

CROWN AND ROOT ROT OF BIRDSFOOT TREFOIL IN ALBERTA

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Abstract

A rot affecting only the internal tissues of the crown and tap root of birdsfoot trefoil (*Lotus corniculatus*) was found in second year and older stands. Associated fungi were isolated and tested for pathogenicity on seedlings. A single causal organism could not be specified, nor was the incidence of the disease affected by the application of fertilizer.

Introduction

Birdsfoot trefoil (*Lotus corniculatus* L.), a recent introduction to Alberta, shows promise as a perennial pasture legume in the higher rainfall areas of central Alberta. However, older experimental stands have been observed to become thin due to dying of plants. Examination of plants revealed discolored and dead centers of the crown, sometimes extending down the tap root. Other forage legumes in this and other areas have shown similar symptoms (4, 6, 7, 8, 9). Symptoms of crown and root rot were distinctly different from winter crown rot (5) or crown bud rot (3) in being usually internal and in not attacking buds in the spring. The center of the crown of affected plants was dark brown and the discoloration extended varying distances down the tap root (Figure 1). Observations in the field did not reveal any foliage symptoms, such as the chlorosis resulting from infection by *Plenodomus meliloti* Dearn & Sanford (1). In the spring, plants with rotted crowns and dead plants were found but plants that survived the winter appeared to grow as well as those without internal discoloration. An examination of fungi associated with the crown and root of affected plants was undertaken in 1969.

Materials and methods

In the fall of 1968, second year trefoil plants grown in a fertilizer test were examined for root rot by splitting the roots. Isolations were made from living trefoil plants dug from the field, washed with tap water and dissected. Samples were removed with a flamed scalpel and forceps from the margin of the discolored area from roots of plants showing internal symptoms and were plated on potato-dextrose agar (PDA) directly or after the surface was sterilized with sodium hypochlorite solution (commercial Javex diluted to 10%). Isolations were made without surface sterilization in the fall of 1969 and 1970, and the spring of 1970.

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Figure 1. Symptoms of crown and root rot of birdsfoot trefoil; internal discoloration in a 4 year-old-plant in the spring.

Selected fungi and bacteria commonly isolated from the diseased roots were tested for pathogenicity using a water suspension of colonies produced on PDA. The inoculum from each petri plate culture was mixed with sterilized sand and placed in four 300 ml plastic cups with sufficient distilled water to saturate the sand. Fifty surface sterilized seeds of birdsfoot trefoil cv. Leo were placed on the sand in each cup and covered with a 1-cm layer of dry sterile sand, and the cups covered with petri dishes.

Four replicates for each inoculum were incubated in a greenhouse for 13 days. The seedlings were then removed, counted, and examined for lesions with a dissecting microscope.

Fifty seeds from eight sources were also examined for seed-borne pathogens by plating directly, or after surface sterilizing by wetting samples with 70% ethanol and then treating with sodium hypochlorite solution. The seed samples were plated on both HDA and water agar.

Results and discussion

Of the 535 roots examined from the 1968 fertilizer test 340 roots (63%) showed disease symptoms. No differences in severity of symptoms were found among fertilizer treatments. Organisms isolated from 36 surface sterilized samples from these diseased roots showed no differences from those isolated from unsterilized samples removed aseptically from the same roots. Isolations made from diseased roots in the fall of 1969 and 1970 and the spring of 1970 were from unsterilized samples.

Frequency of isolation of fungi from 219 diseased trefoil roots is shown in Table 1. Similar numbers of the various fungi were found at each sampling date except for *Gliocladium* sp., which was rarely found in the spring sample. *Rhizoctonia* sp., commonly reported in association with legume root rots, was not found nor was *Mycleptodiscus terrestris* (Gerd.) Ost., a common pathogen of trefoil and other legume roots in the southern United States (7).

Isolates were tested for pathogenicity to seedlings and the results are shown in Table 2. Two fusaria differing in pigmentation in culture were labelled (pink) and (orange).

