Proof of pathogenicity of Fusarium slani (Mart.) Appel & Wr. to sainfoin (Onobrychis viciifolia Scop.) and development of procedures to select for resistance
by Dick Lindsey Auld

A thesis submitted in partial fulfillment of the requirements for the degree of DOCTOR OF PHILOSOPHY in Crop and Soil Science
Montana State University

Abstract:
Root and crown rot limits the production of sainfoin. This disease reduces longevity and forage yield. The objectives of this study were to: 1) determine the causal organism(s) of root and crown rot; 2) further characterize the host-parasite relationships; and 3) develop and initiate screening procedures to detect potential sources of disease resistance.

The results of this study indicate that the root and crown rot of sainfoin usually observed in Montana is caused by Fusarium solani. This pathogen is not seed transmitted but probably occurs in most agricultural soils as a pathogen of other legumes.

Sainfoin can be successfully screened for disease resistance by soaking injured roots in concentrated suspensions of microconidia of F. solani. Initial screening to locate potential sources of disease resistance has shown differential disease resistance in both the world collection of Onobrychis and current breeding material. Selection within these populations should increase disease resistance.

PROOF OF PATHOGENICITY OF *Fusarium solani* (Mart.) APPEL & WR. TO SAINFOIN (*Onobrychis viciifolia* Scop.) AND DEVELOPMENT OF PROCEDURES TO SELECT FOR RESISTANCE

by

DICK LINDSEY AULD

A thesis submitted in partial fulfillment
of the requirements for the degree

of

DOCTOR OF PHILOSOPHY

in

Crop and Soil Science

Approved:

Raymond L. Patterson
Chairman, Examining Committee

Kurt C. Feltner
Head, Major Department

Henry L. Parsons
Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

February, 1976

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to the following:

Dr. R. L. Ditterline for his professional guidance and friendship while serving as my major professor during the course of this study.

Dr. D. E. Mathre for his patience and personal support in the pathological investigations.

Dr. C. S. Cooper for his professionalism and contributions to this study.

Professor R. F. Eslick, Dr. L. E. Wiesner, and H. E. Holen for serving on my graduate committee.

My wife, Sherry, for her sacrifice and encouragement through my graduate work. And to my two daughters, Shannon and Shelly, for their love and trust.

The Montana Agricultural Experiment Station and the Plant and Soil Science Department for providing me with an assistantship. Without this assistance, attending graduate school would have been impossible.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ABSTRACT

Root and crown rot limits the production of sainfoin. This disease reduces longevity and forage yield. The objectives of this study were to: 1) determine the causal organism(s) of root and crown rot; 2) further characterize the host-parasite relationships; and 3) develop and initiate screening procedures to detect potential sources of disease resistance.

The results of this study indicate that the root and crown rot of sainfoin usually observed in Montana is caused by Fusarium solani. This pathogen is not seed transmitted but probably occurs in most agricultural soils as a pathogen of other legumes.

Sainfoin can be successfully screened for disease resistance by soaking injured roots in concentrated suspensions of microconidia of F. solani. Initial screening to locate potential sources of disease resistance has shown differential disease resistance in both the world collection of Onobrychis and current breeding material. Selection within these populations should increase disease resistance.

INTRODUCTION

Sainfoin (Onobrychis viciifolia Scop.) is a non-bloating perennial legume which has been used for hay production and pasture in Europe for several centuries. Cultivation of sainfoin began in Montana in 1964 with the release of 'Eski'. Sainfoin rapidly gained popularity because of its excellent forage quality, winter hardiness, drought tolerance, palatability, and resistance to the alfalfa weevil (Hypera postica Gyllenhal). It offered an excellent alternative to alfalfa (Medicago sativa L.).

In recent years sainfoin has been attacked by a root and crown rot which limits persistance and reduces forage production. This disease is found statewide and is thought to be caused by Fusarium spp. Sainfoin's popularity has declined because of root and crown rot. Disease resistant varieties must be developed if sainfoin is to remain an economic forage crop.

The objectives of this study were to: 1) determine the causal organism(s) of root and crown rot; 2) characterize the host-parasite relationships; and 3) develop and initiate screening procedures to detect potential sources of disease resistance.

LITERATURE REVIEW

Description of Onobrychis viciifolia Scop.

