Forage Legume Hosts of Races 1 and 2 of *Colletotrichum trifolii*

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ABSTRACT

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Seedlings of 14 species of forage legumes were inoculated in the greenhouse with conidia of race 1 or 2 of *Colletotrichum trifolii*. The study identified certain cultivars of *Medicago sativa, Trifolium pratensis, T. incarnatum, T. subterraneum,* and *Melilotus alba* as hosts for both races and extended the host range to include *Coronilla varia* cv. Chemung. Race 1 was more virulent than race 2 on *T. dubium* and *T. subterraneum* and less virulent than race 2 on *T. incarnatum* and *Medicago sativa* cv. Saranac AR. *Medicago sativa* cv. Arc was confirmed to be resistant to race 1 and susceptible to race 2 of *C. trifolii*.

Additional key words: *Lespedeza cuneata, Lotus corniculatus, Trifolium hybridum, T. repens, T. resupinatum, T. vesiculosum, Vicia villosa*

Anthracnose, caused by *Colletotrichum trifolii* Bain, occurs sporadically throughout the warm, humid areas of the world (1,5) and limits yield and persistence of alfalfa (*Medicago sativa* L.) (3) and red clover (*Trifolium pratense* L.) (7). Procedures have been developed to screen for resistance to *C. trifolii* (2,4,7,8,11), and cultivars resistant to the pathogen are available. In addition to alfalfa and red clover, *C. trifolii* is known to infect sweet clover (*Melilotus alba* Desr.) (6,7), subterranean clover (*T. subterraneum* L.), crimson clover (*T. incarnatum* L.), and bur clover (*Medicago hispida* Gaertn.) (7). These host range studies were done before physiologic races of *C. trifolii* were known.

The objective of this study was to determine the reaction of several forage legumes to races 1 and 2 of *C. trifolii*. This is important for determining sources of inoculum and potential variation in pathogenicity.

MATERIALS AND METHODS

Scarified seeds were planted in 10-cm-diameter pots (experiment 1 and 2) or wooden flats 50 × 38 × 7 cm (experiment 3) in a 1:1 mixture (v/v) of pasteurized loam soil or Metromix-200 (Florist Products, Inc., 780 W. Oakton St., Des Plains, IL 60018) and sand. Plants were watered and fertilized to maintain vigorous growth in a greenhouse at 18–30 C. In experiments 1 and 2, plants were thinned to 10 seedlings per pot; in experiment 3, 25 seeds were planted in furrows 6 mm deep and 2 cm apart and covered with pasteurized sand.

Experiments 1 and 2 were arranged in a randomized complete block with two replicates. Experiment 3 was arranged in a split plot with six replicates, with the whole plot (a flat) being race of the pathogen and the subplot the legume species. Analysis of variance and means separation using Duncan's new multiple range test (10) were performed for the data from experiment 3; data for controls were not analyzed.

Isolates of *C. trifolii* were cultured from diseased alfalfa. The sources of race 1 (isolate PA) and race 2 (isolate NC-4) were previously described (14). Both races were maintained on potato-dextrose agar at 3–4 C. Conidia harvested from lima bean agar cultures incubated 7 days at room temperature were suspended in distilled water supplemented with Tween 20 and orange juice (14) and adjusted to 1×10^6 conidia per milliliter using a hemacytometer. Control plants were sprayed with distilled water supplemented with Tween-20 and orange juice.

In the first study, seedlings of *Medicago sativa* cv. Saranac, *M. sativa* cv. Arc, *M. sativa* cv. Saranac AR, *Melilotus alba* cv. Floranna, *Coronilla varia* L. cv. Chemung, *Vicia villosa* Roth (common cultivar), *Lespedeza cuneata* (Dumont) G. Don. (common cultivar), *Lotus corniculatus* L. cv. Viking, *T. pratense* cv. Kenland, *T. subterraneum* cv. Mt. Barker, *T. resupinatum* L. cv. Abon, *T. dubium* Sibth. (common cultivar), *T. hybridum* L. (common cultivar), *T. repens* L. cv. Tillman, *T. incarnatum* (common cultivar), and *T. vesiculosum* L. cv. Yuchi were inoculated when the first trifoliolate leaf was almost fully expanded (2–3 wk old). In the second study, seedlings of the same 14 species were inoculated when about five true leaves had emerged and expanded (7 wk old). In the third study, seedlings from 10 of these 14 species were inoculated when the first trifoliolate leaf was expanded (3 wk old).

