



The crown-root rot complex in sainfoin (*Onobrychis viciaefolia* Scop.)
by Rollin George Sears

A thesis submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE in Agronomy
Montana State University
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Abstract:

The crown-root rot complex was studied in sainfoin. Experiments were conducted to determine the causal organism(s) of crown and root rot, its disease severity and prevalence in Montana and the host parasite relationship.

Crown and root rot found in sainfoin appears to be primarily caused by *Fusarium solani*. The fungus was isolated from decayed crown tissue in both dryland and irrigated fields at ten locations throughout the state. Two major symptoms were identified; crown rot and vascular discoloration in the roots. Vascular discoloration was not directly associated with any soil-borne pathogen. However, *F. solani* and *F. oxysporum* were isolated from discolored vascular tissue sporadically. This symptom may be caused by a microbial toxin or associated with a physiologic reaction by the plant root to different environments. Vascular discoloration was associated with irrigated fields. Crown rot and vascular discoloration appear to be associated with an increased susceptibility for sainfoin plants to winterkill.

Pathogenicity tests indicated *Rhizoctonia solani*, *F. solani* and *F. oxysporum* could infect sainfoin. *R. solani* was the most virulent fungus tested to developing seedlings reducing seedling vigor significantly. *F. solani* stimulated plant growth in seedlings. This response may be associated with production of gibberellin by the fungus. *F. solani* did not cause crown rotting symptoms in seedlings.

F. solani rapidly infects seedling sainfoin plants. The seed hull wounds the developing root allowing infection; hullless seed reduced incidence of infection. Physiologic crown splitting was associated with initiation of crown rotting symptoms. Invasion by *F. solani* appears to occur either at the hull wound or within old decayed stems which allow the fungus to invade the top crown.

Alternaria sp. appeared to be seed-borne on sainfoin hulls. Infection by *Alternaria* sp. in autoclaved soil resulted in reduced seedling vigor or seedling death. *F. oxysporum* and *F. solani* were not transmitted by seed.

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by

ROLLIN GEORGE SEARS

A thesis submitted to the Graduate Faculty in partial
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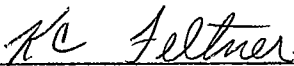
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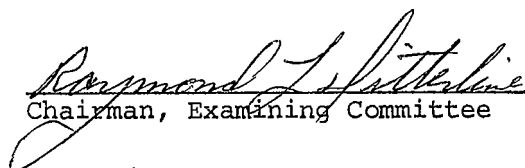
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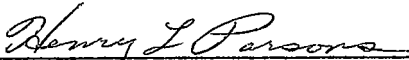
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ABSTRACT

The crown-root rot complex was studied in sainfoin. Experiments were conducted to determine the causal organism(s) of crown and root rot, its disease severity and prevalence in Montana and the host parasite relationship.

Crown and root rot found in sainfoin appears to be primarily caused by Fusarium solani. The fungus was isolated from decayed crown tissue in both dryland and irrigated fields at ten locations throughout the state. Two major symptoms were identified; crown rot and vascular discoloration in the roots. Vascular discoloration was not directly associated with any soil-borne pathogen. However, F. solani and F. oxysporum were isolated from discolored vascular tissue sporadically. This symptom may be caused by a microbial toxin or associated with a physiologic reaction by the plant root to different environments. Vascular discoloration was associated with irrigated fields. Crown rot and vascular discoloration appear to be associated with an increased susceptibility for sainfoin plants to winterkill.

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F. solani rapidly infects seedling sainfoin plants. The seed hull wounds the developing root allowing infection; hullless seed reduced incidence of infection. Physiologic crown splitting was associated with initiation of crown rotting symptoms. Invasion by F. solani appears to occur either at the hull wound or within old decayed stems which allow the fungus to invade the torn crown.

Alternaria sp. appeared to be seed-borne on sainfoin hulls. Infection by Alternaria sp. in autoclaved soil resulted in reduced seedling vigor or seedling death. F. oxysporum and F. solani were not transmitted by seed.

INTRODUCTION

Sainfoin has been reported to be a long-lived perennial legume. It has relatively high yields and is gaining in popularity as a hay and pasture crop in the Rocky Mountain States and Canada. It does not cause bloat and is not affected by the alfalfa weevil.

In Montana sainfoin began to be cultivated with the release of 'Eski' in 1964. The main asset of this crop at that time was its resistance to the alfalfa weevil and its long-lived nature. Recently, however, irrigated fields have not remained productive for more than three years. Dryland fields have shown a decline in production after four to five years. Lack of persistence in stands has resulted in poor yields. This appears to be due primarily to susceptibility to crown and root rot. This disease is one of the major limiting factors to sainfoin cultivation in Montana.

Future varieties of sainfoin must contain crown and root rot tolerance or resistance. Presently, very little is known about the crown and root rot complex in sainfoin. The objectives of this study were to: 1) determine the causal organism(s) of crown and root rot; 2) determine the severity and prevalence of crown and root rot in Montana, both on dryland and irrigated fields; 3) determine the host-parasite relationships; and 4) identify techniques which would be helpful in selecting for tolerance to crown and root rot within sainfoin varieties.

LITERATURE REVIEW

Description and Agronomy of Sainfoin

Sainfoin is classified as a long-lived, deep rooted perennial (47); however, stand depletion is frequently observed after three to four years when grown under irrigation or where there is a high water table (65). The root system consists of a main tap root and numerous secondary roots (73). Root systems may extend 6.1 m into the ground (46). Nodulation usually occurs on the fine secondary roots. Despite numerous investigations, however, a Rhizobium species has not been found that consistently produces effective nodules (2,58,71).

Sainfoin has tall erect stems that originate from a branched crown. Leaves are pinnately compound with 11-29 leaflets per leaf (47,73,69). Sainfoin seed is born in a pod which is bean-shaped and bilaterally compressed with a rough, net-vein appearance (6). The seed is kidney shaped, and olive green to dark brown in color (46).

Sainfoin has excellent forage quality, good drought tolerance and winterhardiness and does not cause bloat in ruminant animals (48,80). Sainfoin hay is similar in quality to alfalfa. Sainfoin hay is lower in crude protein, crude fiber and ash but higher in nitrogen free extract (40). Alfalfa and sainfoin hay fed to cattle, had equal feed efficiencies, digestibilities, feed consumption and average daily gains (40). Pasture trials in Canada (30) and observations in

Montana (15,34) have shown cattle and sheep prefer sainfoin over alfalfa and other popular forage legumes.

Forage yields in sainfoin have been variable, depending on the location and environmental conditions (5,31,59,67). Yields are usually similar to alfalfa the first two to three years, but thereafter sainfoin yields decrease annually due to reduction in stand (13). Stand loss has been attributed to the crown and root rot complex found in sainfoin (5,54).

Seed yields in sainfoin are good. Yields as high as 1,300 kg/h in Montana (7), 1,000 kg/h in Idaho (59) and 700 kg/h in Nevada (40) have been reported. The seed also has the potential as being an excellent protein supplement (13).

Diseases in Sainfoin

Sainfoin has been cultivated in European and Asian countries for centuries. Shain (69) reported that sainfoin was used as a forage crop in Russia over 1,000 years ago. Despite sainfoin's long history as a cultivated crop, very little work has been done on diseases affecting sainfoin.

Soil-borne Diseases of Sainfoin

Root and crown rot is a serious malady in all legumes and sainfoin seems to be especially susceptible. Cooper (10) and Ditterline (14)

feel it is a major limiting factor involving sainfoin cultivation in Montana.

Root rot caused by Fusarium species, believed to be F. solani (Mart), Snyd and Hans. has been observed in sainfoin plants after the year of establishment. Primary symptoms are a dry brown rot of the crown. It has been suggested that infection may cause increased susceptibility to winterkill (54). Verticillium albo-atrum Reinke and Berth. has been isolated from sainfoin in England and Germany (39). The disease is visually identified by the wilting of the leaflets along the mid-rib and is most commonly observed during warm, dry periods in the summer. Verticillium has not been isolated from sainfoin in Montana to date (55). A root and stem rot caused by Sclerotinia trifoliorum Erikss. has been observed in England (36) and Montana (54). Diseased plants usually die quickly under Montana conditions.

Foliar Diseases of Sainfoin

Many leaf diseases have been observed in sainfoin in Europe (36,37,38,49). Sainfoin black stem, caused by Aschochyta onobrychidis, has been found in England (36), Czechoslovakia (49) and Montana (54). Although the fungus is common under Montana conditions and may be epidemic under moist conditions, it does not appear to be causing significant economic damage (54). The disease is confined to

the lower stems where imbedded pycnidia develop in irregularly shaped lesions. Two leaf spotting fungi, Ramularia onobrycidis Allescher. Trans., found in most European countries (37) and Septoria onobinia Saac. Trans., found in England (38), require moist, humid climates. They have not been observed in Montana and are not believed to be of major economic importance. Other leaf and stem diseases found in Europe include; rust (Uromyces onobrychidis), powdery mildew (Erysiphe polygoni DC) and chocolate spot (Botyris conerea) (36,54).

Seed and Seedling Diseases of Sainfoin

Several diseases have been associated with the seed hull of sainfoin. In Russia, pod contamination by Alternaria causes low germination by attacking the seed and sprout. Removal of the hull increased germination 9% and seedling vigor 68% (57). Antagonists of root nodule bacteria are also contaminants found on the pod. Removal of the pod increased effective nodulation (84). In Montana, Wiesner (81) and Cooper (10) have observed the hull to pinch and possibly wound the seedling root. This may cause an increased susceptibility to disease.

Soil-borne Diseases of Other Forage Legumes

Crown and root rot diseases in legumes are extremely complex (21,82). Sclerotinia trifoliorum was reported to cause clover sickness (root rot) in Germany as early as 1857 (66). This fungus attacks

many species of forage legumes and is particularly destructive in northern climates.

Bacterial wilt, caused by Corynebacterium insidiosum (McCull.) Jenson., was first described in 1925 by Jones and McCulloch (43). The bacterium invades the root system causing vascular discoloration and wilting of the top growth (48). Alfalfa varieties resistant to bacterial wilt have been developed. Resistant clones are positively correlated with resistance in their progeny allowing rapid development of resistant varieties through wilt screening methods (16,26,64,79).

For many years bacterial wilt was considered to be the primary cause of root rots. Development of resistant varieties, however, revealed that other pathogens were causing crown and root rot and stimulated plant pathologists and plant breeders to look for them (18).

Phytophthora root rot caused by (Phytophthora megasperma Drechs.) was first identified in 1954 as a disease of alfalfa by Erwin (20) in California. Since then it has been reported in Illinois (3), Ohio (68), Mississippi (42), Minnesota (23), Wisconsin, Iowa, Kansas, Nebraska, South Dakota (24), Arizona (35), and Washington (17). Phytophthora root rot develops in soils that remain wet for ten days or longer (25). Root infection is accomplished by zoospores which swim about and contact roots. The most frequent points of infection are the tips of small roots and the spongy-phellum cells at the base

of the fine lateral roots (52). Root symptoms include lesions which are initially yellowish brown and later develop into dark brown necrotic lesions with yellowish margins (25). In severe cases, taproots are rotted off, usually at the free water level in the soil. Top symptoms in severe cases include a green wilt, followed by death; plants may also become stunted and yellow. Once top symptoms are visible, the plant usually does not recover (25). Resistance is governed by one tetrasomic gene with incomplete dominance. The nulliplex and simplex conditions offer high and intermediate levels of resistance, respectively (16,70). Selection within recommended varieties for resistance has been suggested (70).

Fusarium root rot in forage legumes has been recognized for at least fifty years, but there seems to be little agreement as to its importance (50). In 1926 Fergus and Valteau (22) reported Fusarium species to be pathogenic to red clover. They believed the root rot complex in red clover to be directly caused by Fusarium and indirectly caused by unfavorable environmental conditions. Staten and Leyendecker (75) obtained isolates of F. solani in New Mexico that were pathogenic and could cause typical root rot symptoms in alfalfa after roots were artificially inoculated. F. solani was more virulent if entrance was through natural or artificial wounds rather than by direct penetration. Diseased plants were stunted and leaves were

light green to yellowish in color. Others have reported F. solani as being very virulent to seedlings, causing damping-off (8,9,19,63).