Sainfoin is a deep rooted, perennial, forage legume. It has a branched tap root system which may extend to a depth of several meters and be 5 cm in diameter (60). Nodulation occurs on the fine lateral roots and the young tap roots (3,54,69).

Multiple erect stems, 90-150 cm tall, arise from a branched, prostrate crown. These stems are usually hollow and bear pinnately, compound leaves with 13-15 leaflets per leaf (18,60).

Sainfoin's influorescence is an erect raceme with from 5-80 florets. Each floret gives rise to single seeded pod (6,42). The pods are brown, indehiscent, lenticular, and reticular on the surface (60). The seed are brown to black in color and are approximately 2.5 mm long, 2.0-3.5 mm wide, and 1.5-2.0 mm thick (6). The seed pod comprises approximately 30% of the weight of the pod-intact seed (6,16).

History and Distributions of Onobrychis viciifolia Scop.

Sainfoin has been utilized as a forage crop in Russia for over 1,000 years and in Europe for over 400 years (60,68). Early immigrants brought plants of this species to the North American Continent with introduction into Montana dating back to before 1900 (18).

Sainfoin was evaluated as a potential forage crop in the early part of this century (60), but failed to develop into a major crop

because of disease problems; failure to recognize its forage quality, palatability, and non-bloating characteristics; and due to testing in areas not well adapted to this crop. In recent years, the increasing losses of alfalfa to the alfalfa weevil, and the need for a higher yielding, non-bloating forage legume, has increased the interest in sainfoin.

In 1964 the variety Eski was released by the Montana Agricultural Experiment Station. This variety was selected from plant introductions from Turkey (19). Other varieties such as 'Onar' and 'Melrose' are selections from plant introductions from Russia (28). Currently, plant breeders working in sainfoin are continuing to evaluate both new plant introductions and the earlier introductions which have become adapted to this country.

Agronomic Characteristics of *Onobrychis viciifolia* Scop.

Sainfoin is drought tolerant, winter hardy, and adapted to coarse calcarous soils, which are not well suited for other legumes (31,37,60). It has been utilized for hay production, seed production, and pasture under both dryland and irrigated conditions. However, many researchers report there is a lack of effective nodulation and that stand persistence is poor under certain conditions (3,48,54,66, 69).

Sainfoin has high quality forage and has never been reported to cause bloat (6,16,18,28,60). Feeding trials conducted in Canada, Montana, Idaho, and Nevada have shown sainfoin hay to equal alfalfa hay in nutritive value and to have even greater palatability (4,7,27, 28,36,55). Sainfoin seed also has the potential of becoming a protein supplement in animal diets (16).

Hay yields of sainfoin have been variable. When grown under proper conditions, yields of sainfoin are competitive with the best adapted alfalfa varieties (4,7,27,28,36,55). Best yields are obtained on well-drained, basic soils with a good moisture-holding capacity (4,18,28,60). Sainfoin is sensitive to frequent cuttings. Most existing varieties produce maximum forage in the first cutting (4,18,55). Many stands begin to fail in the third year when irrigated, and in the fourth or fifth year under dryland conditions (4,28,31,62). This stand depletion is thought to be the result of a disease which severely damages the crowns and roots of mature plants (48,66).

Seed yields often approach 1,500 kilograms of clean seed per hectare (27,55). In colder climates, seed is usually not harvested until the second year of establishment. Maximum seed yields are obtained if the first cutting is allowed to set seed (6,28,55).

Sainfoin's role as a pasture legume is enhanced by its lack of bloat, good feed value, and broad range of adaptation (18,28,43). It can either be pastured alone or in mixtures with grasses such as

Russian wild rye-grass or crested wheatgrass. However, it does not seem to compete well with aggressive, rhizomatous grasses such as brome grass or pubescent wheatgrass (28,43). The use of sainfoin in the hay-stockpile management regime distributes forage production and allows an increase in total carrying capacity (12).

Diseases of Onobrychis viciifolia Scop.

Foliar diseases. Most work on foliar diseases has been done in Europe. Mathre (48) in a review, found reports of several foliar diseases of sainfoin. A leaf spot caused by the fungus Ramularia onobrychidis Allescher, forms dark brown spots on the upper surface of leaves. This disease is most severe under moist conditions and occurs in large areas of Europe (33,48).

