Myrothecium roridum and *M. verrucaria* Pathogenic to Roots of Red Clover and Alfalfa

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ABSTRACT

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Myrothecium roridum and *M. verrucaria* were associated with rot sites on roots of field-grown red clover and alfalfa. Both species of *Myrothecium* caused root rot of red clover and alfalfa in controlled inoculations with or without wounding. No differences in cultivar responses were detected. Chlorosis, purpling of leaflet margins, and death of leaves and petioles occurred on inoculated plants but *Myrothecium* spp. could only be recovered from diseased roots. These fungi should be considered as additional causal agents in the root-rot complex of red clover and alfalfa.

Additional key words: *Medicago sativa*

Myrothecium roridum Tode ex Fr. and *M. verrucaria* (Alb. & Schw.) Ditm. ex Fr. are pathogens of several plant species (1–7,9–12,17,19–21,23–27). Although Wensley (26) implicated *M. verrucaria* in a transplant problem of peaches, these soilborne fungi generally attack aboveground plant organs. Both species caused leaf spot of red clover in artificial inoculations (7), and *M. verrucaria* caused leaf spot on birdsfoot trefoil under natural conditions (3). Pathogenicity of *M. verrucaria* isolated from red clover seed in Finland (22) was not determined.

We have frequently isolated both species of *Myrothecium* from diseased roots of field-grown red clover and alfalfa. We have considered them to be saprophytic or secondary invaders without testing them for pathogenicity. Isolation of *M. roridum* as the only fungus associated with a rot site on red clover prompted us to evaluate both species of *Myrothecium* for pathogenicity to red clover and alfalfa. A portion of this research has already been reported (15).

MATERIALS AND METHODS

Both isolates of *M. verrucaria* were from red clover in Wisconsin. *M. roridum* was isolated in Pennsylvania: isolate 1 from *Lotus corniculatus* L., isolate 2 from *Trifolium tembense* Fres., and isolate 3 from *Medicago sativa* L. *M. verrucaria* and *M. roridum* were evaluated on Saranac-AR alfalfa and Kenland red clover grown on slant-boards or in pots. Inoculum consisted of hyphae and conidia grown on autoclaved polyester cloth pieces (1 cm^2) placed on the surface of vegetable-juice agar (18) as described previously (14,16). For one experiment, inoculum was grown on washed, autoclaved, cotton threads 1 cm long by 1 mm wide to reduce inoculum load. In another experiment, to avoid phytotoxic effects of sporodochial fluid (8), autoclaved cloth squares were dipped in an aqueous suspension of washed conidia (150,000 spores per milliliter) and used as inoculum. All fungi were cultured at 21 ± 1 C with 50 $\mu E/m^{-2}/sec^{-1}$ continuous cool-white fluorescent light.

Use of plants grown in slant-board nutrient solution culture to evaluate pathogenicity of root-rotting fungi was described previously (13,14). Plants were 4 wk old when inoculated, and individual roots were inoculated by placing cloth squares or cotton threads against the roots (2 cm above the tips). Either one or two replicates were made on a single plant, depending on available roots. An autoclaved cloth square or thread without fungus served as a control. Three experiments were done to provide 30 red clover and 36 alfalfa roots inoculated with each isolate. Evaluations of rot symptoms and root elongation were made 6 days after inoculation. Slant-board cultures were maintained in a growth chamber with a 14-hr photoperiod (fluorescent and incandescent light at 300 $\mu E/m^{-2}/sec^{-1}$) at 25 ± 1 C and a 10-hr dark period at 15 ± 1 C.

The severed-taproot pathogenicity test for root-rotting fungi was described previously (14,16). Plants of Arlington, Florie, Pennscott, and Redland red clover plus Conestoga, Saranac-AR, and Vernal alfalfa were grown for 7 mo in commercial peat-vermiculite potting mix in 10-cm-diameter clay pots in a greenhouse. Five plants per cultivar per treatment were used. Inoculation consisted of placing a square of polyester cloth bearing a test fungus against the cut surface of a taproot severed 5 cm below the crown. Inoculated plants were repotted and randomized on a greenhouse bench. Untreated plants and plants with severed taproots and autoclaved cloth squares served as controls. The crown and subtending taproot segment were split longitudinally and vertical rot was measured with a ruler 1 mo after inoculation. Plants were observed daily for development of leaf or stem symptoms.