Some observers believe factors other than Fusarium are the primary cause for root rot in red clover. Emphasis has been placed on nutritional, entomological and environmental factors (12,27,28).

Several alfalfa varieties, such as 'Mesilla', 'New Mexico 11-1', 'Teton' and 'Zia' have been described as resistant to Fusarium root rot. These varieties were developed through recurrent selection programs (44). Gene frequencies in alfalfa for resistance are probably low initially, but substantial progress can be made with only a few cycles of recurrent selection (44).

Ideal environmental conditions for Fusarium root rot are: dry soils (11), low or unbalanced soil nutritions (63) and temperatures of 15 to 25C (11).

Effective nodulation in any legume is extremely important. Not only is the legume--Rhizobium interaction important, but also the Rhizobium--soil microflora and soil microflora and soil microflora--legume interactions. In studying the effect of pH on Rhizobium bacteria and Fusarium species Mew (56) found that a pH of 6-7 inhibited Fusarium disease development while both legumes and Rhizobium thrived. A pH between 4-6 produced the opposite effect. Johnson (41) reported that the presence of Rhizobium prior to infection of Fusarium spp.

reduced the incidence of root rot under laboratory conditions. Colonization of Fusarium prior to colonization of Rhizobium increased disease severity, reduced plant vigor and effectively prevented nodulation.

Rhizoctonia solani Kuehn. has been associated with crown bud necrosis (19,32,33), stem and root canker (72) and damping-off (77) in forage legumes. To date, resistant germplasm has not been reported (44). Thielaviopsis basicola Berk and Br. is an important pathogen in cotton (45,53,74) causing damping-off and root rot. It has also been reported on alfalfa (1). The importance of this disease in sainfoin is not known, but it has been isolated from plants in Montana (55).

Plants within most sainfoin varieties vary for many characteristics. This variability is a result of sainfoin being a cross-pollinated, tetraploid plant where relatively little selection pressure for any particular trait has been applied. For an accurate estimation of a cross-pollinated plant's resistance to a particular pathogen, it is necessary to characterize its resistance according to the frequency of plants in each population. No legume species contains all resistant plants to any of the major alfalfa pathogens (44). To improve resistance to a pathogen in sainfoin, the frequency of disease-resistant plants in a population must be increased.

Kehr et al. (44) has outlined eight important factors that should be considered when breeding for resistance: 1) knowledge of the host, 2) knowledge of the pathogen, 3) host-parasite interaction, 4) induced epiphytotics, 5) sources of resistance, 6) selection and recombination, 7) inheritance of resistance, and 8) variety evaluation. Alfalfa varieties with increased tolerance to crown and root rotting organisms have been developed using the above eight guidelines. Recurrent selection utilizing mass screening of young seedlings has been most effective (44). A similar approach should be successful in breeding sainfoin varieties with increased tolerance to crown and root rot.

Experiment One:

Initial investigation into the crown-root rot complex in sainfoin.

MATERIALS AND METHODS

During the fall of 1972, approximately 100,000 'Eski' sainfoin plants were dug from a six year old irrigated stand located at the Field Research Laboratory, Bozeman, Montana. These plants were cut open at the crown and examined for crown and root rot. Forty plants, selected for the fewest visual symptoms of crown and root rot, were transplanted into 20.3-cm clay pots containing a mixture of 75% sand and 25% peat and grown in the greenhouse.

In January, 1973, these plants were again examined visually for evidence of crown and root rot. Plants were removed from pots and washed carefully with tap water to minimize secondary root loss. Roots were cut open longitudinally and severity of disease noted. Decayed tissue from crown and roots, discolored vascular tissue and water soaked secondary roots were surface sterilized in .5% NaOCl for two minutes, rinsed in sterile distilled water and placed on general and specific media. General media used were 'Difco' potato dextrose agar (PDA), acidified Difco potato dextrose agar (HPDA) and acidified Difco corn meal agar (HCMA) (Appendix I). Specific media included Nash and Snyder's (60) pentachloronitrobenzene (PCNB) medium which is selective for Fusarium sp., and an antibiotic medium (PPV) selective for Phytophthora (62) containing Difco corn meal agar,

10 mg/ml pimarin, 100 mg/ml pentachloronitrobenzene and 200 mg/ml vancomycin (Appendix I). Fusarium cultures were identified to species using Toussoun and Nelson's (78) procedure.

RESULTS AND DISCUSSION

The saprophytic fungi isolated in this experiment, primarily Penicillium, Trichoderma and Aspergillus, were not considered important in the crown-root rot complex in sainfoin (55). Isolations from surface sterilized root tissue placed on PDA did not indicate the problem to be associated with bacteria. Fusarium spp. and R. solani were the only known pathogenic fungi isolated from sainfoin roots. P. megasperma was not isolated.

Fusarium spp. were the predominant fungi isolated (Figure 1). Fusarium spp. were associated with all areas in roots where decay and vascular discoloration was observed. R. solani was isolated from root lesions on the outside of the main taproot and on secondary roots, but was not isolated from internal root tissue, indicating the fungus was probably not associated with root decay or vascular discoloration. F. solani was also isolated from root lesions from which R. solani was isolated, indicating that these lesions may be a source of entry for F. solani or other soil-borne pathogens.

The majority of the 40 plants selected for lack of crown and root rot symptoms in the field displayed these symptoms after being

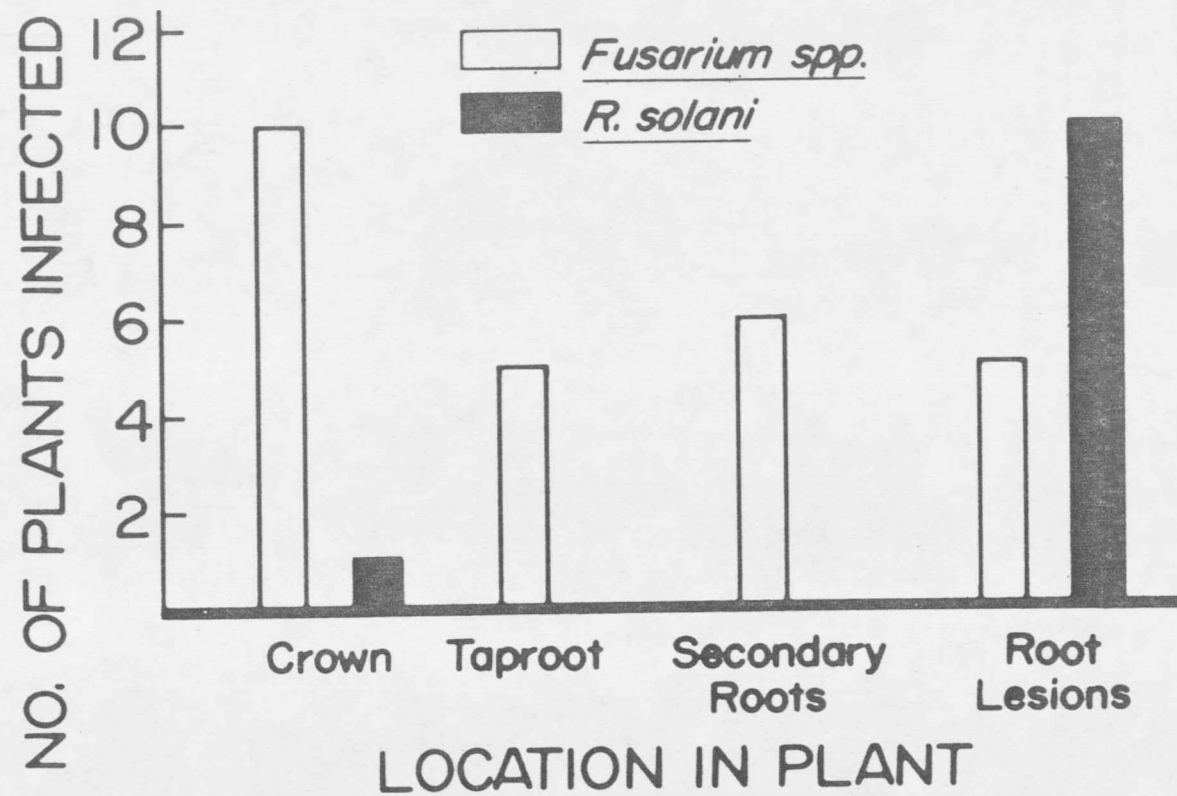


Figure 1: Location and prevalence of *Fusarium* spp. and *Rhizoctonia solani* within the roots of 25 sainfoin plants.

transplanted for six months in the greenhouse. Roots were severely decayed in the crown and vascular discoloration was extensive throughout the taproot and secondary roots. F. solani was isolated from areas having crown and root decay (Figure 2). Discolored vascular areas observed in all but 3 plants did not yield any pathogen consistently. F. solani was associated with vascular discoloration in 4 plants, however, the fungus grew from only 5% of the pieces planted on media. F. oxysporum Schlect. was associated with vascular discoloration in the secondary roots of 2 plants. F. oxysporum was not isolated from other plants with vascular discoloration.

CONCLUSIONS

F. solani is associated with the crown-root rot complex in sainfoin. F. solani was isolated from all areas of the plant root, but most consistently from decayed crown tissue. F. oxysporum could be isolated from areas of vascular discoloration in two of 25 plants examined. In most cases, however, no pathogen could be isolated from the discolored vascular tissue. Vascular discoloration may be a result of crown decay, caused by a pathogen or it may be a physiologic response by the plant to environmental conditions. Vascular discoloration was not associated with wilt symptoms in the foliage.

R. solani could be isolated from small lesions on mature sainfoin roots but was not recovered from within the roots. R. solani

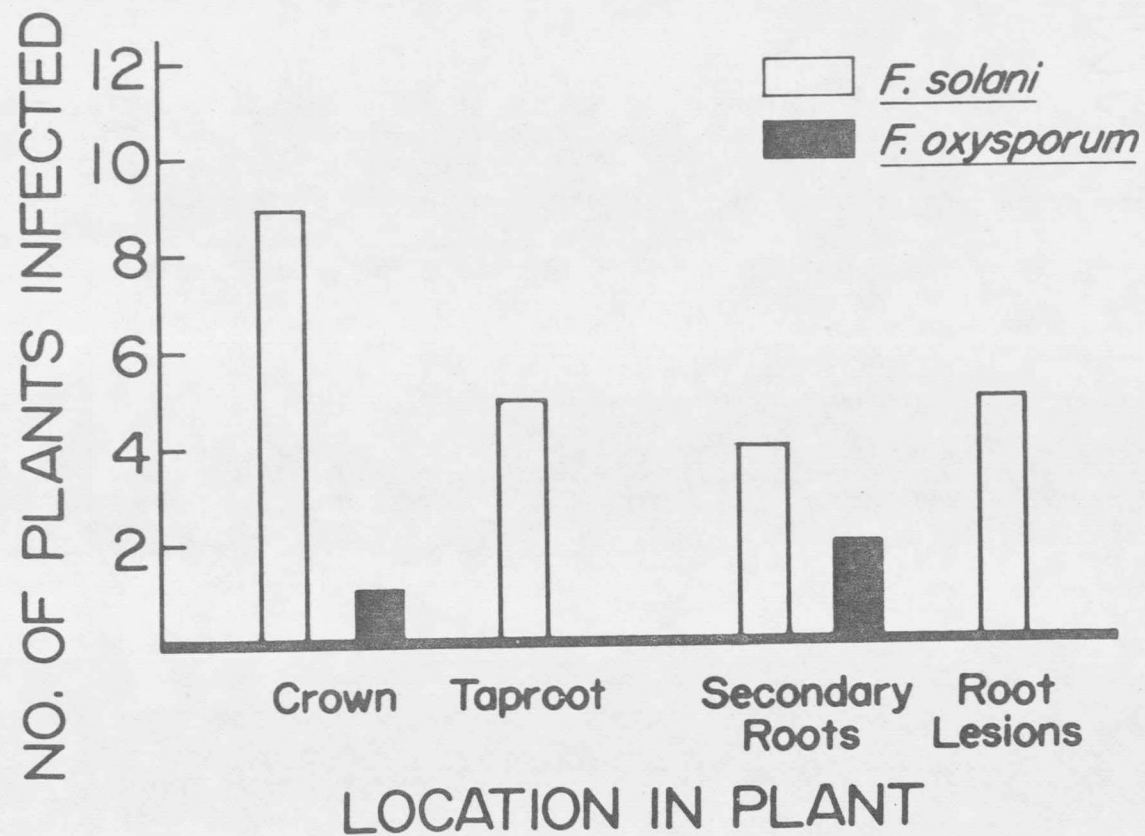


Figure 2: Location and prevalence of *Fusarium solani* and *Fusarium oxysporum* within the roots of 25 sainfoin plants.

probably causes damage to small secondary roots, but is not associated with crown rot or vascular discoloration. Lesions associated with R. solani may act as an entry site for other soil-borne, root rotting pathogens.