Another leaf spot, which occurs primarily in England, is caused by the fungus Septoria orobina Saac. It forms fawn-colored spots on both the leaves and young pods. This disease may be disseminated by contaminated pods (34,48).

Ring spot, which occurs in both England and Montana, is caused by the fungus Pleospora herbarum (Pers.) Rabenh. The symptoms are similar to those of Septoria leaf spot. Conidia form under moist conditions to promote rapid spread. The disease does not appear to cause economic damage in Montana (32,48).

The fungus Aschochyta onobrychidis forms spots on both the leaves and stems. It is found in Europe and Montana, where it occurs primarily as blackened stem lesions. This fungus appears to be seed borne and persists on crop residue. It is not considered to be causing economic damage (32,48).

Other diseases attacking the foliage of sainfoin are: a rust caused by Uromyces onobrychidis; chocolate spot caused by Botrytis conerae; and powdery mildew caused by Erysiphe polygoni D.C. (32,48). None of these foliar diseases are thought to be of economic importance in Montana (48).

Soil-borne diseases. Diseases, that attack the crowns and roots of sainfoin, are the primary cause of stand reduction. Verticillium wilt of sainfoin (Verticillium albo-atrum Reinke and Berth) has been observed in Europe and causes wilting and vascular discoloration (35). It is most severe during the warmer months when the plant is often under moisture stress. This pathogen has not been isolated from diseased sainfoin plants in Montana (48,66).

The fungus, Sclerotinia trifoliorum Erikss, causes root, crown, and stem rot in Montana and Europe (32,48). Infected plants wither and turn brown during the middle of the growing season and sclerotia can be found in the pith tissue. Under high moisture conditions, white or tan mycelial mats appear near the soil line. The damage in infested

fields is usually light in Montana, with only 1% of the plants showing symptoms (48).

Rhizotonia solani Kuehn. has been isolated from secondary roots and darkened lesions on tap roots. R. solani has never been isolated from the internal portion of the crown but could possibly act as a source of entry for other pathogens (66).

Sainfoin is extremely susceptible to the northern root-knot nematode, Meloidogyne hapla Chitwood. Damage was more severe at higher temperatures. Unlike alfalfa, both mature and young roots of sainfoin are susceptible (24).

The most serious disease of sainfoin in Montana is root and crown rot though to be caused by Fusarium spp. (48,66,67).

Seed and seedling diseases. The seed and seed pod of sainfoin has been shown to carry Alternaria, Fusarium, Mucor, and numerous bacteria (66,74). Many of the bacteria were antagonistic to nodulation (74). Contaminated seed have been shown to spread Fusarium pathogens in beans (Phaseolus vulgaris L.) and mimosa (Albizia julibrissin) (22,58).

Sainfoin seedlings are susceptible to Alternaria spp., Rhizoctonia solani and Pythium spp. (66). Symptoms often appear as classical 'damping off'.

Development of Root and Crown Rot in Onobrychis viciifolia Scop.

Occurrence of the disease. Sears (66,67) in an extensive study of Montana found the root and crown rot of sainfoin in nine of ten sampled locations. While both F. solani (Mart.) Appel & Wr. and F. oxysporum Schlecht. were isolated, F. solani was present in over 90% of the diseased plants. This disease also causes severe losses in New Mexico and may occur in other areas where sainfoin is grown (52).

When Eski was grown under normal field conditions, F. solani was isolated from 60% of the seedlings by the end of the first growing season. The rapid entry of this fungus was thought to be enhanced by the seed pod wounding the young tap root as it began to thicken and elongate (66). The seed pod reduces emergence in the field and germination in the laboratory (5). Removal of the pod prior to planting may be beneficial (5,66).

Symptoms begin to appear during the second year. The crown appears to be unable to support the increasing number of stems and splits. This tearing may be a source of entry to pathogens. F. solani has been consistently isolated from this area. The disease appears to develop first in the stem, just above the crown where it forms a darkened streak which extends into the vascular tissue of the crown. As the disease progresses, the crown tissue is destroyed resulting in the eventual death of the plant (66). Fumigation of field plots increased vigor and regrowth, and reduced the frequency of root and crown rot in

sainfoin (66). The crown splitting does not result in destruction of crown tissue in the absence of pathogens.

