mycelium which gives rise to the common name. As the disease progresses the roots decay and the cortex sloughs off.

Rhizoctonia solani Kühn causes both damping-off, and root canker as well as stunt and collar rot. Many workers have reported R. solani as highly pathogenic to seedlings in both pre-emergence and post-emergence damping-off. They do not agree as to its prevalence in the field or to the age at which alfalfa plants become immune. McDonald (68) in Canada found it of little importance except in month-old plants and then only as one of several fungi involved. Benedict (6) reported that it killed many small roots without causing lesions. Several workers reported some isolate of _R_. _solani_ to be the most pathogenic of any found. Erwin (27) found it causing damping-off and crown rot, but many of the isolates he made were not pathogenic.

This fungus has been one of the most serious in damping-off of cuttings used for asexual reproduction of breeding lines in the greenhouse. Granfield _et al_. (36) reported losses of 90%. Kernkamp _et al_. (59) had essentially the same experience with the fungus evidently carried over in trays and benches. Infection advanced inward from the tray sides toward the center. They reported damping-off especially severe at 85°F and above and recovered _R_. _solani_ from 28 of 70 platings. Good control was achieved by sterilization of flats, benches, soil and rooting media. Haskett _et al_. (44) reported good control with Captan 50W and Panodrench 4 when applied to the rooting sand one day before cuttings were set out.

Addoh (1) found _R_. _solani_ the most pathogenic to alfalfa cuttings of any organism isolated and achieved good control with a drench of Captan 75 or Panodrench.

Benedict (6) reported what he called "stunt" in Ontario of older alfalfa plants caused by _R_. _solani_. Smith (87,88) described in 1943 in California a root canker of established alfalfa plants caused by _R_. _solani_. Infection seemed closely related to high soil temperature from June to September. Cankers began as dark sunken areas, usually circular and sometimes with brownish borders. They generally began at the base of small roots. There was little spread up and down the root. Temperature studies measured by both diameter of colonies and cankers produced on four month old plants showed optimum growth at 30°C. Lesions healed over in the winter and few cankers developed in the field before soil temperature was 20°C or warmer three inches below the surface.

Pythium

Several species of Pythium have been found to be important in pre-emergence and post-emergence damping-off. Halpin et al. (41) found four species, P. irregulare Buis., P. debaryanum Hesse, P. ultimum Trow and P. splendens Braun highly pathogenic to young seedlings. P. paroecandrum Drech. caused moderate damping-off, P. arrhenomanes Dresch. only a slight amount and P. rostratum Butler none. Little difference in pathogenicity was found in the temperature range from 16° to 28°C.

Table 2. Effect on alfalfa seedlings of seven species of Pythium at different temperatures for 15 days. Data from Halpin et al. (41).

	16°C	20°C	24°C	28°C	Ave.
Check	90 (0)	92 (0)	90 (0)	92 (0)	91 (0)
P. rostratum	92 (0)	91 (0)	90 (0)	91 (0)	91 (0)
P. arrhenomanes	93 (1-)	93 (1-)	92 (1-)	93 (1-)	93 (1-)
P. paroecandrum	25 (2+)	40 (2+)	30 (3+)	14 (4-)	23 (3)
P. splendens	0 (4)	0 (4)	0 (4)	0 (4)	0 (4)
P. ultimum	0 (4)	0 (4)	0 (4)	0 (4)	0 (4)
P. debaryanum	0 (4)	0 (4)	0 (4)	0 (4)	0 (4)
P. irregulare	0 (4)	0 (4)	0 (4)	0 (4)	0 (4)

First figure is % stand and the second in () is disease severity with 0 for no disease and 4 meaning all plants killed.

In a later study Halpin and Hansen (40) tested the effect of age of seedling on damping-off by five species of Pythium and concluded that "age had a profound effect ... alfalfa plants were extremely susceptible to some species of the fungus when inoculated at the time of seeding or within one day thereafter. However by the time the plants were three days old they had developed resistance."

Chi and Hansen (15) in a follow-up study did not find immunity so soon acquired but agreed that it increased with age of the plant. Most were immune at five days but a few were still susceptible at two weeks and 1% became infected when inoculated at 29 days of age. In studying environmental effects on immunity development, they found that resistance developed more slowly at 16°C and most rapidly at 20°C. Soil type seemed to have little effect but pre-emergence damping-off appeared most often in silt loam and post-emergence damping-off in silty clay loam. Plants in nutrient solution developed resistance more slowly than those in sand or soil.

Gregory et al. (38) found P. ultimum to be the most pathogenic of this genus especially in pre-emergence damping-off. P. debaryanum caused extensive post-emergence damping-off. They reported P. irregulare and P. vexans DeBary only mildly parasitic. High daily maximum temperatures of 85° to 90°F decreased damping-off and increased seedling survival over 70° to 75°F maximums. They too concluded that resistance increased with age of the seedlings and found little or no loss of seedlings inoculated 14 or 20 days after seeding.