Experiment Two:

Prevalence and severity of crown and root rot in sainfoin at ten locations in Montana and the pathogens involved.

MATERIALS AND METHODS

Irrigated and dryland sainfoin fields in 10 areas in Montana were surveyed to determine the primary pathogen causing crown and root rot and its prevalence. Areas surveyed included: Bozeman, Broadview, Conrad, Decker, Heath, Hilgar, Huntley, Kalispell, Lewistown and Moccasin, Montana. Field histories were obtained where possible and visual symptoms of crown and root rot were recorded.

Sainfoin plants were dug at random using a sharpshooter shovel. Plant samples were shaken vigorously to remove soil, placed in a plastic bag and stored in a styrofoam cooler containing ice until isolations from the roots could be made. Maximum storage under these conditions was 5 days.

In the laboratory, roots were scrubbed thoroughly and rinsed with tap water. Plants were cut open at the crown and split longitudinally down the length of the taproot. Severity of disease was noted. Root pieces of diseased tissue from the crown and secondary roots and discolored vascular tissue were surface sterilized for two minutes in 0.5% NaOCl, rinsed in sterile distilled water and placed on PCNB, HCMA and PPV media (Appendix I). Marks and Mitchell's technique (51) for isolating Phytophthora megasperma was also

attempted using 'Ladak-65' alfalfa seedlings as bait. All cultures were incubated at 22C. Fungi growing from diseased tissue were identified approximately 25 days after placement on media. Fusarium isolates were identified using Toussoun and Nelson's procedure (78).

Root pieces were also tested for possible infection by bacterial pathogens, with particular emphasis on Corynebacterium insidiosum. Diseased tissue was placed in 95% ETOH for 10 seconds, 0.5% NaOCl for 3 minutes and washed with sterilized distilled water. Tissue was then mascerated with a scalpel in sterilized distilled water and 0.1 ml streaked on a medium containing 1.5% glucose, 2.0% agar, 1.0% yeast extract dialysate and 0.5% calcium carbonate (76). Cultures were incubated at 4C in a refrigerator and at 22C.

RESULTS AND DISCUSSION

With one exception, the crown and root rot complex in sainfoin was observed in every field examined (Table 1). This field was a two year stand of Remont growing near Decker, Montana. At other locations, F. solani was isolated from the crowns of diseased plants. This fungus appears to be widespread and associated with crown rot in sainfoin under many different environments. F. oxysporum and F. solani were isolated from discolored vascular tissue in both dryland and irrigated fields. P. megasperma and C. insidiosum were not isolated from sainfoin roots in this survey.

Table 1. Prevalence of crown and root rot in sainfoin and the pathogens involved at 10 locations in Montana.

Location	Variety	No. fields observed	Dryland (D) or irrigated (I)	Est. No. plants observed	% plants with crown & root rot	Plants sampled	No. plants with crown rot and pathogen isolated	No. plants with vascular discoloration and pathogen isolated
Decker	Remont	1	D	10	* ¹	10	0	0
	Eski	1	D	10	*	10	2, <u>F. solani</u>	0
Moccasin	Eski	3	D	100	75%	25	25, <u>F. solani</u>	4 <u>F. oxysporum</u> 3 <u>F. solani</u>
Conrad	Remont	4	I	100	60%	20	15, <u>F. solani</u>	2 <u>F. oxysporum</u> 2 <u>F. solani</u>
	Eski ⁴	3	I	100	60%	20	20, <u>F. solani</u>	8 <u>F. solani</u>
	Eski	1	D	50	75%	10	4, <u>F. solani</u>	0
Lewistown ⁵	Eski	1	D	30	100%	5	† ²	1 <u>F. solani</u>
	Eski	1	I	20	100%	5	†	0
Hilger ⁵	Eski	2	D	50	90-100%	15	†	2 <u>F. solani</u>
	Eski	1	I	25	100%	10	†	0
Heath ⁵	Eski	1	D	30	75%	5	†	1 <u>F. solani</u>
Huntley	Eski	2	I	40	+ ³	40	18, <u>F. solani</u>	0

Table 1 (continued)

Location	Variety	No. fields observed	Dryland (D) or irrigated (I)	Est. No. plants observed	% plants with crown and root rot	Plants sampled	No. plants with crown rot and pathogen isolated	No. plants with vascular discoloration and pathogen isolated
Broadview ⁶	Eski	2	D	100	50%	30	14, <u>F. solani</u> 1, <u>F. oxysporum</u>	0
Creston	Eski ⁷	5	D	50	75%	20	5, <u>F. solani</u>	0
	Eski ⁸	1	I	50	75%	50	10, <u>F. solani</u>	0
	Melrose	1	I	30	+	30	0	0
Bozeman	Eski	4	I	100,000	90-100%	75	50, <u>F. solani</u> 8, <u>F. oxysporum</u>	5 <u>F. solani</u> 3 <u>F. oxysporum</u>
	Eski	1	D	50	50%	10	8, <u>F. solani</u>	0
	Remont	2	I	10	*	10	6, <u>F. solani</u>	0
	Eski	1	I	300	+	150	112, <u>F. solani</u>	
	Remont	1	I	300	+	150	75, <u>F. solani</u>	

1. Observation was not made
2. Made no isolations
3. Seedling stands show no obvious visual symptoms of disease
4. Isolations from discolored vascular tissue are from plants grown in wet spots
5. At Lewistown, Hilger and Heath, crown rot was present but isolations were made only from taproots with severe vascular discoloration
6. Vascular discoloration was not present; stunted plants were associated with F. solani
7. Plant roots dried out during transport
8. Vascular discoloration severe, isolates were obtained.

Crown and root rot was more severe in irrigated than in dryland fields. Plants grown in dryland fields with more than 50 cm of annual rainfall near Lewistown and Creston were less severely diseased than plants grown under irrigation, but were more severely diseased than plants grown in other dryland areas.

Crown and root rot was uniformly prevalent within irrigated and dryland fields. Generally, 50-75% of the plants were diseased in most fields. Fields near Bozeman, Lewistown and Creston had a higher incidence of crown and root rot, with 90-100% of the stands diseased.

Plants grown in irrigated fields or moist environments were usually decayed at the crown, and vascular discoloration was severe (Figure 3). The discoloration appears to move down from the crown into the taproot and secondary roots. Severely diseased plants did not have well developed secondary roots and some were stunted with yellowish-green foliage. Foliage symptoms were not consistently observed, however, and most plants in fields appeared healthy.

F. solani was the predominate fungus isolated from decayed crown tissue inside the roots (Table 1). Other pathogenic organisms were not consistently isolated from crown tissue. All efforts to consistently isolate a pathogen from the discolored vascular tissue in the taproot and secondary roots failed. However, both F. solani and F. oxysporum were sporadically isolated from this region. At

Conrad, Montana, Eski plants growing in wet areas from irrigation were yellowish in color and stunted. Vascular discoloration and crown rot was severe and, in this case, was associated with F. solani (Figure 4).

Sainfoin plants grown on dryland were less severely diseased. Vascular discoloration of these plants was less than for irrigated plants and occurred primarily in the taproot. Diseased plants had decayed crowns. Symptoms were a dry, brownish-black rot that tapers downward in the shape of a 'V', 2.5-7.0 cm into the taproot. Crown buds and stems generally had vascular discoloration adjacent to the crown associated with F. solani (Figure 5).

Sainfoin persists better on dryland than irrigated fields. At Creston and Lewistown, plants in irrigated fields developed vascular discoloration in the second year of production and severe stand loss was observed the following year. Vascular discoloration may disrupt vascular functions and cause plants to become extremely susceptible to winterkill. Vascular discoloration does not appear to be associated with wilting symptoms. Under dryland conditions, crown rot was more prominent as a disease symptom, but did not cause severe damage until the third or fourth year of production. Crowns decayed more slowly under these conditions. Farmers reported yield reductions by the third production year, but did not observe stand depletion until the fourth or fifth year.



Figure 3: Typical crown decay and vascular discoloration in Eski sainfoin grown under irrigation.

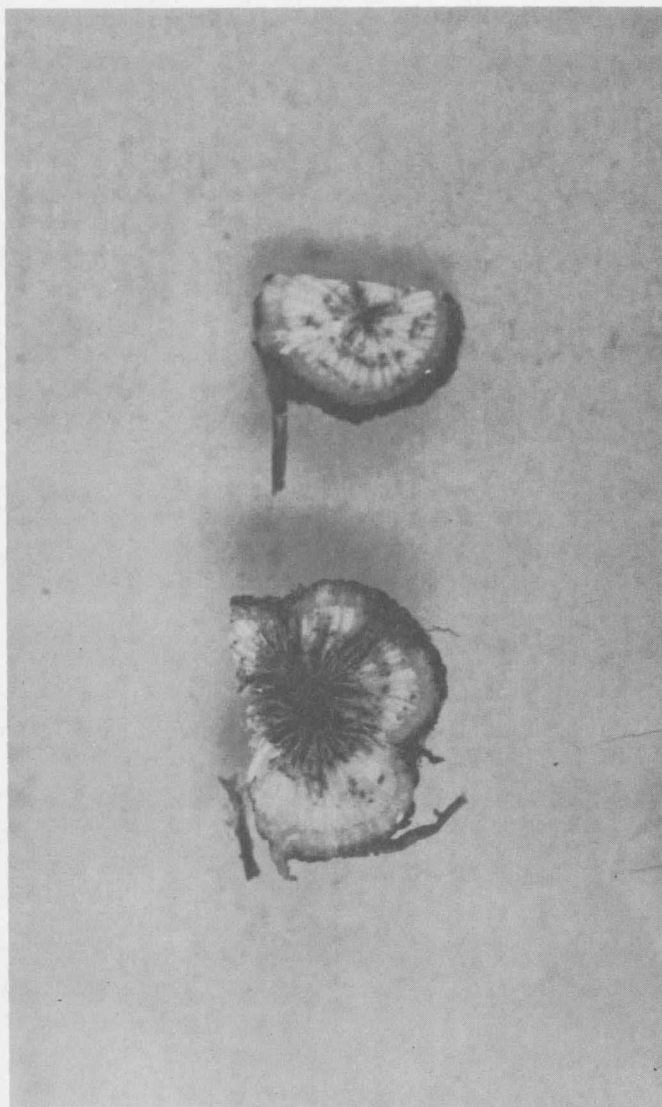


Figure 4: Cross sections of an Eski sainfoin root with vascular discoloration associated with F. solani. Plants were growing in standing water and were stunted with yellowish-green foliage.