Table 1. Bacteria and fungi isolated from roots of birdsfoot trefoil affected by crown and root rot

Isolate	No. of isolates
Bacteria	79
<i>Gliocladium</i> sp.	56
<i>Stemphylium loti</i>	22
<i>Fusarium</i> sp.	15
<i>Phoma</i> sp.	6
<i>Rhizopus</i> sp.	3
<i>Alternaria</i> sp.	2
<i>Papulaspora</i> sp.	2
<i>Penicillium</i> sp.	2
<i>Pyrenochaeta</i> sp.	1
Unidentified fungi	46
Total	234

Two isolates of bacteria were also tested, a yellow colony and a white colony. *Stemphylium* sp., a common foliar pathogen, was pathogenic on seedlings. *Fusarium* (pink) caused the greatest reduction in emergence. In Ontario *Fusarium solani* (Mart.) App. & Wr. has been reported from diseased basal parts of birdsfoot trefoil (2). *Pyrenochaeta* sp. was a weak pathogen on birdsfoot trefoil seedlings. In Manitoba *Pyrenochaeta terrestris* (Hansen) Gorenz, Walker & Larson has been associated with crown and root rot of alfalfa (6).

Table 2. Effect of bacteria and fungi isolated from birdsfoot trefoil roots on emergence and seedling injury

Isolate	% reduction in emergence	% surviving seedlings lesioned	Total % injured plants
<i>Fusarium</i> (pink)	100	0	100
<i>Stemphylium loti</i>	27	67	94
<i>Fusarium</i> (orange)	51	15	66
<i>Papulaspora</i>	34	13	47
Bacteria (yellow)	20	11	31
<i>Gliocladium</i>	14	8	22
<i>Pyrenochaeta</i>	4	2	6
Bacteria (white)	0	5	5

The testing of seedlings gives only an indication of the possible pathogenicity of various isolates on mature plants, but the difficulties involved in producing axenic mature plants precluded such tests. The isolates were not tested in combinations which may occur in the field. Also the disease may be compounded by winter injury since no root rot was observed in first year plantings.

In the test for seed-borne root pathogens, common contaminants such as Alternaria sp. and Penicillium sp., were found. Only one seed in 800 yielded a suspected root pathogen, Papulaspora sp. Apparently the more pathogenic isolates from roots are not commonly seed-borne.

The study indicated that birdsfoot trefoil, in common with other forage legumes, was affected by a crown and root degeneration which caused reductions in stand and longevity. Several fungi found in association with the disease were shown to be pathogenic on seedlings. Mature plants were not inoculated due to the questionable validity of field inoculations, and to difficulties in producing mature axenic plants. The cause of the disease could not be attributed to a single pathogen or to the influences of climate or cultural practice.

Literature cited

1. Berkenkamp, B., and H. Baenziger. 1968. The reaction of sweet clover varieties to brown root rot. *Can. J. Plant Sci.* 49: 181-183.
2. Gordon, W. L. 1959. The occurrence of Fusarium species in Canada. VI. Taxonomy and geographic distribution of Fusarium species on plants, insects and fungi. *Can. J. Bot.* 37:257-290.
3. Hawn, E. J., and M. V. Cormack. 1952. Crown bud rot of alfalfa. *Phytopathology* 42:510-511.
4. Jones, F. R. 1928. Winter injury of alfalfa. *J. Agr. Res.* 37:189-211.
5. Lebeau, J. B. 1966. Pathology of winter injured grasses and legumes in western Canada. *Crop Sci.* 6:23-25.
6. McDonald, W. C. 1955. The distribution and pathogenicity of the fungi associated with crown and root rotting of alfalfa in Manitoba. *Can. J. Agr. Sci.* 35:309-321.
7. Petit, R. E., O. H. Calvert, and J. D. Baldrige. 1969. Pathogenicity and virulence of Mycoleptodscus terrestris to birdsfoot trefoil. *Phytopathology* 59:1203-1208.
8. Weimer, J., L. 1927. Observations on some alfalfa root troubles. *U. S. Dep. Agr. Circ.* 425.
9. Willis, C. B. 1966. Internal breakdown of red clover in Prince Edward Island. *Can. Plant Dis. Surv.* 46:83-84.