Bucholtz (9, 10, 11) in Iowa identified P. debaryanum as the main incitant of alfalfa damping-off and noted its attack any time from the germinating seed to the time when the plant developed secondary roots. Germinating seeds became brown, soft and gelatinous. Seedlings were attacked on the hypocotyl and root and showed a brown discoloration at the soil line. Older plants showed infection only in the cortex. He noted a definite association of soil acidity and Pythium with 16 to 48% disease of plants at pH 6.2 and only 6 to 7 % diseased at pH 6.8 to 7.0.

Liming soils helped reduce infection some but was generally ineffective. The dry summer of 1934 seemed to free some soils of Pythium. There was less disease incidence in acid soil at 9°C than at 20° to 25°C. Early planting and deep plowing reduced damping-off and increased the stand.

Granfield et al. (37) studied the relation of fallowing to damping-off caused by Pythium. They noted a decrease in vigor of plants growing in land fallow the previous three to five years. Seedlings showed stunting, lack of vigor, yellowing, decay of the primary root below the soil line and localized lesions on the hypocotyl prior to emergence. After four years in fallow 55% of the plants were diseased. Roots of plants on continuously cropped land were longer. Sterilization of soil reduced damping-off to 6 to 7%. Isolations from roots were predominately Pythium. It was most virulent at lower temperatures. Heavy applications of lime decreased damping-off somewhat.

Kreitlow et al. (61) working with P. debaryanum found pre-emergence damping-off did not increase in thick stands but post-emergence disease did increase. Fertilization and pH showed little effect. Damping-off increased with soil moisture. Fungicidal seed treatments gave good control of Pythium in the greenhouse but field studies did not give consistent improvement in stands.

Stuteville (92) in Kansas investigated resistance to Pythium irregulare of various breeding lines and selections of alfalfa. Plants that grew from larger seeds were more resistant. At 60°F the damped-off plants died slowly and some formed adventitious roots and survived. At 82°F the plants died quickly. Highly significant differences in resistance were shown in 16 varieties. Survival expressed in percent of controls ranged from 35.9% in Buffalo down to 3.42 % in Zia. Pythium tolerance did not seem to be closely associated with plant vigor or climatic adaptation. Introductions of 15 varieties from 13 countries showed variations in suceptibility but none were more resistant than commonly cultivated varieties.

Gregory et al. (38) tried several antibiotics and antagonistic organisms against Pythium induced damping-off in alfalfa. Actidione as a seed treatment injured seedlings severely enough to preclude its use as a protectant. Fradicin soil treatments were ineffective. In another report Gregory and his co-workers (39) made 65,000 isolations of micro-organisms from soil, peat and chaff. From these they found 29 actinomycetes, 31 bacteria, and 14 fungi which produced antibiotics inhibitory to the strain of P. debaryanum tested. An antibiotic, gliotoxin, extracted from Trichoderma lignorum prevented damping-off of alfalfa seedlings by P. debaryanum when added to the soil three days ahead of the inoculum.

Weindling (102) studied the relationship of Trichoderma lignorum (Tode) Harz. to other soil fungi and found it parasitizes Rhizoctonia solani, Phytophthora parasitica, Pythium spp., Rhizopus spp. and Sclerotium rolfsii. When growing submerged in the medium the inhibitory action appeared to be diffusible.

Allen and Haensler (2) reported Trichoderma lignorum reduced damping-off of cucumbers when added with the Pythium and Rhizoctonia inoculum. A sterile filtrate inhibited Rhizoctonia and Pythium debaryanum. This toxin was destroyed by heat of 100°C and by oxidation which occurred on standing 10 days.

Meredith and Semeniuk (73) isolated a Streptomyces from the soil near Ames, Iowa which inhibited the growth of Pythium graminicola. Teakle (95) reported a root rot of lucerne from Australia caused by P. myriotylum Drech. It appeared in low nutrient water logged virgin soil containing a large amount of decomposing roots and other parts of native plants. Almost all plants were lost by four months after seeding.

Fusarium

Many species of Fusarium have been found associated with damping-off, root rot and wilts of alfalfa but there is not universal agreement as to their pathogenicity. This springs in part from lack of agreement on the taxonomy of this large and varied genus. This variability makes it difficult to duplicate experimental results.

As early as 1921 Cottam (23) reported a dry rot of alfalfa roots caused by a Fusarium. It attacked the base of the lateral roots destroying them, then infected the parenchymatous tissue and xylem vessels of the tap root. This rot was most active during the hottest period of the summer and seemed confined to hot dry localities of Utah. Plants withered and died in a random scattering over the field. Spots developed from centers at these plants and spread into areas several rods in diameter with active margins three feet wide. No pathogenicity studies were made.

Cormack (19) collected five parasitic species of Fusarium from alfalfa and sweet clover roots in widely scattered fields in Alberta. F. avenaceum (Fr.) Sacc. and F. arthrosporiodes Sherb. were the most common in early spring as well as summer. F. culmorum (W. G. Sm.) Sacc. was very virulent in summer but not in spring. F. poae (Peck) Wr. and F. scripi

Lamb et. Fautr. var. acuminatum (Ell. et. Ev.) Wr. were weak pathogens. All five had wide growth ranges of cardinal temperatures from a minimum between -2° and $3^{\circ}C$ to an optimum of 20° to $27^{\circ}C$ and a maximum of 34° to $37^{\circ}C$. They penetrated tissue directly and appeared to have advance toxic action.