Occurrence and severity of root and crown rot of sainfoin could be aggravated by root damage caused by nematodes or insects. In alfalfa, the infection of F. oxysporum was increased from 15 to 90% by the addition of northern root-knot nematodes (Meloidogyne hapla Chitwood)(51). In red clover (Trifolium pratense L.) insect root injury increased the incident of crown rot (23). Non-parasitic nematodes such as Diplogaster lheritieri Maupas may spread plant pathogens. These nematodes injest spores of F. oxysporum and Verticillium spp. without reducing their viability (37).

The suspected pathogen. F. solani is a common pathogen of most agronomic crops (2,17,47). This fungi exists in the soil and in crop residue as chlamydospores. Chlamydospores are resting structures produced by hyphae and conidia through the condensation of their contents and the formation of a thick wall (56,72). Root exudates of susceptible plants stimulate the chlamydospores to germinate and produce mycelium which seek the surface of the root (11). The fungus forms a small surface thallus and penetrates the plant through the middle lamella at a junction of epidermal cells (9,11). It also invades roots through mechanical and natural wounds. Once established, hyphae invade the living cortex intercellularly until they are stopped by the endodermis (11).

When the hyphae are exposed to daylight they produce macroconidia and microconidia. Macroconidia are large sickle-shaped condia with three to seven cells and are usually formed on sporodichia. Microconidia are produced on the aerial mycelium and are usually composed of only one or two cells (72). The principle function of the spores is dissemination, but they may also be directly infective (57). When macroconidia are added to the soil they form a short hyphae which then forms chlamydospores (11,56).

Isolates of F. solani may produce a hormonal growth response in plants that are grown in infested soil (11,25,56,66) The asexual forms are capable of genetic recombination through the processes of heterokaryosis and parasexuality (50,72).

The genus Fusarium is divided into nine taxonomic species in a system developed by Snyder and Hansen (72). This system uses the shape and morphology of macroconidia as its principle index. Other characteristics such as colony morphology and pigmentation are undependable and subject to rapid mutation (72). F. solani has been divided into nine formae species on the basis of selective pathogenicities to their hosts (50). This selectivity is subject to rapid mutation and may be simply inherited (73). Some investigators feel the division into formae species is highly artificial and question its validity (61).

F. solani has been a severe pathogen on beans (2,49,56), peas (Pisium sativum L.)(47,49), and many other legumes (20,49,56). This fungus produces a sexual stage (Hypomyces) that is composed of individuals which are self-sterile, interfertile, and usually hermaphroditic. Ascospores are found in perithecia which develop in nature only on mulberry leaves under very humid conditions. Investigators recently induced the sexual stage in vitro (49,50).

Crown Rot in Other Forage Legumes

Red clover has historically been limited by a lack of stand persistence due largely to root and crown diseases (15,45). Isolates of Pythium, Fusarium, Rhizoctonia, Phoma, and Gliocladium have been associated with this disease, but Fusarium spp. are the prevalent pathogens. Strains of these pathogens exhibited a wide range of virulence when tested on 'Midland' red clover (40,41). Infection is thought to occur in the seedling stage (41). Stands often fail to recover after the first cutting of the second year. The root and crown rot was more severe in years with high temperatures, limited soil moisture, and when the plants were grown under nutritional stress (8,17).

Pathogens associated with root and crown rot of alfalfa are Phytophthora (63), Aphanomyces (46,65), Pythium (65), Rhizoctonia (65), and Fusarium (1,13,65,70). Root and crown rot is aggravated by moisture and nutritional stress (10,55).

Nitrogen fixing bacteria, Rhizobium, are less effective in the presence of Fusarium spp. (38,45,53). The relationship between the bacteria and fungus is dependent upon soil pH, soil microflora, and time of inoculation (53). Plants with diseased roots have fewer nodules which may lead to a nitrogen stress and increased disease susceptibility.

Development of Selection Procedures

Techniques do not exist for selecting sainfoin plants with resistance to root and crown rot caused by Fusarium spp. After six years, only 3 of 100,000 plants were free of disease symptoms indicating that resistance is rare in existing populations (66). Therefore, selection procedures must be found that will screen large number of plants in short periods of time.