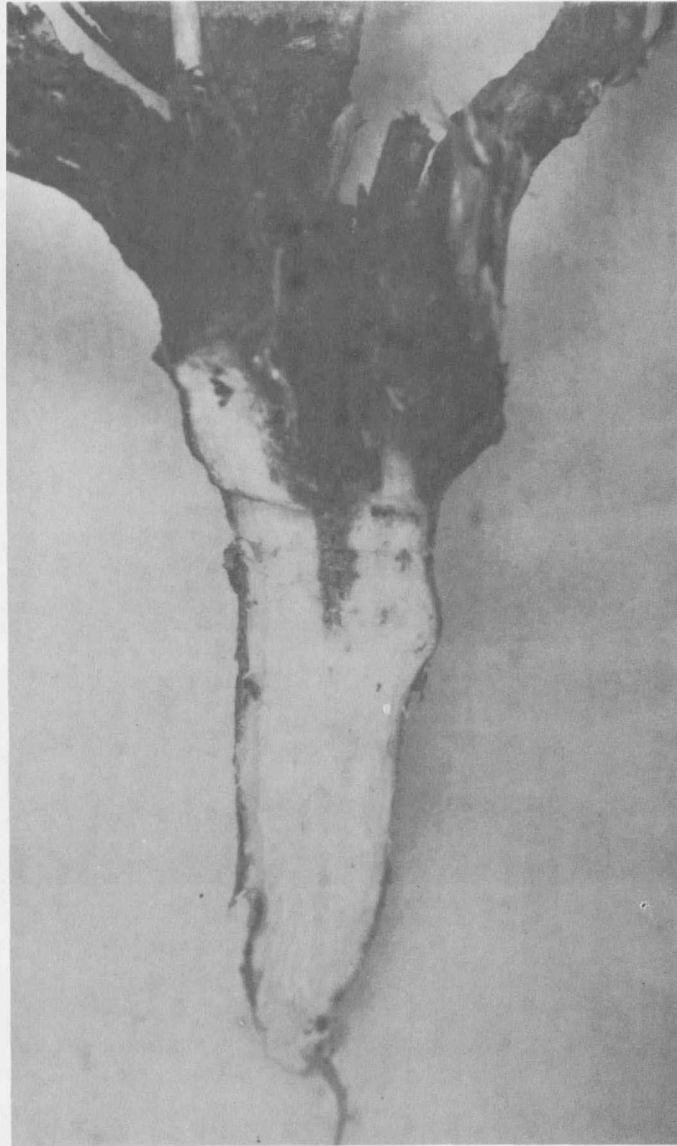


Figure 5: Initial 'V' shape crown decay found in a second year production field of dryland Eski. The vascular discoloration, appearing as black dots at the base of the stems is consistently associated with F. solani.

Management practices did not seem to reduce or delay crown and root rot except when fields were cut once for hay, then allowed to set seed. This particular management practice appeared to maintain stands longer at Conrad, Moccasin and Hilger, Montana. Prevalence of crown and root rot was not reduced in these fields. Cropping practice prior to seeding sainfoin did not appear to influence prevalence of disease. Sainfoin planted in soil previously sown to grain or grass developed crown and root rot as rapidly as sainfoin sown to fields previously growing alfalfa.

Disease development associated with F. solani appears to develop first in the stem, just above the main root. Symptoms begin as a darkened streak in the vascular tissue or in the crown tissue beneath the old decayed stem from the previous year's growth (Figures 5 and 6). F. solani moves into the crown after the seedling year. Invasion appears to coincide with the physiologic tearing of the inner crown as the sainfoin plant develops (Figures 6 and 7). The sainfoin crown does not appear to be strong enough to support the increasing number of stems and, as a result, tears or splits. This tear could be an ideal invasion site. Figure 6 shows an old decayed stem where F. solani was isolated and the crown physically splitting down the center of the root. At this time, usually in the second year, symptoms of crown rot appear. In Montana it has been

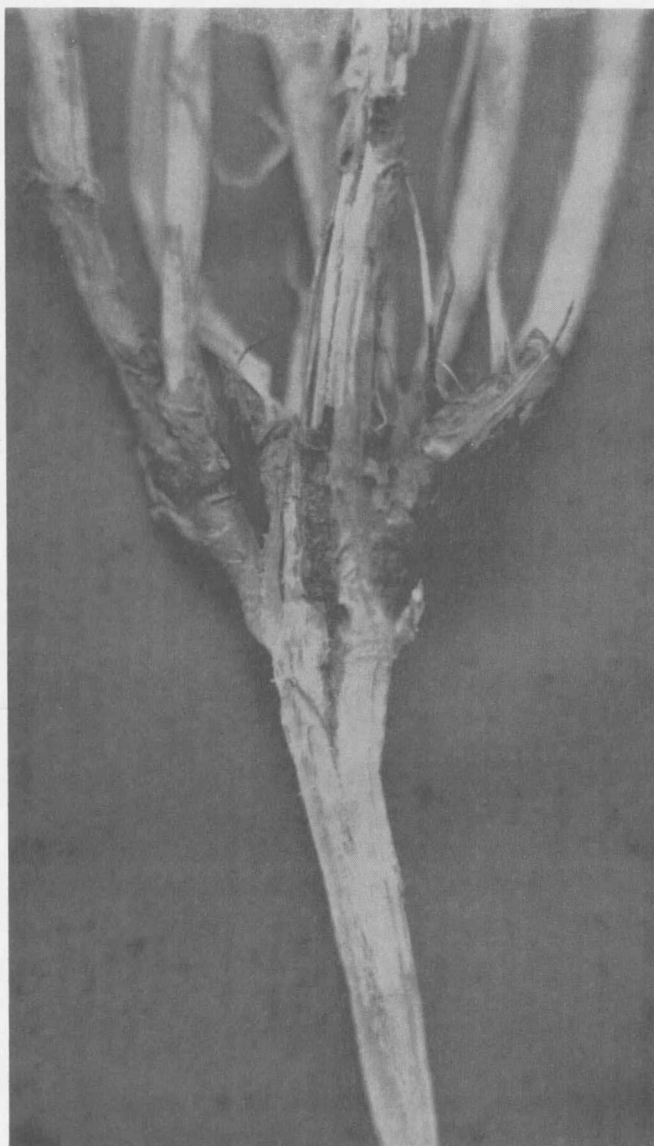


Figure 6: Eski sainfoin with an old decayed stem from the previous year's growth. F. solani was isolated from the brown, decayed hollow stem. Physiologic tearing is also beginning in the crown.

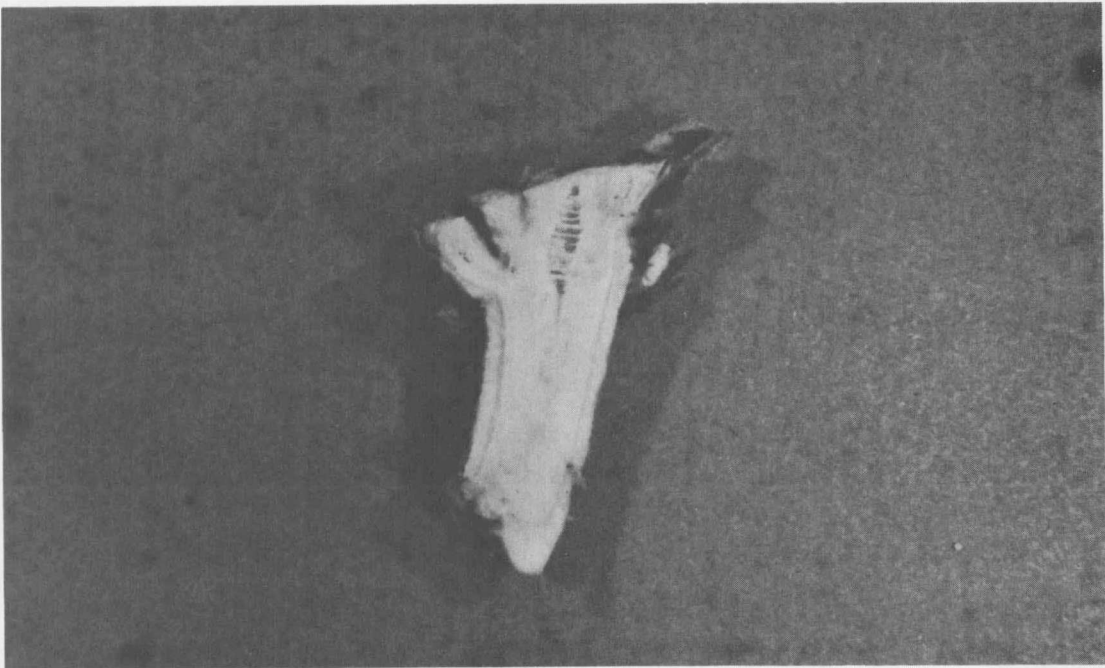


Figure 7: Initial physiologic tearing of the root tissue in the crown of a seedling Remont sainfoin plant.

shown that higher seeding rates and denser plant spacings increased yields in sainfoin (4,14). Higher plant densities probably decrease tillering, which may, in turn, reduce physiologic crown tearing and delay disease development allowing plants to remain in stand longer.

CONCLUSIONS

Crown rot in sainfoin appears to be caused by F. solani. The fungus is widespread, and is associated with yield reductions in many areas in the state. Symptoms of crown rot associated with F. solani, when observed, were similar to those described in alfalfa by Staten and Leyendecker (75); (i.e., stunted plants that are yellowish-green in color). These symptoms were not present in all diseased plants, however. Stand reduction in crown rotted plants may be due to increased susceptibility to winterkill.

Vascular discoloration observed in the taproots and secondary roots of diseased sainfoin plants could not be directly attributed to F. solani or F. oxysporum. Isolations from this root tissue were consistently negative. Vascular discoloration appeared to be associated with more rapid stand loss in sainfoin grown under irrigation or in moist environments. This symptom could be a physiologic reaction or may be caused by a microbial toxin.

Experiment Three:

Rapidity of Infection

MATERIALS AND METHODS

To determine when Fusarium solani initially infects sainfoin, 10-row plots of Eski and Remont sainfoin were seeded May 12, 1973 in a Bozeman silt loam soil at the Field Research Laboratory. Rows were 6 m long and 30 cm apart. Plants were irrigated twice during the growing season. Fifty seedlings were dug at random from each plot on June 25, July 10, July 23, and December 2, 1973. Roots were washed and examined visually for disease symptoms. Root pieces 1 cm, 2 cm, and 5 cm from the crown were surface sterilized one minute in 0.5% NaOCl, rinsed in sterilized distilled water and placed on PCNB and HCMA media (Appendix I). Cultures were incubated 14 days at 22C before fungi were identified. Fusarium species were identified using Toussoun and Nelson's technique (78).

RESULTS AND DISCUSSION

F. solani and F. oxysporum rapidly infected sainfoin roots shortly after seeding (Table 2). There appeared to be no difference in susceptibility between Eski or Remont (Figure 8). F. solani was found to be more prevalent in infected seedlings than F. oxysporum, in both varieties (Figure 9). Sixty and 45% of the Eski and Remont roots, respectively, were infected with F. solani at the end of the

Table 2. Time of infection of Remont or Eski Sainfoin Roots by Fusarium solani and F. oxysporum in the field at Bozeman, Montana in 1973.

Days after planting	Eski			Remont		
	Infected		Noninfected	Infected		Noninfected
	<u>F. solani</u>	<u>F. oxysporum</u>		<u>F. solani</u>	<u>F. oxysporum</u>	
45 (June 26)	4 ¹	1	45	5	0	45
59 (July 10)	7	0	43	5	0	45
72 (July 23)	19	7	24	17	5	28
204 (Dec. 2)	12	3	5	9	3	8