In 1949 Staten (90) reported a root rot of alfalfa caused by F. solani (Mart.) Appel and Wr. It caused "reddish brown vascular discoloration which, in the majority of cases, followed from the point of origin in lateral roots". Typical crown rot was sometimes seen. Plants were stunted and leaves were light green to yellow.

Four Fusarium species were prominent among the fungi found on alfalfa roots in Manitoba by McDonald (68). F. solani caused some post-emergence damping-off, F. acuminatum Ell. & Ev. caused stunting and F. oxysporum pre-emergence damping-off. F. oxysporum Schlecht var. redolens (Wr.) Gordon was only slightly pathogenic.

Fusarium roseum and F. solani were found by Erwin (27) to be associated with a condition he called "dark crown necrosis" but field tests did not prove pathogenicity. He stated that there was "no indication that F. roseum was pathogenic on alfalfa past the seedling stage".

Weimer (100, 101) working in Kansas in 1927 - 28 reported a wilt of alfalfa caused by F. oxysporum Schlecht var. medicaginis Weimer. Upper leaves turned yellow, faded to buff and then straw color. Basal leaves sometimes were tinged with rose or pink. Tips of stems wilted in the heat of the day. A single stem was first affected and several weeks elapsed before the whole plant was killed. Vascular bundles darkened sometimes on one side of the stem only. With the bark removed the vascular region showed cinnamon or mummy brown. This compares to the yellow to light brown of bacterial wilt with which it can easily be confused. Infection occurred during late spring or summer on year-old plants as well as seedlings invading through lateral roots.

Nematodes played a significant role in Fusarium wilt of alfalfa according to research of Walters and Slack (98) and McGuire and Walters (69) in Arkansas. They inoculated alfalfa plants with combinations of F. oxysporum var. vasinfectum and several species of Meloidogyne.

Table 3. Relationship of rootknot nematodes to the development of Fusarium wilt in alfalfa. Data from McGuire and Walters (69)

	% Infected	%Dead
Meloidogyne hapla and F. oxysporum var. vasinfectum	95	40
Meloidogyne javanica " " " " "	60	5
Meloidogyne incognita" " " " "	50	10
Meloidogyne arenaria " " " " "	50	4
Fusarium only	15	0
Meloidogyne spp. only	0	3
Check	0	0

Nematodes alone reduced root size and killed a few plants. Fusarium alone gave slight infection. Fusarium plus Meloidogyne hapla caused 95% infection and killed 40% of the plants. They concluded that "nematodes injure alfalfa roots, allowing ever-present Fusarium fungus to enter".

Very little information was found in the literature on soil conditions necessary to permit Fusarium to become a serious root disease agent. Menon and Williams (72) studied the effect of four different crops and their residues, temperature and moisture on soil mycoflora. Fusarium and Aspergillus appeared to build up more under alfalfa than in corn, oats or wheat ground.

Colletotrichum

Anthracnose caused by Colletotrichum trifolii Bain and Essary is usually a disease of the aerial parts of alfalfa. But several workers have reported it causing crown and root rot. Bain and Essary (5) described this new species in 1906 which they found on alfalfa and sweet clover the previous year. It caused a crown rot just at or below the soil surface. They called it the "most serious plant disease in the state". Freeman (31) mentioned the disease in his Kansas bulletin in 1908 and said it was "in Tennessee".

Monteith (74) made the first comprehensive study of anthracnose in 1928 on clover but stated that it was common on alfalfa where it appeared on clover. Any aerial part as well as the upper taproot was affected. In the crown it caused wilting on one side of the plant then eventually girdled and killed the plant. Tissues became brittle and plants broke off at soil level. Pathological histology studies revealed direct penetration of the epidermis with intra and intercellular mycelial growth. Phloem collapsed and xylem and pith were invaded. Anthracnose was only serious during dry summer weather but required high humidity for infection. Optimum growth temperature was 25° to 30°C.

Henderson and Smith (47) reported a crown rot of alfalfa caused by C. trifolii in the summer and fall of 1947. Dying alfalfa showed stem rot and root rot downward from the crown appearing to have entered through the shoot. Affected tissue was yellowish to reddish brown.

An epiphytotic of anthracnose in New York in the summer of 1958 was reported by Roberts et al. (81). C. trifolii was identified as the incitant. Symptoms were wilting, bleached foliage, brittle stems and bluish black necrotic tissue. Half of the diseased plants had crown rot. No explanation was given of possible conditions causing an outbreak of this disease.

Sclerotinia

Sclerotinia trifoliorum Eriks. was reported on Medicago lupulina in Europe in 1897 by Tubeuf (97) but with no description. Freeman (32) reported a basal stem rot caused by S. libertiana in Arizona in 1914. Cormack (18) reported Sclerotinia sp. pathogenic on clover and less so on alfalfa roots in Canada in 1934. It was more pathogenic in early spring than in summer.