Early work on alfalfa root diseases was done on bacterial wilt, Corynebacterium insidiosum (McCull.) Jensen (14,39). Techniques developed to screen for resistance to this organism have been adapted to screen for other diseases caused by fungi such as Fusarium (13,14,21, 30,44,64). Techniques which appear favorable are inoculation of single roots, bare-root-soak, root-ball-soak, crown-soak, and inoculation of cut stems (13,14,45,63). The percentage of infected plants can be increased by wounding the roots prior to, or in conjunction with, inoculation (13,14,59,64). Because of the slow action of most root rotting pathogens, researchers visually score the spread of infection

three to six months after inoculation. This scoring system is usually based on five to seven categories and is used as the principal index of disease resistance (13,14,45).

It is possible to control the severity of a test by manipulating environmental factors. These factors include: age of plants at time of inoculation (2,14,47), water status (14,21), concentration of inoculum (14,45), the length of the trial period (14,21,47), and the temperature (14,21,45). These factors should be manipulated to create severe disease symptoms in inoculated sainfoin plants in a minimum amount of time. The development of the proper combination of these factors will require extensive investigation.

Chapter I

MATERIALS AND METHODS

General

The purpose of these experiments was to determine if: 1) the pathogens causing root and crown rot were seed-borne, 2) microconidia of F. solani could be used as inoculum, and 3) fungicides or seed treatments could reduce the severity of root and crown rot in sainfoin. Plants were grown either in the greenhouse or at the Field Research Laboratory, Bozeman, Montana. Plants in the greenhouse were grown with a 16-hour photoperiod provided by supplemental lighting from incandescent lights and were watered with a nutrient solution (Appendix Table 1). Seed and root pieces used for isolation were surface-sterilized by soaking in a 0.5% NaOCl solution for three minutes.

Data were analyzed by analysis of variance and means were separated by Duncan's New Multiple Range Test. Correlations were determined among indices within experiments.

Experiment 1: Determination of potentially pathogenic fungi associated with sainfoin seed

Samples of eight commercial seed lots of sainfoin were obtained from the Montana State Seed Testing Laboratory to determine if seed-borne pathogenic fungi were affecting sainfoin. The seed of each sample were divided into three groups and treated as follows:

Group I: Pod-intact - no treatment.

Group II: Pod-intact - surface-sterilized

Group III: Pod-removed - surface-sterilized

Eighty seed of each group were placed in petri dishes containing acidified potato dextrose sugar (HPDA)(66)(Appendix Table 1) and 80 seed were placed in petri dishes containing pentachloronitrobenzene agar (PCNBA)(57)(Appendix Table 1). HPDA was used for general fungal isolations and PCNBA was used to isolate Fusarium spp. The HPDA and PCNBA plates were incubated at 27 C for four and eight days, respectively. Fungal cultures were then identified by microscopic examination (72). The percentage of seeds contaminated with Alternaria spp. and/or F. oxysporum were analyzed by contingency chi square.

On the basis of laboratory isolations, two seed lots were chosen for further evaluation in the greenhouse. The seed pods were removed from one-half of the seed of both the seed lot heavily contaminated (#1538) and the seed lot lightly contaminated (#405) with Alternaria spp. One-half of both the 'pod-intact' and the 'pod-removed' seed were then surface-sterilized. One hundred seed from each of the four treatments of both seed lots were incubated for nine days at 25 C in germination trays to determine if treatments had affected germination. Treated seed were planted in Bozeman silt loam that had been either autoclaved at 121 C for six hours or pasturized for 16 hours with unpressurized steam. The experiment was conducted in a split-split-plot randomized complete block design with four

replications. The two seed lots were assigned to main plots, the two soil treatments to subplots, and the four seed treatments to sub-subplots. Each sub-subplot contained 50 seed.

After 24 days, seedling emergence was determined and the roots of four seedlings from each sub-subplot were harvested. A root piece was removed 1 cm below the crown of each seedling, surface-sterilized for 45 seconds, and placed on HPDA (66) to determine the percentage of seedlings infected with Alternaria spp.