1. Number of plants infected

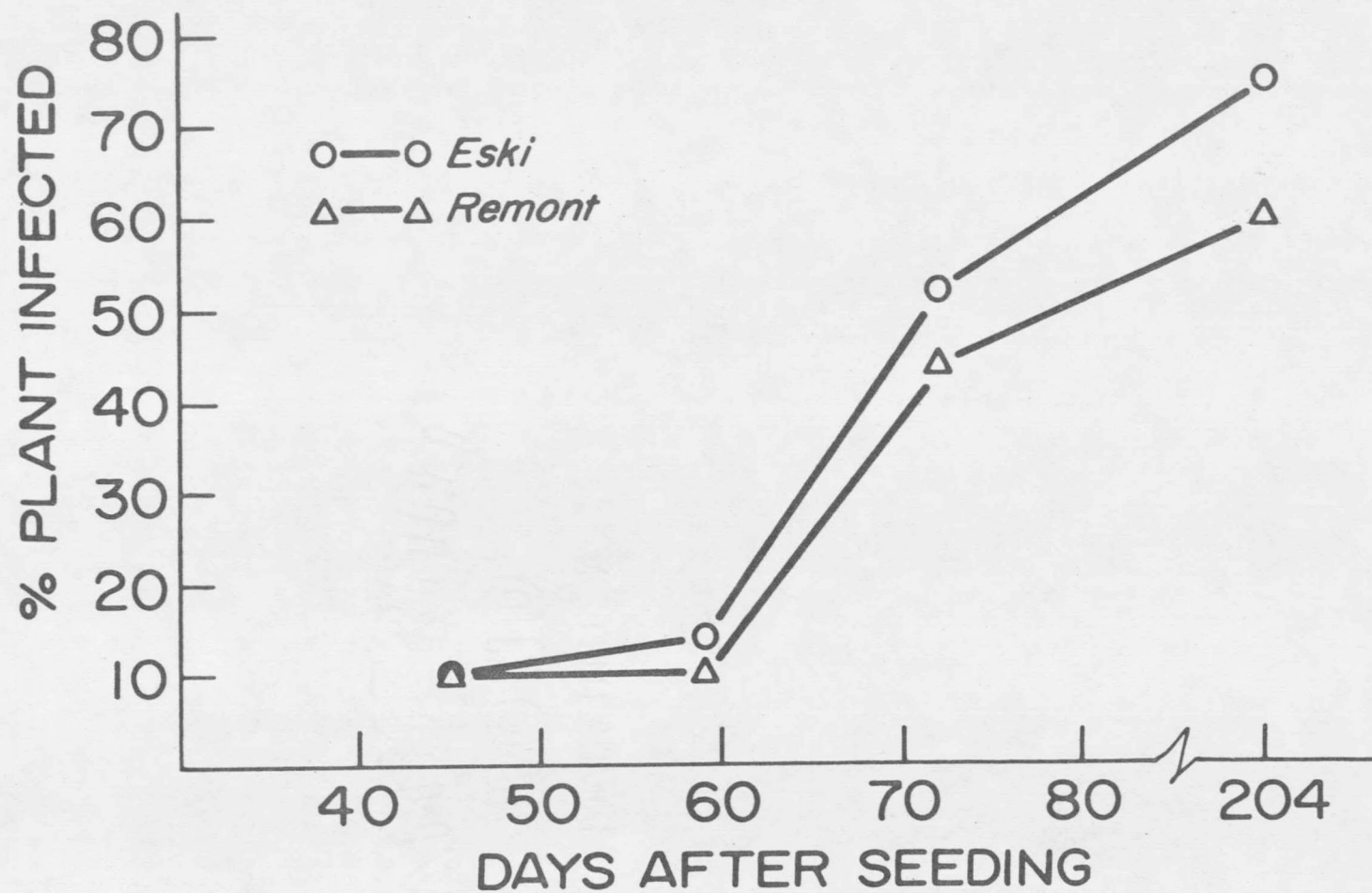


Figure 8: Time of infection of Fusarium spp. in Remont and Eski sainfoin.

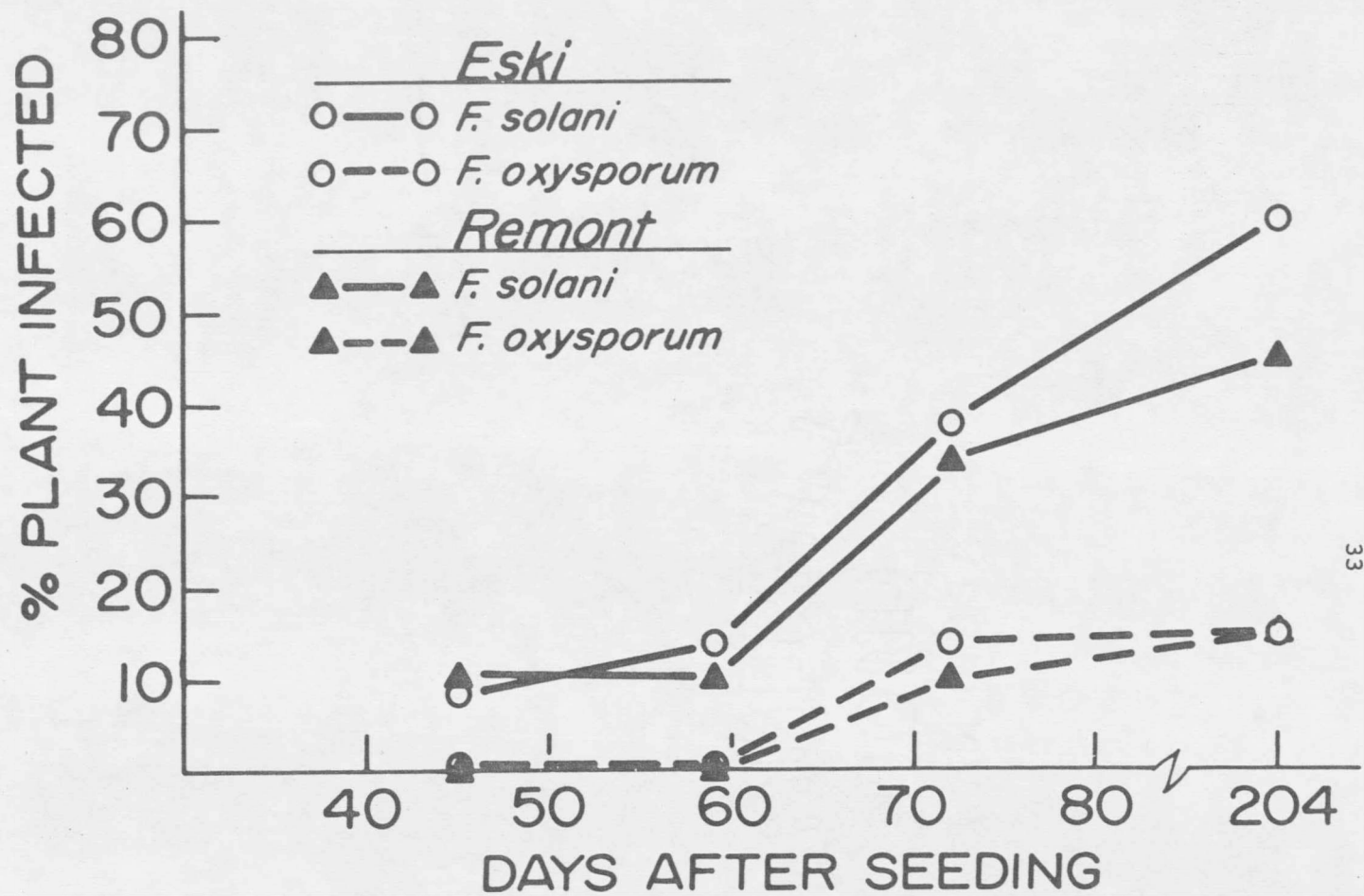


Figure 9: Time of infection of *Fusarium solani* and *Fusarium oxysporum* in Eski and Remont sainfoin.

growing season. F. oxysporum infected 15% of the roots of both varieties by the end of the growing season. Infected seedlings were discolored around the seed hull or where it had been attached to the root.

The sainfoin hull usually does not split bilaterally upon germination of the seed thus forcing the radicle to grow through the net veination of the hull. When this occurs, the hull usually remains attached to the developing root (Figure 10). As the root enlarges in circumference, the hull cuts into the root (hereafter referred to as hull cut) (Figure 11). The rapid increase in infection by F. solani in both Remont and Eski between July 10 and July 23 is believed to be due to the hull damaging or cutting the root. The hull cut was not observed after close examination of seedlings, on July 10, but was observed on July 23. Most of the Fusarium isolates were obtained from this hull cut region. Root tissue above the hull was infected with Fusarium spp. only when the hull cut was infected, indicating that Fusarium spp. may move upward into the crown from the hull cut region.

CONCLUSIONS

Eski and Remont sainfoin seedlings are extremely susceptible to invasion by F. solani and F. oxysporum shortly after seeding. Infection increased rapidly between the 59th (July 10) and 72nd



Figure 10: Eski sainfoin with seed hull attached to the developing root (right) and the seed hull removed (left).

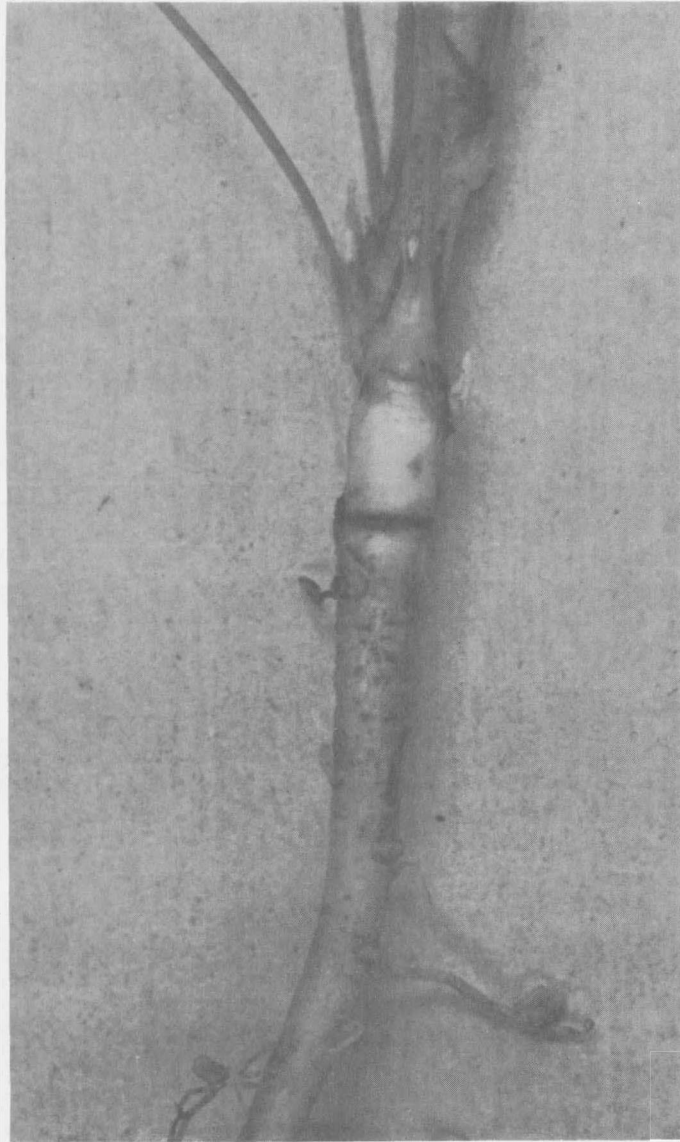


Figure 11: Eski sainfoin seedling
with the wound caused
by the sainfoin hull.

(July 23) day after seeding. Injury to the developing root by the seed hull may increase incidence of Fusarium spp. infection.

Experiment Four:

Fumigation

MATERIALS AND METHODS

To determine if the crown-root rot complex found in sainfoin was physiologic in nature or caused by a pathogen, a 3 m² plot of Bozeman silt loam soil at the Field Research Laboratory, Bozeman, Montana was fumigated with chloropicrin (Picfume, Dow Chemical Company) at the rate of 535.5 kg/h on June 1, 1971. The fumigant was applied with a MacClean fumigun (Neil A. MacClean, Inc., Belmont, California) using three ml chloropicrin on a 30 cm grid. An adjacent 3 m² plot was not fumigated. Thirty days after fumigation Eski sainfoin was planted with a cone seeder 1.3 cm deep in each of these two plots. Plots were irrigated twice every summer.

Plants from fumigated and unfumigated plots were dug at random and examined for crown and root rot on August 25, 1973. Diseased root tissue was surface sterilized in 0.5% NaOCl for two minutes, rinsed in sterilized distilled water and placed on PCNB and HCMA media (Appendix I). Cultures were incubated at 22C for 14 days and then identified.

RESULTS AND DISCUSSION

Plants growing in the fumigated soil appeared healthier, were more vigorous, and grew more rapidly following clipping than plants

growing in the non-fumigated soil. Plants in the fumigated plots displayed only slight symptoms of crown and root rot (Figure 12). Crown decay was beginning to occur in some plants in association with the physiologic splitting of the crown. Vascular discoloration was not observed in any of the plants examined. Plants in non-fumigated plots had developed both crown rot and vascular discoloration (Figure 13), which was observed in all plants examined. Vascular discoloration was not severe but had extended downward from the crown into the taproots.

Plants in both plots were infected with F. solani and had rotting crowns. F. oxysporum was isolated from crown tissue in two plants in fumigated plots. This fungus was not isolated from root tissue of plants growing in non-fumigated plots. F. solani was isolated from discolored vascular root tissue from one plant. Isolations from this tissue in other plants did not yield any pathogenic fungi or bacteria.