Cappellini (14) in New Jersey in 1960 observed 100% losses in newly seeded alfalfa from S. trifoliorum. It caused 20% to 30% loss from crown rot in the spring on alfalfa seeded the previous fall. Isolates from several different crops varied considerably in pathogenicity on alfalfa. Some were not pathogenic. Optimum temperature for growth was 15° to 20°C.

Little further reference was found in the literature to any work done specifically on alfalfa with any species of Sclerotinia.

Plenodomus

Plenodomus meliloti D. and S. was described by Dearness and Sanford (82) in 1930 on sweet clover in Canada. It was serious only in late winter or early spring. Optimum temperature was 15° to 17°C for growth but severe lesions could still be caused at 2° to 3°C. Alkaline soils were unfavorable for its development. Symptoms were brown necrotic lesions of the taproot or lateral roots which appeared when the surface of the soil thawed. Pycnidia were usually abundant on the lesion surface.

Four years later Cormack (18) reported P. meliloti causing a destructive root rotting following winter dormancy. Lesions were arrested in June when the fungus ceased to be pathogenic. The fungus was able to penetrate wounded and unwounded roots alike. It advanced within and between cells killing the cells ahead of advancing hyphae.

McDonald (68) found P. meliloti an important root inhabitant only in the spring in Manitoba. At this season it made up 30% of the isolates obtained. Summer isolates were only 2 or 3% Plenodomus. This correlates well with the cool temperature observations of other workers. This fungus is one of those doing damage at the first spring thaw and their damage was usually attributed to winter injury.

No reports of this fungus on alfalfa were found from pathologists in the United States.

Cylindrocarpon

Cylindrocarpon ehrenbergii Wr. was reported in 1937 by Cormack (20) as a root parasite of sweet clover and alfalfa in Alberta. Damage occurred at the end of winter dormancy. Three other species of *Cylindrocarpon* were mildly pathogenic.

Symptoms appeared early in the spring. As soil thawed it was permeated with whitish mycelium. Water-soaked lesions on the root enlarged and turned brown. The root was often completely rotted off in a week or two. Salmon orange colored brittle sclerotia-like stromata developed in cracks of ruptured cork. Infection was rare during the summer season. Wounding facilitated entry of the fungus but was not necessary for infection. Alfalfa appeared less suceptible than sweet clover.

Both Cormack (20) and McDonald (68) found year-old plants more susceptible than seedlings. Temperature studies showed *C. ehrenbergii* growing on frozen agar at -2°C. Optimum growth was reached at 20°C. Periodic observations on roots inoculated with this fungus in late fall showed that infection did not occur until the soil started to thaw in April but then it proceeded rapidly. However relatively light infection occurred when roots were inoculated shortly after the soil thawed out. More infection occurred in April than later in the season after the plants passed their most susceptible stage.

While *C. ehrenbergii* was not as specifically limited to early spring injury as *Plenodomus* its damage had usually been attributed to winter injury and fits into the group of cold loving parasites.

Snow Mold

Canadian plant pathologists have done considerable work on another cold-loving fungus growing under snow cover which they have called a "low temperature basidiomycete". It is of major importance in western Canada. Broadfoot and Cormack (8) published a description in 1941 although it was first studied on turf grass a decade earlier. Cormack (21) pointed out that it usually had been called winter kill because it attacked under the snow and showed up after the first thaw. Lebeau and Dickson (64) discovered that the toxic principle was hydrogen cyanide which was formed in association with alfalfa and built up in high enough concentrations under snow to kill alfalfa plants. In 1959 Lebeau and co-workers (65) proved that infection must occur in the fall and the fungus must grow under the snow for some time before HCN is produced in toxic quantities.

Symptoms appeared at the first thaw in the spring as irregular patches of dead plants. There was a partial rotting causing brown areas on crowns and less commonly on lower roots. Incidence appeared more in dry than wet soil and old stand than in younger plants.

This "low temperature basidiomycete" was very slow growing in culture requiring 10 to 14 days incubation at 1°C to produce observable growth. Ward _et al_. (99) reported several isolates with widely varying growth rates, pathogenicity and HCN production indicating the possibility that this is a complex of several entities. No conidial or perfect stages have been positively identified but characteristic clamp connections in the mycelium show its basidiomycete affinity. Cormack and Lebeau (22) in 1959 identified a _Typhula_ sp. causing injury to alfalfa similar to this fungus but it produced no HCN and so their relationship was not conclusive.

Phytophthora

First report of root rot of alfalfa caused by Phytophthora cryptogea Pethybridge and Lafferty came from Erwin (28) in California in 1954. Taproot, crown and lateral roots first showed brown irregular areas. Leaves yellowed and wilted then plants died. Inside the root red brown necrotic areas in water-soaked tissue were common. A yellowish region showed two to three cm. above and below lesions but the fungus could not be isolated from these. It was extremely slow growing in culture. Optimum growth temperature on solid and liquid media was 25°C. Seed treatment with Ceresan improved emergence but did not protect plants long enough to survive. Untreated seeds did not even emerge in artificially infested soils.

