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

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; hulless 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 tom 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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Date March 20, 1974

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Approved:

Head, Major Department

Chairman, Examining Committee

Graduate Dean

MONTANA STATE UNIVERSITY
Bozeman, Montana

June, 1974

ACKNOWLEDGMENTS

I wish to express my sincere appreciation to the following:

Dr. R. L. Ditterline whose guidance and friendship as my major professor were invaluable during the course of study and preparation of this manuscript.

Dr. D. E. Mathre for his assistance in conducting this study.

Dr. C. S. Cooper, Dr. L. E. Wiesner and Dr. E. O. Skogley for serving on my graduate committee.

My wife, Donna, whose endless patience, cooperation and love supported me throughout my graduate work.

The Montana Agricultural Experiment Station for providing me with an assistantship. Without this assistance, graduate school would have been impossible.

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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, digestabilities, 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 contaminates 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 Valleau (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 pimaricin, 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

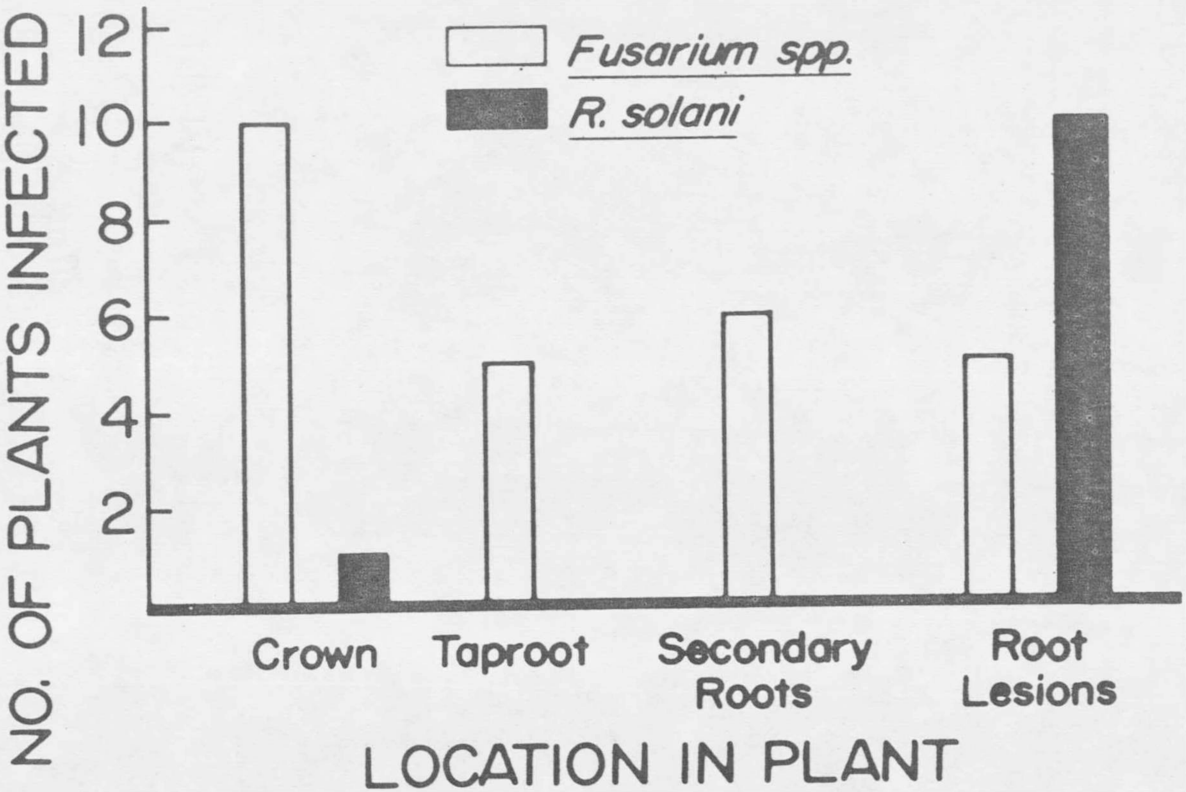

Figure 1: Location and prevalence of Fusarium spp. and Rhizoctonia solani within the roots of 25 sainfoin plants.

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*