Experiment II: An evaluation of the relative pathogenicity of microconidia and macroconidia of four isolates of F. solani

Three surface-sterilized, pod-removed 'Remont' seed were planted in 10.2 cm square plastic pots which contained Bozeman silt loam soil that had been autoclaved at 121 C for six hours. Seedlings were thinned to one per pot at emergence. Eighty-three days after emergence the seedlings were inoculated with F. solani by piercing the crown with a sterile dissecting needle and applying approximately 4×10^6 conidia to the wound.

The experiment was conducted in a split-plot randomized complete block design with three replications. Inoculation with either micro- or macroconidia was assigned to main plots. Microconidia were produced in Modified Eckert's Broth (66) and a mixture of micro- and macroconidia were produced in Snyder and Nash's sporulation medium (56)

Appendix Table 1). Inoculations with each of the four isolates of F. solani and with sterile water were assigned to subplots. There were 10 seedlings in each subplot.

Ninety days after inoculation, roots of seedlings were harvested, split longitudinally, and visually scored for disease severity. A single section of each taproot was removed 2 cm below the crown, surface-sterilized, and placed on HPDA (66) to determine the percentage of plants infected with F. solani.

Experiment III: Evaluation of soil fumigation and seed treatments for the control of root and crown rot of sainfoin.

A split-plot randomized complete block design with three replications was used to evaluate the effect of planting either pod-intact or pod-removed seed into pots which had been treated with fungicides. Surface-sterilized Remont seed with either the pod-intact or the pod-removed were randomly assigned to main plots. The fungicide treatments were assigned to subplots. These were:

Control: No treatment.

Chloropicrin Soil Fumigation: Chloropicrin (trichloronitromethane) was injected 10 cm into the soil in a 15.4 cm grid pattern with a Fumigun at the rate of 647 kg/ha. The treatment was applied on June 10, 1974, and the plots were sealed with plastic tarps for seven days.

Benlate Soil Drench: A 50% active-ingredient, wettable powder of the fungicide 'Benlate' (Methyl-(butylcarbanoyl)-2 benzimidazole carbamate) was incorporated 5 cm into the soil with a rake at the rate of 340 kg/ha. This treatment was applied on July 1, 1974.

Benlate Seed Treatment: Benlate was dusted on the seed at the rate of 2.5 g of wettable powder of the fungicide per 1000 g of seed immediately prior to planting.

The study was seeded at the Field Research Laboratory on July 9, 1974, at the rate of 24 and 34 kg/ha for the pod-removed and the pod-intact seed, respectively. Plots were 3.1 m long, 1.8 m wide, and contained 12 rows spaced 15.4 cm apart. The border surrounding each plot was 92 cm wide and seeded to crested wheatgrass. The plots were irrigated 20 minutes daily for 12 days to permit seedling establishment. Thereafter, irrigation water was applied as needed.

Five times during the course of the study a minimum of eight seedlings were removed from the margins of each plot and scored for nodulation (Table 1-1) and disease severity. A section of root from each plant was removed 3 cm below the crown, surface-sterilized, and placed on PCNBA (57) to determine the percentage of seedlings infected with Fusarium spp. Fusarium isolates were identified to species by cultural characteristics on slants of fresh potato dextrose agar (FPDA) (Appendix Table 1) and microscopic examination of macroconidia (72).

Table 1-1. The visual rating system used to assign nodulation scores to sainfoin seedlings.

Score	Description
1	No nodules formed
2	Nodules on 1% of the secondary roots
3	Nodules on 5% of the secondary roots
4	Nodules on 25% of the secondary roots
5	Nodules on 50% of the secondary roots
6	Nodules on 75% of the secondary roots

Forage yield, plant height, stand density, and seedling vigor were measured on a 1 m wide strip in the center of each plot. Table 1-2 lists the indices and the dates on which they were measured in this study.

RESULTS AND DISCUSSION

Experiment I: Determination of potential pathogenic fungi associated with sainfoin seed.

Helminthosporium, Nigrospora, Fusarium, Alternaria, and numerous saphrophytic fungi were found on sainfoin seed. Fifty-seven percent of the untreated seed were contaminated with Alternaria spp. (Table 1-3). Other fungal species occurred on less than five percent of the seed. Seed treatment reduced contamination with Alternaria spp. ($X^2 = 594.2$; $P < .005$). With the exception of one seed lot (#1538),

Table 1-2. The date of sampling and the indices measured on the soil fumigation and seed treatment experiment at the Field Research Laboratory, Bozeman, Montana.