Schmitthenner and Williams (83) found crop rotation ineffective in controlling P. cryptogea.

Bushong and Gerdemann (13) found P. cryptogea in eight counties in Illinois in 1959. It was the most prevalent in heavy poorly drained soils. Cool rainy weather permitted rapid development of the disease with plants quickly wilting and dying. Warm dry conditions slowed the development of the disease with the result that plants were stunted and chlorotic. In some cases there was a soft rot of the crown and root.

Duffield (25) found P. cryptogea on alfalfa roots in Kansas in 1958 to 1960. Simmonds (85) reported P. parasitica causing crown and root rot of alfalfa in Queensland, Australia in 1959.

Phymatotrichum

Cotton root rot was first described in Texas by Pammel (77) in 1888. He had been hired by the new experiment station specifically to study this disease as one of their first projects. "Few people believe it to be due to a parasitic fungus" he stated in his first report. Acid, alkali, heavy soil, iron sulfate and various other things had been blamed. He consistantly isolated Ozonium auricomum Lk. from diseased roots and concluded that it was the causal agent. Four years later in 1892 Curtis (24) in Texas studied the new alfalfa root rot and established that it was caused by the same fungus.

"Brown root rot" was mentioned on alfalfa in Arizona in 1906 by Thornber (96) but the causal organism was not identified. His description fitted the Texas disease. In 1908 Freeman (31) mentioned it in a Kansas bulletin saying it occurred in Texas and Arizona. He called it Texas root rot and identified the fungus as O. auricomum. Duggar (26) in 1916 described the pycnidial stage and transferred it to Phymatotrichum omnivorum (Shear) Duggar. Stewart (91) in 1926 stated that it occurred in Kansas as well as Texas, Arizona and California. A search of the literature on distribution has failed to substantiate this claim. It appears to be limited on the north to southern Oklahoma except for an isolated area near Sayre in west-central part of the state. Surveys showed it to be indigenous to virgin soils of southwest United States.

Ezekiel (30) studied the distribution and found its northern limit followed rather closely the isotherm line where the lowest recorded temperature was -10°F between 1899 and 1938. Correlation was also rather close with the northern limits of where the average January temperature is 40°F or higher and the July average is 80°F or more and the frost-free period is 200 days or more. Exposures of more than 24 hours at -13°C killed all stages of the fungus in his experiments.

Symptoms on alfalfa resembled Rhizoctonia, killing plants in a well-defined usually circular area which enlarged in time to a hundred yards or more in diameter. Plants turned yellow, wilted and usually died in the course of a few days. The disease was abundant on wet soils. The attack of the fungus usually stopped one to three inches below the soil surface. Roots were covered with a brown cushion of mycelium with small roots completely rotted away.

No later intensive studies of this disease on alfalfa were found in the literature.

Sclerotium

"Charcoal rot" is the name coined by Taubenhaus (94) in 1913 for the disease caused by Sclerotium bataticola Taub. on sweet potatoes. According to Ashby (4) Maublanc had described a pycnidial stage from beans as Macrophoma phaseoli Maub. in 1905. Ashby later transferred it to Macrophomina phaseoli (Maub.) Ashby. Hensen et al. (48) reported it mildly pathogenic on red clover in Kentucky in 1937. No record was found of charcoal rot of alfalfa until Hoffmaster et al. (49) reported it as a new host in 1943. It caused damping-off, root decay, stem rot, precocious ripening, low yield and premature death. It was "moderately and variably agressive" and "invasion was favored by devitalization, characteristic of plants subjected to environmental extremes of continental climate". High temperature favored its development. This agrees with investigation by other workers on its development in other plants.

No reference was found to any recent specific research into this disease on alfalfa. Norton (76) checked the relationship of *M. phaseoli* with several other soil fungi including three species of *Aspergillus*, *Thielavia terricola*, *Trichoderma lignorum* and *Bacillus cereus*. All these organisms inhibited growth of *M. phaseoli* in sterile, unamended and amended soil. *Thielavia* and *Trichoderma* invaded the *Macrophomina* colony and apparently parasitized it. Norton concluded that the charcoal rot fungus could not compete with other soil micro-organisms. In another study he observed that high temperature did not increase its pathogenicity but lowered host plant resistance.

Allison (3) found alfalfa susceptible to the southern blight fungus, *S. rolfsii*. Hot humid weather of 25° to 30°C favored its development.

Verticillium

References to research on *Verticillium* wilt of alfalfa were found only from Britain and Germany. According to Isaac (50), Richter and Klinkowski reported it in Germany in 1941. Noble *et al.* (75) found it in Britain in 1953 where it had been confused with bacterial wilt. Isaac *et al.* (50, 51, 52) in a series of research reports from Britain said it became widespread in England and Wales from 1954 to 1956. *V. albo-atrum* Reike & Berthold was named as the causal organism.