CONCLUSIONS

Soil fumigation appeared to increase vigor and regrowth in sainfoin plants. Plants growing on non-fumigated plots had much more crown and root rot than those grown on fumigated plots. This indicates that crown and root rot found in sainfoin does not appear to be caused by internal or physiological breakdown, which has been reported



Figure 12: Eski sainfoin grown in fumigated Bozeman silt loam soil at Bozeman, Montana. Crown rot has developed in conjunction with the physiologic tearing of the crown. Most plants did not show symptoms of crown rot.

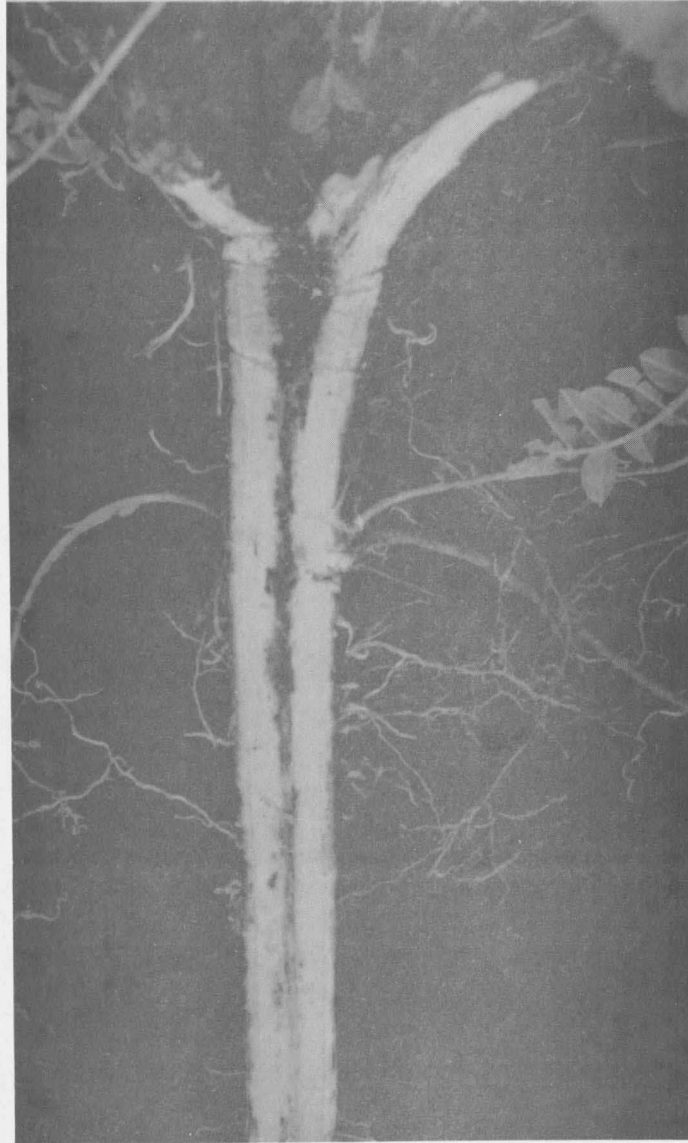


Figure 13: Eski sainfoin grown in Bozeman silt loam soil at Bozeman, Montana. Crown rot and vascular discoloration have developed in this root.

to occur in red clover (12,27,28). Crown rot appears to be caused mainly by F. solani, with some indication that F. oxysporum may also play a role in this disease. Vascular discoloration occurred only in non-fumigated plots but a pathogen(s) could not be isolated from the discolored areas.

Experiment Five:

Pathogenicity Tests

MATERIALS AND METHODS

Pathogenicity of fungi isolated from sainfoin crown roots was determined using 4 sainfoin varieties. Sainfoin varieties included: 'Eski', derived from a single plant introduction from Turkey; 'Remont', a composite of 17 plant introductions from Iran; 'Melrose', a Canadian variety derived from plant introductions from the U.S.S.R.; and 'Hall', a cultivar subjected to 70 years of natural selection at Hall, Montana. The fungi used were F. solani, F. oxysporum, Rhizoctonia solani, and Thielaviopsis basicola.

A split-plot randomized complete block design was used with 4 replications in the greenhouse. Varieties were assigned to main plots and fungi to sub-plots. Plants were potted in a mixture of 25% Bozeman silt loam, 50% sand and 25% peat in 20.3-cm clay pots which had been washed with tap water. Pathogens were incorporated into the soil mixture by hand mixing prior to potting.

Fungi used in this experiment were isolated from diseased sainfoin plants. F. solani and F. oxysporum were grown on autoclaved 'Park' oat kernels. Ninety-five grams of oats were autoclaved in a one liter Roux flask, moistened with 56 ml sterile distilled water and inoculated with 10 ml of a heavy spore suspension of each fungus. Ten Roux flasks were used for each Fusarium species. Cultures were

incubated at a room temperature at 22C for 25 days and shaken daily to prevent formation of mycelial mats.

Inoculum of T. basicola was prepared by placing one ml of a spore suspension into 80 ml fresh potato dextrose broth in a one liter Roux flask. The cultures were grown for 100 days at 22C. Ten flasks were used. Each culture was combined, communitied in a Waring blender and incorporated into the soil mixture.

R. solani was grown on Difco PDA for one week in 24 150 x 15 mm Petri dishes. The mycelium and agar were cut into 3 mm squares and mixed into the soil mixture.

Pots infested with Fusarium contained approximately 63 g of infested oats, pots infested with T. basicola contained 50 ml of a heavy spore suspension and pots infested with R. solani contained 1.5 Petri dishes of the fungus. All pathogens were incubated in the soil for 23 days prior to planting. The soil was kept moist by watering daily with a complete nutrient solution.

Six hulled sainfoin seeds were planted per pot at a depth of 1.3 cm with equidistant spacing on July 12, 1973. Two weeks after emergence, seedlings were thinned to three seedlings per pot. Visual symptoms were noted throughout the experiment. Plants were grown 80 days and harvested by carefully removing the plants from the pots, and rinsing the soil from the roots with tap water. Tops and roots

were weighed separately. Roots were examined for visual evidence of disease symptoms.

Reisolation of F. solani and F. oxysporum was attempted using PCNB and HCMA media. Reisolation of R. solani was attempted using water agar (2% Difco agar in distilled water) while reisolation of T. basicola was attempted using Yarwood's carrot disc technique (83).

RESULTS AND DISCUSSION

Infection occurred in all varieties of sainfoin grown in pots artificially infested with F. solani, F. oxysporum, and R. solani. T. basicola was not successfully reisolated in this experiment.

R. solani reduced growth of all varieties (Table 3); F. oxysporum and T. basicola did not affect growth. Plants grown in soil infested with F. solani yielded more tops and roots than plants grown in other treatments. However, differences were not significant.

Visual phenotypic differences in plant growth were observed in F. solani treatments. In all varieties, the stems elongated more and flowering occurred 2-3 weeks earlier than in plants grown in other soil treatments.

Root growth was not significantly affected (Table 3) by soil treatments but F. solani appeared to cause the greatest root growth. Varieties had similar root growth.

Table 3. The effect of four soil-borne pathogens on forage growth and root growth in four sainfoin varieties grown in clay pots in the greenhouse.

Pathogens	Mean forage growth(g)	Mean root growth(g)
<u>F. solani</u>	33.21 a ¹	31.48 a
Check	27.50 ab	26.27 ab
<u>F. oxysporum</u>	25.83 b	24.93 b
<u>T. basicola</u>	21.33 b	25.54 ab
<u>R. solani</u>	13.50 c	19.98 b

1. Means not followed by the same letter differ significantly (P < .05)

Melrose produced more forage growth than other varieties (Table 4). Hall normally yields less than Eski in yield trials. Its good performance in this study may be due to its 70 years of natural selection which could have increased the gene frequency of disease resistant genes. Carleton (4) noted that semi-native selections such as Hall and Augusta have lower yields than Eski in the first three years of production, but better persistence enables them to equal Eski yields in the fourth year at Bozeman. Melrose may also contain some degree of resistance to the soil-borne pathogens used in this experiment.

Table 4. Effect of four soil-borne pathogens on forage growth of four sainfoin varieties.

Varieties	Mean forage growth(g)
Melrose	30.75 a ¹
Hall	22.97 b
Remont	22.32 b
Eski	21.05 b

1. Means not followed by the same letter differ significantly ($P < .05$)
2. Varieties x pathogens interaction was insignificant ($P < .05$)

R. solani was found associated with secondary roots almost exclusively. Lesions had developed in some cases and small secondary roots were water soaked and beginning to decay. R. solani was not found to be directly associated with the hull cut.

F. oxysporum was isolated from secondary roots and from tissue surrounding the hull cut. In the varieties Hall and Melrose, F. oxysporum was isolated primarily from the hull cut while in Remont and Eski, isolates were frequently obtained from the secondary roots. Darkening around the hull cut and dark brown areas on secondary roots were associated with infection, but consistent symptoms of this type were not always observed. F. oxysporum did not affect top or root growth. Nelson (61) has suggested that this isolate appears to be

a cultural variant and, consequently, may be less virulent than the original wild-type isolate. Further tests using various isolates may be useful in detecting a more virulent strain.

F. solani was associated with the hull cut in all varieties. Generally, the roots were discolored in this region. Discoloration may occur internally within the root at the hull cut. Isolates were not obtained from secondary roots, indicating that this isolate of F. solani may need an artificial or natural wound for infection. F. solani increased growth of tops and roots in all varieties and caused plant hormone-like effects. This isolate may produce gibberellin or a hormone similar in action to gibberellin. Griffiths and Lim (29) have reported similar responses to infection by strains of F. solani in Malayan crop plants. They suggested that these "overgrowth" symptoms in infected plants were caused by gibberellin. The isolate of F. solani used in this experiment may be less virulent than other isolates. The response could also be associated with the oats used to infest the soil with F. solani.

CONCLUSIONS

Infection with isolates of F. solani, F. oxysporum and R. solani indicated that these fungi could be pathogens involved in the crown-root rot complex. Symptoms of crown or root rot were not

observed in F. solani infected plants. These symptoms usually do not occur in the field until the second or third year of production.

R. solani was the most virulent pathogen tested and reduced top growth significantly. R. solani causes damping-off and reduces seedling vigor in many crops. In sainfoin it damaged secondary roots and decreased seedling vigor. This effect might continue into the second year of production.

F. solani and F. oxysporum did not reduce top or root growth. F. solani increased seedling vigor of all varieties and may be less virulent than other strains. This isolate may produce gibberellin which could cause the increased stem elongation and earlier flowering observed in infected plants.

Varieties may differ in disease resistance, but results indicate that detecting levels of resistance, especially to F. solani, may be extremely difficult.

Experiment Six:

Seed Test

MATERIALS AND METHODS

A greenhouse study was conducted to determine: 1) if Fusarium sp. were infesting sainfoin seed; and 2) if the sainfoin seed hull was wounding the developing root and thereby allowing invasion by Fusarium sp. or other pathogens. A split-plot randomized complete block design was used with 4 replications. Soil treatments were assigned to main plots and seed treatments to sub-plots. Soil treatments included: 1) untreated Bozeman silt loam (BSL); 2) BSL artificially infested with Fusarium solani; 3) BSL artificially infested with Fusarium oxysporum; and 4) sterilized BSL. The sterilized BSL was autoclaved 45 minutes in an AMSCO soil autoclave at 250C. Seed treatments included: 1) hulled seed; 2) hulled, surface sterilized seed; 3) hulless seed; 4) hulless, surface sterilized seed. Seed from the variety Eski was used. Seed in treatments 2 and 4 were surface sterilized by placing them in 0.5% NaOCl for 2 minutes and rinsing with sterilized distilled water.

Fusarium cultures used to infest the soil treatments were grown one week at 22C in 250-ml flasks containing modified Eckerts broth (Appendix I). Agitation was provided with a wrist action shaker. Cultures were then communitied with a Waring blender and the spore mycelial suspension incorporated into the soil before potting.

Each 10.3-cm clay pot received approximately 63 ml of inoculum. Spore density was 2×10^7 macroconidia/ml for F. solani and 4×10^7 for F. oxysporum. Pots were maintained after potting under moist conditions for 30 days before planting to allow conversion of macroconidia into chlamydospores (60).

Five seeds were planted in each pot. Twenty-one days after emergence, plants were thinned to two per pot. Seedlings removed in the thinning process were washed with tap water and root pieces surface sterilized two minutes in 0.5% NaOCl and rinsed in sterilized distilled water before being placed on PCNB and HCMA media.