Date		Index
1974	Aug. 26	Number of plants showing visual wilt symptoms
		Number of plants with visual root symptoms
		Percentage of plants infected with F. spp.
	Aug. 30	Stand density
		Seedling vigor scored
	Sept. 13	Number of plants with visual root rot symptoms
		Percentage of plants infected with F. oxysporum
		Percentage of plants infected with F. solani
	Oct. 8	Nodulation scored
		Percentage of plants infected with F. oxysporum
		Percentage of plants infected with F. solani
1975	June 5	Nodulation scored
		Disease severity scored
		Percentage of plants infected with F. oxysporum
		Percentage of plants infected with F. solani
	July 3	Forage yield
	Aug. 13	Plant height
	Sept. 29	Forage yield
	Oct. 8	Disease severity scored
		Percentage of plants infected with F. oxysporum
		Percentage of plants infected with F. solani

Table 1-3. The effect of pod removal and surface-sterilization of seed on the percentage of seed contaminated with either *Alternaria* spp. or *F. oxysporum* from eight commercial seed lots of sainfoin.

Seed lot	Contamination with *Alternaria* spp. 1/			Contamination with *F. oxysporum* 2/		
	PI 4/ Untreated	PI Surface 3/ sterilized	PR 5/ Surface sterilized	PI Untreated	PI Surface sterilized	PR Surface sterilized
	%	%	%	%	%	%
007	98.8	1.3	0.0	0.0	0.0	0.0
403	42.5	2.5	0.0	1.3	0.0	0.0
405	23.8	0.0	0.0	1.3	0.0	0.0
469	42.5	0.0	0.0	1.3	0.0	0.0
509	61.3	1.3	0.0	3.8	0.0	0.0
699	11.3	13.8	0.0	9.8	0.0	0.0
1005	75.0	0.0	0.0	0.0	0.0	0.0
1538	100.0	51.3	4.8	0.0	0.0	0.0

[1]Based on isolations made on HPDA (66).

[2]Based on isolations made on PCNBA agar (57).

[3]Seed surface sterilized by soaking in a 0.5% NaOCl solution for 3 minutes.

[4]Seed pod intact.

[5]Seed pod removal.

removing the pod and surface-sterilization of the seed eliminated this fungus (Table 1-3).

Two percent of the untreated seed were contaminated with F. oxysporum (Table 1-3). Surface-sterilization of the seed pod completely eliminated this fungus. Seed lots responded the same to seed treatments ($X^2 = 3.4$; $P > .995$). F. solani was never isolated from the pod or seed of sainfoin. This pathogen does not appear to be seed transmitted.

When planted in the greenhouse, seedling emergence of the seed lot heavily infested with Alternaria spp. (#1538) was reduced (Table 1-4). The degree of seedling infection was proportional to the amount of Alternaria spp. contamination on the seed. Since both seed lots had very similar germination in the laboratory, the reduction in emergence was probably due to seed rot or pre-emergence damping-off caused by Alternaria spp.

Seedling response was the same in both the pasteurized and autoclaved soils. Sears (66) reported sainfoin losses to Alternaria occurred only in heat treated soils. More information would have been gained if an untreated soil had been used as one of the soil treatments. Surface-sterilizing the pod, removing the pod, and surface-sterilizing the seed reduced the percentage of seedlings infected with Alternaria spp. but did not result in increased seedling emergence (Table 1-5).

Table 1-4. Sainfoin seedling emergence as affected by seed-borne Alternaria spp.

Seed lot	Seedling emergence 24 days %	Seedlings infected with Alternaria spp.[1] %	Seed germination laboratory[2] %
1538	68.8 a [3]	11.7 a	85.8
405	80.3 b	4.7 b	86.0

[1] Based on isolations made on HPDA (66).

[2] Means based on 400 seed.

[3] Means within a column not followed by the same letter differ at the .05 level of probability.

Table 1-5. The effect of pod removal and seed surface-sterilization on the percentage of seedlings infected with Alternaria spp. and percentage of seedling emergence.