External symptoms were typical of vascular wilts with flagging of upper leaves during the heat of day, pale yellow to whitish and desiccated shoots. New shoots sometimes appeared from crowns and in turn wilted and died. The basal two to six inches of the stem were often covered with grayish mycelium which later turned dark with microsclerotia.

Roots showed no external symptoms but had dark brown discoloration of xylem due to deposition of gum-like material in vessels and tracheids and browning of cell walls. This vascular discoloration could be traced from small lateral roots through the taproot up into stem apices and occasionally into leaf petioles and flower pedicles.

Pathogenicity studies indicated this was a distinct strain of V. albo-atrum as isolates from other plants were nonpathogenic on alfalfa. Indications were that the fungus was not borne in the seed itself but with seed in pieces of infested plant parts. Isaac also proved that the fungus could be transmitted from infected to healthy plants by cutting tools at harvest with the open cut stem the infection court. Verticillium also could penetrate the root directly.

Seed dressing and fumigants gave promise in killing the fungus in accompanying plant parts and so would prevent introduction to new fields. In a follow-up report Zaleski (105) found all strains of alfalfa susceptible to Verticillium wilt but with some variation in susceptibility.

No reference was found to Verticillium wilt occurring naturally on the North American continent but in 1961 Smith (89) from New Mexico was able to get some infection of alfalfa artificially inoculated with V. albo-atrum isolated from cotton.

Cephalosporium

Roberti (80) claims first report of Cephalosporium on alfalfa from Rhode Island in 1960. McDonald (68) however, had reported it in Manitoba five years before but of little importance. Neither identified the species. Roberti (80) observed severe infection over a two year period in which 90 to 98% of the seedlings were infected. Symptoms showed up as black specks or hair-like lesions starting in the epidermis and expanded into sunken areas of the cortex. Root hairs were killed and taproots died back. The fungus invaded the xylem and pith forming reddish brown streaks extending to the crowns.

Stagonospora

Stagonospora is usually associated with leaf spot of alfalfa. Jones and Weimer (55) in 1938 reported Stagonospora meliloti (Lasch.) Petr. (Phoma meliloti Allescher) as the incitant of a root rot in California and Wisconsin. The ascigerous stage is Leptosphaeria pratensis Sacc. & Briard. Dark brown to black lesions appeared only on the upper part of the taproot, crown and branches. These lesions are first moist then become rough, dry and cracked. Progress of the disease was slow, requiring several months to rot the taproot. Inside the root there was a distinct reddish wedge extending from the cortex through the rays into the pith.

The Phoma stage appeared in the fall in cool temperature and the Leptosphaeria the following spring and summer. The ascigerous stage was rare. Pathogenicity studies indicated that the fungus entered only through wounds, progressed through parenchymatous ray cells and did not enter vessels.

Erwin (27) in a 1954 study of S. meliloti noted the same reddish flecking in "wood and bark". No symptoms were observed in seedlings but appeared when plants were two to three months old. The fungus did not remain viable long in soil, being inhibited by competitive microflora. McDonald (68) found S. meliloti mostly as a vascular isolate in three year old plants growing in high lime soils. It was not very important in his survey in Manitoba. Duffield (25) mentioned Leptosphaeria pratensis as a common isolate from Kansas alfalfa roots.

Miscellaneous Fungi

Several other fungi have been reported to be associated with alfalfa root and crown rot. Most have been isolated cases.

Diplodia. Sleeth (86) in 1951 found *Diplodia gossypina* Cke. (*D. natalensis* Pole and Evans) to be the cause of crown rot in Arizona. It was isolated from three and four year old plants and caused dark brown to black dry rot. Pathogenicity was demonstrated on two year old plants. Injuries were necessary for the fungus to gain entrance. The author said it was of "minor importance".

Rosellinia. Harris (42) reported a root rot of alfalfa from California in 1941 caused by *Rosellinia necatrix* (Hartig) Berlese. This was the first report in the United States. It killed all plants in circular spots in the field. Fumigation of the soil halted the advance of the disease.

Aphanomyces. Linford (67) found alfalfa susceptible to the pea root rot fungus, *Aphanomyces euteiches* Drech. It attacked very young seedlings which were weakened and killed. Only a small fraction of the stand was affected.

Ascochyta. Several researchers (25, 27, 36, 63, 68, 70) have reported *Ascochyta imperfecta* Pk. (*Phoma medicaginis* Malbr. & Raum.), the black stem fungus, sometimes caused seedling rot. McDonald (68) and Leach (63) both found it in the steles of taproots. Granfield *et al.* (36) ranked it the most destructive of any disease on cuttings for asexual propagation in the greenhouse. Root infection came from the black stem lesions already on stems before cutting. It is of doubtful importance in field seeded alfalfa.

Leptodiscus. Gerdemann (35) in 1953 established a new genus and species, *Leptodiscus terrestris* Gerdemann, for the fungus found causing root rot of alfalfa and red clover in Illinois. It caused both pre-emergence and post-emergence damping-off. Penetration was direct causing a dark decay of roots. Sclerotia were formed in the field and the pycnidial stage in the greenhouse. Optimum temperature for growth was 30°C.