Top growth was harvested 85 days after planting and the forage yield from each plant weighed immediately. Roots were removed carefully from the pot, and isolations made as described above. Cultures were incubated 14 days at 22C and Fusarium species identified using Toussoun and Nelson's technique (78).

RESULTS AND DISCUSSION

Rate of infection by F. solani and F. oxysporum was affected by the absence or presence of the seed hull. Plants from hulled seed (hulls intact) yielded 12% less forage after 85 days of growth than plants from hullless seed (hulls removed), but differences in yield were not significant ($P < .05$). It is possible that had the

experiment run longer, the detrimental effects of the hull cut may have been more pronounced.

Plants growing in soil infested with F. solani yielded significantly more foliage than plants growing in other treatments (Table 5). This is in agreement with data from the pathogenicity tests and could be due to production of gibberellin by F. solani. Plants grown in autoclaved Bozeman silt loam (BSL) had lower mean forage yields than other treatments because of seedling death in some pots. R. solani was isolated from two dead seedlings while Alternaria sp. was isolated from 5 dead seedlings. R. solani was isolated from dead seedlings in one unsterilized hulled and one unsterilized hulless treatment. This fungus was either a soil contaminate after autoclaving or was seed or hull-borne. Alternaria sp. was isolated from dead seedlings grown from unsterilized hulled seed in all five cases. Conidia or mycelium of this fungus may contaminate the hull and attack developing seedlings. Alternaria sp. was not associated with seedling death in any of the other soil treatments. Sterilization of the soil may have removed inhibitory fungi or bacteria and allowed Alternaria sp. to become more virulent to sainfoin seedlings. Reduced germination and seedling vigor caused by Alternaria sp. has been reported in Russia (57) and is a major reason for their seeding hulless seed.

Table 5. Effect of four soil treatments on mean forage weight of Eski sainfoin grown in Bozeman silt loam soil in the greenhouse for 85 days.

Treatment	Weight (g)
<u>F. solani</u>	11.16 a ¹
<u>F. oxysporum</u>	8.14 b
Unautoclaved	7.0 b
Autoclaved, noninfested	6.4 b

1. Means not followed by the same letter differ significantly (P < .05)

Frequency of F. solani and F. oxysporum infection was influenced by soil treatments and seed treatments. Plants grown in Fusarium spp. infested pots were infected with Fusarium spp. with greater frequency than other treatments (Table 6). There was no difference in frequency of infection between F. solani and F. oxysporum infested soils. Under these greenhouse conditions, both fungi infected 50% of the plants in each soil treatment. These results compared well with results in Experiment Three.

Fusarium spp. infection was greatest in seedlings grown from hulled seed (Table 7). The hull appears to allow increased infection as a result of its wounding effect previously described in Experiment Three. In the unautoclaved BSL treatment, 9 plants of 32 growing were infected with Fusarium spp.; three were infected with F. solani and

six with F. oxysporum. Two plants were infected in the autoclaved BSL treatment, one with F. solani and one with F. oxysporum. Both isolates were obtained from plants grown from surface sterilized hulled seed. Neither F. solani or F. oxysporum in this experiment appeared to be seed transmitted.

Table 6. Effect of four soil treatments on infection by F. solani and F. oxysporum in Bozeman silt loam soil.

Treatment	No. plants infected per pot ¹
<u>F. solani</u>	1.5 a ²
<u>F. oxysporum</u>	1.5 a
Unautoclaved	.57 b
Autoclaved, noninfected	.13 b

1. There were two plants in each pot
2. Means not followed by the same letter differ significantly (P < .05)

CONCLUSIONS

The hull of sainfoin seed often wounds or damages the developing root and allows increased infection by F. solani and F. oxysporum. Removal of the hull before planting may reduce infection by F. solani and F. oxysporum in the year of seedling development.

F. solani and F. oxysporum do not appear to be transmitted on the hull. These results may be misleading because of the small

sample size and further tests should be conducted. Alternaria is carried on the seed and under sterile conditions appears to be pathogenic to developing seedlings.

Table 7. Effect of hulled and hulless sainfoin on frequency of infection by Fusarium spp.

Treatment	No. plants infected per pot ¹
Sterilized hulless seed	.57 a ²
Unsterilized hulless seed	.75 ab
Sterilized hulled seed	1.20 b
Unsterilized hulled seed	1.20 b

1. There were two plants in each pot
2. Means not followed by the same letter differ significantly (P < .05)

F. solani stimulated seedling vigor. Data from this experiment and Experiment Five support the conclusion that gibberellin or a similar plant hormone probably is produced by the fungus and causes the increase in seedling vigor.

Successful infection by F. solani and F. oxysporum was accomplished by mixing conidial suspensions into unfumigated soil. Negative growth response to infection by either F. solani or F. oxysporum was not detected. Screening for Fusarium spp. resistance using a seedling test may be extremely difficult using the described methods.

SUMMARY AND CONCLUSIONS

The crown-root rot complex in sainfoin is one of the limiting factors to production of this crop. It is associated with reduced yields and rapid stand decline in fields throughout Montana. The objectives of this study were to: 1) identify the causal organism of crown and root rot; 2) determine the severity and prevalence of crown and root rot in the state; 3) determine host-parasite relationships; and 4) identify techniques which would be helpful in selecting for tolerance to crown and root rot within sainfoin varieites.

The results of this study indicate two major symptoms are expressed in diseased sainfoin roots; crown rot and vascular discoloration. Crown rot appears to be caused by F. solani. This fungus was found in decayed crown tissue at ten locations in the state under a wide variety of environmental conditions. Infection with this pathogen was obtained under greenhouse conditions, but symptoms of crown and root rot were not observed during the duration of these tests. Vascular discoloration occurred rarely in dryland fields, but frequently in irrigated fields of sainfoin. Vascular discoloration was not directly associated with any soil-borne pathogen, although F. solani and F. oxysporum were isolated sporadically from this tissue. The symptom may be a result of a physiologic reaction by the plant to different environments or possibly due to the production of a microbial toxin.

F. solani rapidly infects seedling sainfoin roots. Observations and tests indicated that in Bozeman, more than 50% of the plants were infected six months after seeding. The seed hull often wounds the developing root during this period and a positive relationship was established with hull wounds and incidence of infection by F. solani. F. oxysporum also invaded hull wounds, however, tests indicated infection may also occur in secondary roots and seedling infection is much less frequent.

F. solani and F. oxysporum were not shown to be seed-borne or transmitted by seed in this study, however, further investigation of possible seed transmittal is needed. Alternaria sp. was a contaminate on the sainfoin hull and may reduce seedling vigor. R. solani may also contaminate hulls. R. solani was shown in pathogenicity tests to be the most virulent seedling pathogen tested, and significantly ($P < .05$) reduced seedling vigor.

Development of crown rot was associated with the physiologic tearing of the crown. F. solani was consistently isolated from old decayed stems at the crown, which originated directly above the crown tear area. Crown tearing may enable F. solani to invade the crown-root system and this may be an important step in the development of crown and root rot in sainfoin. Selection of plants not subjected to this tearing of the crown may reduce or delay crown rot in sainfoin.

Seedling vigor was not affected by infection of F. solani or F. oxysporum. Isolates of F. solani used in tests stimulated seedling vigor. Gibberellin may be produced by F. solani and may cause this hormone-like response. A seedling screening technique to find Fusarium spp. resistant plants may be difficult.

LITERATURE CITED

LITERATURE CITED

1. Anonymous. 1960. Index of plant diseases in the United States. Agr. Handbook No. 165, USDA, 530 p.
2. Burton, J.C., and R.L. Curley. 1968. Nodulation and nitrogen fixation in sainfoin (Onobrychis sativa Lam.) as influenced by strains of Rhizobia. Mont. Agr. Exp. Sta. Bull. 627.
3. Bushong, J.W., and J.W. Gerdemann. 1959. Root rot of alfalfa caused by Phytophthora crytozea in Illinois. Plt. Dis. Reprtr. 43:1178-1183.
4. Carleton, A.E. 1971. Annual Report. Montana State University. Unpublished data.
5. _____, C.S. Cooper, C.W. Roath, and J.L. Krall. 1968. Evaluation of sainfoin for irrigated hay in Montana. Mont. Agr. Exp. Sta. Bull. 627.
6. _____, _____, and L.E. Wiesner. 1968. Effect of seed pod and temperature on speed of germination and seedling elongation of sainfoin (Onobrychis viciaefolia Scop.) Agron. J. 60:81-84.
7. _____, L.E. Wiesner, A.L. Dubbs, and C.W. Roath. 1967. Yield and quality of sainfoin seed as related to stage of maturity. Mont. Agr. Exp. Sta. Bull. 627.
8. Chi, C.C., and E.W. Hanson. 1961. Nutrition in relation to the development of wilts and root rot incited by Fusarium in red clover. Phytopathology, 51:704-711.
9. _____, W.R. Childers, and E.W. Hanson. 1964. Penetration and subsequent development of three Fusarium species in alfalfa and red clover. Phytopathology, 54:434-437.
10. Cooper, C.S. 1974. Personal communications. USDA-ARS, Montana State University.
11. Cormack M.W. 1937. Fusarium spp. as root parasites of alfalfa and sweet clover in Alberta. Can. J. Res., 15:493-510.
12. Cressman, R.M. 1967. Internal breakdown and persistence of red clover. Crop Sci., 7:357-361.

13. Ditterline, R.L. 1973. Yield and yield components of sainfoin (Onobrychis viciaefolia Scop.) seed and evaluation of its use as a protein supplement. Ph.D. thesis. Montana State University.
14. _____. 1973. Annual Report, Montana State University. Unpublished data.
15. _____. 1974. Personal communication. Montana State University.
16. Donnely, E.D. 1952. Breeding for wilt resistance in alfalfa. Agron. J., 44:562-568.
17. Elgin, Jr., J.H., D.W. Evans, and J.M. Kraft. 1972. Occurrence of Phytophthora root rot of alfalfa in Washington. Plt. Dis. Repr., 56:830-831.
18. Elliot, E.S., R.E. Baldwin, and R.B. Carroll. 1969. Root rots of alfalfa and red clover. W. Va. Agr. Exp. Sta. Bull. 385-T.
19. Erwin, D.C. 1954. Relation of Stagnospora, Rhizoctonia and associated fungi to crown rot of alfalfa. Phytopathology, 44:137-144.
20. _____. 1954. Root rot of alfalfa caused by Phytophthora cryptogea. Phytopathology. 44:700-704.
21. _____. 1954. Crown rot of alfalfa in California. Alfalfa Improvement Conf. Report. 14th (Davis, California), 32 p.
22. Fergus, E.N., and W.D. Valleau. 1926. A study of clover failure in Kentucky. Ky. Agr. Exp. Sta. Bull. 269.
23. Frosheiser, F.I. 1967. Phytophthora root rot of alfalfa in Minnesota. Plt. Dis. Repr., 51:679-681.
24. _____. 1969. Phytophthora root rot of alfalfa in the upper mid-west. Plt. Dis. Repr., 53:595-597.
25. _____, and D.K. Barnes. 1973. Field and greenhouse selection for Phytophthora root rot. Crop Sci., 13:735-738.