Seed treatments	Seedling emergence 24 days %	Seedlings infected with Alternaria spp. [1] %
Pod-intact	79.6	18.8 a [3]
Pod-intact Surface-sterilized [2]	70.4	7.8 b
Pod-removed Surface-sterilized	76.2	1.6 b
Pod-removed	72.2	4.7 b

[1] Based on isolations made on HPDA (66).

[2] Surface-sterilized by soaking in a 0.5% NaOCl solution for three minutes.

[3] Means within the column not followed by the same letter differ at the .05 level of probability.

Experiment II: An evaluation of the relative pathogenicity of microconidia and macroconidia of four isolates of F. solani.

The microconidial inoculum infected a higher percentage of plants than did the macroconidial inoculum, but both inocula produced similar disease symptoms (Table 1-6). Both types of conidia are infectious, but the smaller microconidia may have more readily entered the wounds caused by the inoculation technique. The four isolates of F. solani produced different amounts of macroconidia in the Snyder and Nash's sporulation medium (56) (Table 1-7). The macroconidial inoculum was a composite of both microconidia and macroconidia, and probably did not accurately estimate the performance of an inoculum composed entirely of macroconidia.

Table 1-6. The effect of inoculating sainfoin seedlings with either microconidia or macroconidia of F. solani on the mean disease severity score and the percentage of plants infected.

Inoculum	Mean disease severity score [1]	Plants infected with F. solani [2]
Microconidia	2.05 a [3]	30.0 a
Macroconidia plus microconidia	1.87 a	21.6 b

[1] Disease severity scored as follows: 1 = no spread of discoloration; 2 = spread of discoloration less than 1 cm; 3 = spread of discoloration more than 1 cm; 4 = extensive vascular necrosis; and 5 = dead plant

[2] Based on isolations made on HPDA (66).

[3] Means within a column not followed by the same letter differ at the .05 level of probability.

The isolates caused similar disease symptoms and infected a similar number of seedlings (Table 1-7). Inoculation with any of the isolates resulted in more severe disease symptoms than inoculation with sterile water, but only one isolate infected a higher percentage of the seedlings than that observed in the control. Greater differences in the percentage of plants infected could probably have been detected had a more efficient inoculation procedure been used.

Table 1-7. The effect of inoculating sainfoin seedlings with four isolates of F. solani on the mean disease severity score and the percentage of plants infected. Also shown is the Percentage of microconidia produced in Snyder and Nash's sporulation medium (56).

Isolate	Mean disease severity score[1]	Plants infected with F. solani[2] %	Microconidia %
11	2.25 a [3]	16.7 ab	35
07	2.18 a	16.7 ab	50
06	2.17 a	18.3 a	5
01	1.98 a	11.7 ab	94
Control	1.20 b	1.7 b	0

[1] Disease severity scored as follows: 1 = no spread of discoloration; 2 = spread of discoloration less than 1 cm; 3 = spread of discoloration more than 1 cm; 4 = extensive vascular necrosis; and 5 = dead plant.

[2] Based on isolations made on HPDA (66).

[3] Means within a column not followed by the same letters differ at the .05 level of probability.

In future studies more resolution could be obtained by: 1) increasing the inoculum level from 4×10^6 to 4×10^8 conidia/plant; 2) using the root-cut-soak inoculation technique; 3) including the composited isolates as a treatment; 4) isolating the treatments to reduce contamination; and 5) developing procedures to obtain inoculum composed entirely of macroconidia.

Experiment III: Evaluation of soil fumigation and seed treatments for the control of the root and crown rot of sainfoin

Field fungicide treatments affected only the number of seedlings with wilt symptoms, seedling vigor, stand density, and plant height. The Benlate soil drench caused several emerging seedlings to wilt and reduced both stand density and seedling vigor (Table 1-8). This indicates that sainfoin is sensitive to high rates of Benlate. During the second year plant height differences were observed. Plants grown in the soil treated with Benlate and Chloropicrin were significantly taller than the control. This could be the initial expression of long-term effects. This study should be continued to determine other possible effects of the Benlate and Chloropicrin treatments.

No differences were detected in the isolation of Fusarium spp., disease severity, nodulation, or forage yield. This could have been the result of limited sample size, rapid invasion of the small plots by soil microbes, or lack of sufficient time for effects to be expressed. In future studies, the use of larger plots would allow the