Thielavia. Burkholder (12) mentioned that alfalfa was susceptible to the bean root rot fungus, *Thielavia basicola*.

Pyrenochaeta terrestris (Hansen) Gorenz, Walker & Larson, the incitant of pink root of onion, was reported by McDonald (68) in Manitoba in 1955 as causing root rot of alfalfa. It was found rather frequently but he rated it weakly pathogenic. This fungus was more prevalent on two and three year old plants in late summer and fall.

Mycorrhiza. Jones (53) in 1924 reported an unidentified phycomycete, which he classed as mycorrhizal, on alfalfa roots. It entered the primary cortex and fed by haustoria causing a greenish-yellow discoloration of the roots and stunting of the plant.

Physoderma. *Physoderma alfalfae* causes crown wart of alfalfa. But since it is a disease of the crown buds and emerging shoots it is not included in this report.

CONTROL

Research results on specific control measures have been reported in the appropriate section with the organism involved. This section is written to tie these together in a meaningful whole.

Resistance

The first defense of a plant against an invading fungus is its anatomical make-up. Seth and Dexter (84) made a detailed study of root structure in bacterial wilt resistant and susceptible varieties. They found no correlation between any structural feature and wilt resistance. Cormack (18) concluded that cork formation on alfalfa roots did not seem to play a significant role in preventing penetration of *Sclerotinia* or *Plenodomus* in the spring. Priestly(78) studied the role of suberin and cutin in disease resistance and found that these materials replaced cellulose in cell walls but drew no conclusions as to their importance. LeClerg (66) studied the vascular structure of alfalfa roots and their plugging as a result of disease. Water flow through roots in which vessels and tracheids were plugged was markedly slowed. He also noted that "tracheids were generally smallest at the crown and therefore more easily plugged".

Stuteville (92) in 1961 found highly significant differences in 16 varieties of alfalfa in their resistance to *Pythium irregulare* with survival ranging from 3.42% in Zia to 35.9% in Buffalo. Less resistant varieties were more subject to pre-emergence damping-off. Post-emergence damping-off killed a proportionately higher percentage of the more resistant varieties. None of the 15 foreign introductions proved more resistant than Rhizoma.

Little reference was found in any study to the biochemical basis of resistance in alfalfa roots. Experiments by Hawn and Lebeau (45) indicate that root exudates of wilt resistant alfalfa varieties stimulate certain bacteria in their root zones to produce a complex substance toxic to the bacterial wilt organism, Cornybacterium insidiosum. Assays showed that resistance of alfalfa varieties to wilt may be related to the amount of bacterial antibiotic they stimulate in their respective root zone.

Antibiosis in Control

The interaction of soil micro-organisms and pathogens is extremely complex. Antagonisms and antibioses of soil fungi and bacteria against alfalfa root invaders have been studied by many researchers (2, 38, 39, 73, 102). Their findings have been reported with the appropriate parasites. Trichoderma lignorum has been the most commonly studied antagonist. Some organisms produce diffusible antibiotics and others actively parasitize the root pathogens. Gregory and his co-workers (39) found 29 actinomycetes, 31 bacteria and 14 fungi from among 65,000 isolates that produced antibiotics against a strain of Pythium debaryanum.

Environmental and Cultural Control

The effect of environment and cultural practices has been reported under specific diseases. Several of the winter crown rots in Canada appear limited to areas of prolonged snow cover. Sclerotium rolfsii occurs in the warm humid southern climate. Texas root-rot, Phymatotrichum omnivorum, is limited to hot semidesert regions of the Southwest where temperatures never fall below -10°F. Charcoal rot attacks only under conditions of high temperature and low moisture stress. Pythium affects only young seedlings and is most severe on cool damp acid soils which have been fallowed.

Crops and their residues significantly affect soil mycoflora which in turn affect pathogenic fungi. Research by Menon and Williams (71) and a follow-up by Williams and Schmitthenner (103) showed that Aspergillus and Fusarium species increased in alfalfa ground and Pythium damping-off was significantly affected by different crops and their residues. Soybeans and their residue reduced alfalfa seedling diseases.

Chemical Control

Several researchers (1, 44, 38, 71) have successfully used chemicals and soil amendments for controlling root disease in the greenhouse but none were very successful in the field.

Bucholtz (11) in 1943 found lime reduced Pythium damping-off but rated it "ineffective". Granfield et al. (37) 1935 used formalin to reduce Pythium damage and noted that lime at high rates reduced damping-off. Kreitlow et al. (61) treated seeds of several legumes with common seed treatment fungicides against Pythium. These gave good results in the greenhouse but were inconsistent in the field. The effect of fungicides on the Rhizobium nitrogen fixing bacteria presents an extra problem. Control of damping-off of asexual cuttings in the greenhouse has been reported by Addoh (1) and Haskett et al. (44) in Kansas using soil drenches of Captan 75, Panodrench, PCNB, and Phaltan.