26. Fulkerson, J.F. 1960. Pathogenicity and stability of strains of Corynebacterium insidiosum. *Phytopathology*, 50:377-380.
27. Graham, J.H., and R.C. Newton. 1959. Relationship between root feeding insects and incidence of crown and root rot in red clover. *Plt. Dis. Repr.*, 43:1114-1116.
28. _____, C.L. Rhykerd, and R.C. Newton. 1960. Internal breakdown in the crown of red clover. *Plt. Dis. Repr.*, 44:59-61.
29. Griffiths, D.A., and W.C. Lim. 1965. "Overgrowth" in Malayan crop plants following infection by Fusarium solani and F. decemcellulare. *Plt. Dis. Repr.*, 49:979-980.
30. Hanna, M.R., and S. Smoliak. 1968. Sainfoin yield evaluations in Canada. *Mont. Agr. Exp. Sta. Bull.* 627.
31. _____, _____, and B.P. Goplin. 1972. Sainfoin for western Canada. *Canada Dept. of Agr. Publ.* 1470.
32. Hanson, C.H., and J.L. Allison. 1951. Studies on the nature and occurrence of stand depletion in alfalfa strains in North Carolina. *Agron. J.*, 43:375-379.
33. Hawn, E.J., and M.W. Cormack. 1952. Crown bud rot of alfalfa. *Phytopathology*. 42:510-511.
34. Holden, J.L. 1968. A producer's evaluation of sainfoin. *Mont. Agr. Exp. Sta. Bull.* 627.
35. Hine, R.B., F.A. Gray, and M.H. Schonhorst. 1971. Phytophthora root rot of alfalfa: a major factor in stand decline. *Progress Agr. Arizona*, 23:4-5.
36. Hughes, S.J. 1945. Studies on diseases of sainfoin (Onobrychis sativa). I. Ring-spot caused by Pleospora herbarum (Pers.) Rabenh. *Trans. British Mycol. Soc.*, 28:86-90.
37. _____. 1949. Studies of some diseases in sainfoin. II. The life history of Ramularia onobrychidis Allescher. *Trans. British Mycol. Soc.*, 32:34-59.
38. _____. 1949. Studies on some diseases of sainfoin (Onobrychis sativa). IV. Leaf-spot caused by Septoria orobinia Saac. *Trans. British Mycol. Soc.*, 32:60-62.

39. Isaac, I. 1946. Verticillium wilt of sainfoin. Ann. Appl. Biol., 33:28-34.
40. Jensen, E.H., C.R. Torell, A.L. Cesperance, and C.F. Speth. 1968. Evaluation of sainfoin and alfalfa with beef cattle. Mont. Agr. Exp. Sta. Bull. 627.
41. Johnson, H.W. 1967. Potential of the Rhizobium - Fusarium interactions on the incidence of alfalfa root rot. Ph.D. thesis. Univer. R.I. Cited in "The Fusarium root rot complex of selected forage legumes in the northeast." 1971. Penn. Agr. Exp. Sta. Bull. 777.
42. _____, and F.L. Morgan. 1965. Phytophthora root and crown rot of alfalfa in the Yazo-Mississippi Delta. Plt. Dis. Reprtr., 49:753-755.
43. Jones, F.R., and L. McCulloch. 1926. A bacterial wilt and root rot of alfalfa caused by Aplanobacter insidiosum L. McC. J. Agr. Res., 33:493-521.
44. Kehr, W.R., F.I. Frosheiser, R.D. Wilcoxson, and D.K. Barnes. 1972. Breeding for disease resistance. In Alfalfa, Science and Technology. C.H. Hanson (ed). pp. 335-354.
45. King, C.J., and J.T. Priestley. 1942. A root rot of cotton caused by Thielaviopsis basicola. Phytopathology, 32:752-761.
46. Knipe, W.J. 1972. Reproduction and genetics of sainfoin (Onobrychis viciaefolia Scop.) as they relate to its breeding. Ph.D. thesis. Montana State University.
47. Krall, J.L., C.S. Cooper, C.S. Crowell, and A.J. Jarvi. 1971. Evaluation of sainfoin for irrigated pasture. Mont. Agr. Exp. Sta. Bull. 658.
48. Kreitlow, K.W. 1963. Infecting seven-day-old alfalfa seedlings with wilt bacteria through wounded cotyledons. Phytopathology, 53:800-803.

49. Kricala, B.A., and E. Morancik. 1964. Anthracnose caused by Aschocyta onobrychidis. A new disease of sainfoin in Czechoslovakia. *Biologia (Bratislava)*, 19:729-734.
50. Leath, K.T., F.L. Lukenzie, H.W. Crittendon, E.S. Elliot, P.M. Halisky, F.L. Howard, S.A. Ostazeski. 1971. The Fusarium root-rot complex of selected forage legumes in the northeast. *Penn. Agr. Exp. Sta. Bull.* 777, 64 pp.
51. Marks, G.C., and J.E. Mitchell. 1970. Detection, isolation and pathogenicity of Phytophthora megasperma from soils and estimation of inoculum levels. *Phytopathology*, 60:1687-1690.
52. _____, and _____. 1971. Penetration and infection of alfalfa roots by Phytophthora megasperma and the pathological anatomy of infected roots. *Can. J. Bot.*, 49:63-67.
53. Mathre, D.E., A.V. Ravenscroft, and R.H. Garber. 1966. The role of Thielavopsis basicola as a primary cause of yield reduction in cotton in California. *Phytopathology*, 56:1213-1216.
54. _____. 1968. Diseases in sainfoin. *Mont. Agr. Exp. Sta. Bull.* 627.
55. _____. 1974. Personal communication. Montana State University.
56. Mew, T.W. 1968. Root rot of soybean (Glycine max) in relation to antagonism of Rhizobium japonicum and Fusarium oxysporum. M.S. thesis, Univ. R.I. Cited in "The Fusarium root-rot complex of selected forage legumes in the northeast. 1971. *Penn. Agr. Exp. Sta. Bull.* 777.
57. Mishustin, E.N., and I.M. Karashchuk. 1955. Epiphitnaya microflora semyan espartseta: povyshenie ego urozhainosti. (The epiphytic microflora of the seeds of sainfoin and increasing the yield of the latter.) *Izv. Akad. Nauk. SSSR: Ser. Biol.* 2:8018. Cited in "A compilation of abstracts of sainfoin literature." 1968. A.E. Carleton and C.S. Cooper (Processed).
58. Murray, G.A. 1968. Plant growth and nodulation of alfalfa and sainfoin plants in relation to manganese concentrations. *Mont. Agr. Exp. Sta. Bull.* 627.

59. Murray, G.A., and E.A. Slinkard. 1968. Forage and seed production potential of sainfoin in northern Idaho. Mont. Agr. Exp. Sta. Bull. 627.
60. Nash, S.M., and W.C. Snyder. 1962. Quantitative estimations by plate counts of propagules of the bean root rot Fusarium in field soils. Phytopathology, 52:567-572.
61. Nelson, P.E. 1974. Personal communication. The Pennsylvania State University.
62. Ocana, G., and P.H. Tsao. 1968. A selective agar medium for the direct isolation and enumeration of Phytophthora in the soil. (Abstr.) Phytopathology, 56:893.
63. O'Rourke, C.J., and R.L. Miller. 1966. Root rot and root microflora of alfalfa as affected by potassium nutrition, frequency of cutting and leaf infection. Phytopathology, 56:1040-1046.
64. Pearson, L.C., and L.J. Ellings. 1960. Predicted disease resistance in synthetic varieties of alfalfa from clonal cross data. Agron. J., 52:291-294.
65. Piper, C.V. 1914. Forage Plants and their Culture. The MacMillian Co., New York. pp. 559-562.
66. Rehm, E. 1872. Die entwicklungsgeschichte eines die kleearten zerstorenden pilzes, Peziza ciborisdes Fries. J.F. Landwirts. Gottingen: 151-176. Cited in "The Fusarium root-rot complex of selected forage legumes in the northeast." 1971.
67. Roath, C.W. 1968. Sainfoin for dryland hay in western Montana. Mont. Agr. Exp. Sta. Bull. 627.
68. Schmitthenner, A.F. 1964. Prevalence and virulence of Phytophthora, Aphanomyces, Pythium, Rhizoctonia, and Fusarium isolated from diseased alfalfa seedlings. Phytopathology, 54:1012-1018.
69. Shain, S.S. 1959. (Moscow State Publishing House of Agricultural Literature), Description of Sainfoin in Agrotechniques of Perennial Forages. 51-54. Trans. R.P. Knowles. Cited in "A compilation of abstracts of sainfoin literature." 1968. A.E. Carleton and C.S. Cooper (Processed).

70. Shyh-Jane, N., D.K. Barnes, and F.I. Frosheiser. 1973. Inheritance of Phytophthora root rot resistance in alfalfa. Crop Sci., 13:714-717.
71. Sims, J.R., N.K. Miun, and A.E. Carleton. 1968. Evidence of ineffective Rhizobia and its relation to the nitrogen nutrition of sainfoin (Onobrychis viciaefolia). Mont. Agr. Exp. Sta. Bull. 627.
72. Smith O.F. 1943. Rhizoctonia root canker of alfalfa (Medicago sativa). Phytopathology, 33:1081-1085.
73. Spedding, C.R.W., and E.C. Diekmahns. 1972. Grasses and Legumes in British Agriculture. Commonwealth Agricultural Bureaux. pp. 405-411.
74. Staffeldt, E.E. 1959. Thielaviopsis basicola, a part of the cotton (Gossypium hirsutum) seedling disease complex in New Mexico. Plt. Dis. Repr., 43:506-508.
75. Staten, G., and P.J. Leyendecker. 1949. A root disease of alfalfa caused by Fusarium solani. Plt. Dis. Repr., 33:254-255.
76. Stobel, G.A. 1970. A phytotoxin glycopeptide from potato plants infected with Corynebacterium sepedonicum. J. Bio. Chem., 245:32-38.
77. _____, and D.E. Mathre. 1970. Outlines of Plant Pathology. Van Nostrand Reinhold Company. New York, pp. 465.
78. Toussoun, T.A., and P.E. Nelson. 1968. A pictorial guide to the identification of Fusarium species according to the taxonomic system of Snyder and Hansen. The Pennsylvania State University Press. University Park and London, 51 pp.
79. Tysdale, H.M., and B.H. Crandall. 1948. The polycross progeny performance as an index of the combining ability of alfalfa clones. Agron. J., 40:293-306.
80. Varga, P. 1968. Aims of sainfoin breeding in Romania. Mont. Agr. Exp. Sta. Bull. 627.

81. Wiesner, L.E. 1974. Personal communication. Montana State University.
82. Wilhelm, S. 1959. Parasitism and pathogenesis of root-disease fungi. In C.S. Holten et al. (ed). Plant Pathology--Problems and Progress 1908-1958, Univ. Wis. Press, Madison, Wis., pp. 356-366.
83. Yarwood, C.E. 1946. Isolations of Thielaviopsis basicola from soil by means of carrot discs. Mycologia, 38:346-348.
84. Zorembo, V.P. 1961. Osobennosti vzaimootnos-Shenii Kulbenkovykh bakterii s mikrofloroi semyan espartseta (Characteristics of the interrelationships of the root nodule bacteria with microflora of the sainfoin family. Trudy Inst. Mikrobiol. Akad. Nauk. SSSR., 11:188-197). Cited in "A compilation of abstracts of sainfoin literature." 1968. A.E. Carleton and C.S. Cooper (Processed).

APPENDIX

APPENDIX I

1. Acidified Potato Dextrose Agar (HPDA)

Difco potato dextrose agar	39g
Distilled water	1000 ml
Lactic acid (25%)*	2ml/l

A general purpose growth medium for many fungi

2. Acidified Corn Meal Agar (HCMA)

Difco corn meal agar	17g
Distilled water	1000 ml
Lactic acid (25%)*	2ml/l

A general purpose growth medium for many fungi

3. PCNB Agar (Nash and Snyder, 1962)

Agar	20.0g
Peptone	5.0g
KH ₂ PO ₄	1.0g
MgSO ₄ · 7H ₂ O	0.5g
Distilled water	1000 ml
Streptomycin*	300 mg
Pentachloronitrobenzene (PCNB), 75% W.P.*	1.0g

* Add after autoclaving

Preparation: Streptomycin and PCNB are added after autoclaving and medium cooled to 42-45C.

A specific medium for isolating Fusarium from the soil and plant tissue.

4. PPV Agar (Ocana and Tsao, 1968)

Difco corn meal agar	17g
Distilled water	1000 ml
Pimaricin	10 ppm
Pentachloronitrobenzene	100 ppm
Vancomycin	200 ppm

Preparation: Pimaricin, PCNB, and vancomycin are added after autoclaving medium cooled to 42-45C.

A specific media used for isolating Phytophthora from the soil and plant tissue.

5. Corynebacterium Agar

Agar	20g
Glucose	15g
Yeast extract dialysate	10g
Calcium carbonate	5g
Distilled water	1000 ml

A general purpose medium for the isolation of Corynebacterium species.

6. Modified Eckerts Medium

Glucose	18g
Yeast Extract	3g
Difco peptone	5g
KH_2PO_4	1.7g
K_2HPO_4	1.4g
MgSO_4	0.5g
Distilled water	1 liter

A general purpose growth medium for many fungi.

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