RESULTS AND DISCUSSION

In the slant-board evaluation, *M. roridum* and *M. verrucaria* caused brown, water-soaked rots with poorly delineated margins visible 3 days after inoculation on both red clover and alfalfa. Test fungi were recovered from brown areas 0.5 cm above inoculation sites; Koch's postulates were fulfilled. Chlorosis, purpling of leaflet margins, and death of leaves and petioles occurred

Table 1. Length of rot and root elongation of red clover and alfalfa inoculated with two species of *Myrothecium* 6 days earlier

	Red clover		Alfalfa	
Treatment	Rot (mm)	Root (mm)	Rot (mm)	Root (mm)
Untreated control	0 a[z]	133 a	0 a	163 a
M. verrucaria (isolate 1)	13 b	61 b	19 b	36 b
M. verrucaria (isolate 2)	16 b	56 bc	22 b	35 b
M. roridum (isolate 1)	36 c	24 d	28 b	30 b
M. roridum (isolate 2)	28 c	36 cd	31 b	31 b
M. roridum (isolate 3)	35 c	27 d	26 b	32 b

[z]Numbers within a column followed by the same letter are not significantly different at $P = 0.05$ as determined by analysis of variance, LSD.

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Table 2. Length of rot in taproots of red clover and alfalfa plants 1 mo after inoculation with two species of *Myrothecium*

Treatment	Length of rot (mm)	
	Red clover	Alfalfa
Untreated control	0.0 a[z]	0.0 a
Severed-root control	1.0 a	0.9 a
M. verrucaria (isolate 1)	8.9 b	7.7 b
M. verrucaria (isolate 2)	9.8 b	6.7 b
M. roridum (isolate 1)	11.9 cd	6.4 b
M. roridum (isolate 2)	10.2 bc	6.4 b
M. roridum (isolate 3)	12.2 d	5.9 b

[z]Numbers within a column followed by the same letter are not significantly different at $P = 0.05$ as determined by analysis of variance, LSD.

frequently on inoculated plants. Foliar symptoms were evident as early as 5 days after inoculation. Neither *Myrothecium* species nor any other fungi could be isolated from affected petioles or leaves.

All isolates caused rot and severe inhibition of root growth in both hosts (Table 1). Significant isolate × cultivar interactions did not occur; therefore, data were pooled for each host species. Root inhibition and rot did not occur in the controls. Pathogenicity of fungi differed significantly in clover but not in alfalfa.

In greenhouse evaluations, rots caused by all fungi were similar. Rots were typically dark brown and occurred across entire roots, with occasional streaks extending upward in the vascular system. Rotted root portions remained firm and were not water-soaked. Foliar symptoms included severe stunting in addition to leaf and petiole symptoms as described for slant-board evaluations. Significant isolate × cultivar interactions did not occur; data were pooled and summarized (Table 2). Rot was significantly greater from inoculations with any isolate of *Myrothecium* than in the controls. Rots caused by the various isolates differed significantly in red clover but not in alfalfa. *Myrothecium* isolates were recovered from rotted root tissue but not from symptomatic leaf or petiole tissue.

With both evaluation techniques, inoculations of red clover with washed conidia produced symptoms typical of those obtained with the standard fungal inoculum. Hence, disease symptoms did not result from toxic sporodochial fluid but more likely from infection of roots and production of toxins in vivo. Failure to isolate either species of *Myrothecium* from symptomatic leaves and petioles also supports this conclusion. Inoculation of roots with fungi carried on narrow threads caused rots similar to but smaller than rots caused by fungi carried on larger cloth squares. Therefore, we concluded that pathogenicity was not merely a function of inoculum quantity.

Based on this evidence, *M. roridum* and *M. verrucaria* must be considered root pathogens of red clover and alfalfa. Although reported to be favored by wounds in some situations (19), these fungi entered intact roots of 1-mo-old plants. They are capable of being primary pathogens rather than merely secondary wound parasites and should be recognized as additional causal agents in the root-rot complex of red clover and alfalfa.

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