Chemical control of damping-off from Pythium and Rhizoctonia appears to be a possibility in the field because plants develop early resistance. But chemical protection against fungi which attack older mature roots appears problematical.

DISCUSSION

It is apparent that root and crown diseases of alfalfa are complex. Many factors affect expression of these diseases - species of organism, age of plant, temperature, moisture, soil type and reaction, cropping sequence, and interaction of soil organisms themselves. Symptoms of many of these diseases are quite similar, requiring careful sampling, isolation techniques, field observations and evaluation in order to reach valid conclusions.

A broad study of the organisms involved would be valuable. It should cover more than one year and should take into consideration several variables - age of plants, time of year, part of the root or crown affected, weather conditions, soil type and moisture, soil amendments used and cropping history. In order to be sure that all fungi involved are identified, a variety of isolation techniques should be used - different media, incubation temperatures and lengths of incubation. This would help eliminate missing some important pathogens such as the Cylindrocarpon which grew on Cormack's (20) frozen agar, Erwin's (28) Phytophthora cryptogea which was extremely slow in starting growth in culture, or the Canadian "low-temperature basidiomycete" which took 10 to 14 days to produce observable growth at 1°C. Age of plants has been shown by many researchers to control growth of pathogenic fungi and should be considered. Marked differences have been found in fungi infecting alfalfa roots at different times of year.

Several researchers have done their work only with highly virulent strains then found their results not applicable under field conditions where these specific strains were not prevalent. Others have carried out investigations in sterile cultures and soil and in so doing failed to measure the importance of interplay of soil microflora.

The extreme complexity of antibiosis offers both a challenge and a stumbling block to the research worker. It is possible that workable biotic controls can be developed from research in this area. But the investigator who fails to consider the interactions of soil micro-organisms under field conditions is liable to draw erroneous conclusions.

An area of study which could yield interesting results is the relationship of nematodes to root and crown fungi as indicated by Arkansas workers McGuire, Walters, and Slack (69, 98). Predatory mycophagous nemas possibly are a controlling force on some soil pathogens.

Another interesting area would be studying factors involved in causing common soil inhabiting fungi to become pathogenic at times. Is this influenced by cropping history, climate, soil amendments or mutations to virulent strains? There are yet many unanswered questions!

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FUNGUS ROOT DISEASES OF ALFALFA

by

WILLIAM G. WILLIS

B. S., Kansas State University, 1951

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ABSTRACT

Root and crown diseases of alfalfa were serious in Kansas during 1958 to 1962. Farmers lost newly seeded and year old stands. Acreage in the state dropped a half million acres from 1955 to 1959.

A survey of the literature was made to summarize research published on fungi causing root and crown diseases of alfalfa to form a basis for further study of this problem. All research papers available on this subject were reviewed and summarized as to symptoms, conditions of disease expression and control.

A large number of organisms have been proved pathogenic to alfalfa crowns and roots. They can be divided into groups according to conditions of the plant or environment under which they occur.

Pre-emergence and post-emergence damping-off was caused by seven species of *Pythium*. *P. debaryanum*, *P. ultimum*, and *P. irregulare* were most often the causal agents. Alfalfa seedlings acquired resistance to *Pythium* 3 to 10 days after emergence. *Rhizoctonia solani* was one of the most pathogenic damping-off organisms on young plants and on cuttings for asexual propagation. Several species of *Fusarium* also damped-off seedlings. *Phytophthora cryptogea* caused serious damping-off of seedlings and year old stands.

Canadian workers found three fungi causing root and crown rots of alfalfa immediately after spring thaw. *Plenodomus meliloti*, *Cylindrocarpon ehrenbergi*, and a "low-temperature basidiomycete" were found causing root and crown decay which had previously been blamed on winter injury.

Phymatotrichum omnivorum, *Sclerotium bataticola* and a species of *Fusarium* caused root and crown injury during hot dry weather.

Warm humid weather was associated with injury from two species of Sclerotinia, Sclerotium rolfsii, and a Fusarium species. Violet root rot caused by Rhizoctonia violaceae occurred mostly in wet soils.

Vascular wilts have been caused by Verticillium albo-atrum, Fusarium oxysporum var. medicaginis and F. oxysporum var. vasinfectum.

Ascochyta imperfecta, Stagonospora meliloti and Colletotrichum trifolii ordinarily cause leaf or stem diseases but under certain conditions caused crown and root decay.

Eleven species of Fusarium were reported causing damping-off, root decay and wilt under a wide variety of conditions.

Other fungi found infecting alfalfa roots and crowns include Cephalosporium sp., Diplodia gossypina, Rosellinia necatrix, Aphanomyces euteiches, Leptodiscus terrestris, Thielavia basicola and Pyrenochaeta terrestris.

Resistance has proved the most successful control. Antagonistic and antibiotic producing organisms in the soil hold many alfalfa pathogens in check. Crop rotations and residues also affect soil pathogens. Chemical control appears feasible only for damping-off fungi for which resistance is acquired early.