Seedlings were sprayed with the conidial suspension until runoff. Plants were placed in a chamber maintained at 18–33 C for 3 days, and the foliage was kept wet by intermittent misting. Plants were observed 8, 12, 20, and 30 days after inoculation in experiments 1 and 2 and after 18 and 27 days in experiment 3. Symptoms were recorded for each host and race at each interval. Survival percentages based on stand count were calculated on the last day of observation.

When inoculated leaves were shed, stems collapsed, and seedlings died during the three experiments, tissue was collected, surface sterilized in 1% sodium hypochlorite for 1–2 min, and either incubated for 1–2 days in a moist chamber or cultured on a variety of laboratory media.

RESULTS AND DISCUSSION

Disease reaction in the first study was variable (Table 1). Symptoms ranged from hypersensitive flecking on leaves and petioles to seedling death. Defoliation in plants that survived ranged from slight to severe, and seedlings were often stunted. In a few species, only inoculated foliage became infected, and new growth was free of symptoms. Because symptoms varied according to host, disease reaction of each species was placed in one of five categories (Table 1): hypersensitive flecking (H), leaf lesions (LL), stem lesions (SL), stem collapse (SC), and death (D).

Race 1 or 2 killed some seedlings of 10 of the 14 species inoculated. Survival ranged from 5 to 100% (Table 1). Symptoms varied on other species from hypersensitive flecking to leaf lesions. Host reactions to each race were similar in range of foliar symptoms, but some differed markedly in the number of seedlings surviving.

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Reactions of older plants in the second study were generally less severe than those of younger plants. Lesions were often smaller, and more time was required for the inoculated plants to die. Hosts that reacted similarly to inoculation with both races at both ages included *Medicago sativa* cv. Saranac, *M. sativa* cv. Arc, *M. sativa* cv. Saranac AR, *T. incarnatum, T. dubium, Coronilla varia* cv. Chemung, *T. vesiculosum* cv. Yuchi, *T. repens* cv. Tillman, *T. resupinatum* cv. Abon, and *Lotus corniculatus* cv. Viking. Hosts that had less severe disease symptoms when older were *V. villosa, Lespedeza cuneata, T. subterraneum* cv. Mt. Barker, *T. hybridum*, and *T. pratensis* cv. Kenland. None of the plants of these last five species were killed when inoculated at 7 wk with either race, but some died when inoculated as 3-wk-old seedlings. No species was found for either race in which inoculated 3-wk-old seedlings survived and inoculated 7-wk-old seedlings died.

Inoculations with conidia of race 1 or 2 were repeated (experiment 3) with 3-wk-old seedlings of 10 species in which *C. trifolii* had killed seedlings in the previous study, including two cultivars of *Medicago sativa* (Table 2). *Coronilla varia* cv. Chemung and *Melilotus alba* cv. Floranna were highly susceptible to both races; *Medicago sativa* cv. Arc was highly susceptible to race 2. The other species or cultivars comprised several statistically overlapping groups depending upon the race used as inoculum. The analysis of variance for the whole plot (race) and split plot (host) showed a significant difference among hosts ($P = 0.01$) and a significant interaction between race and host ($P = 0.01$), but no significant difference between races ($P = 0.05$). This interaction was the result of the differential reaction of *M. sativa* cv. Arc to races 1 and 2, because Arc is used to distinguish these races (9,14).

Despite the absence of other significant interactions, more seedlings were killed by race 2 than race 1, with these differences most apparent on *Lespedeza cuneata, Medicago sativa* cv. Saranac AR, and *T. incarnatum*. Fewer seedlings of *T. subterraneum* cv. Mt. Barker and *T. dubium* survived inoculation with race 1 than race 2. The percentage of seedlings of *T. pratensis* cv. Kenland surviving inoculation with either race was 84% or higher because of the anthracnose resistance to *C. trifolii* in cultivar Kenland. Both races killed only a few seedlings of *T. hybridum* and *T. resupinatum* cv. Abon, and survival rate was 89% or higher.

The forage species included in these tests are cross-pollinated. When they were inoculated with both races of *C. trifolii*, seedling survival ranged from 2 to 99% (Table 2). When alfalfa populations are evaluated for resistance to *C. trifolii*, seedling survival (ie, resistant plants) is often used to designate levels of resistance. Because of their heterogeneous nature, alfalfa cultivars with seedling survival of 0–5% were considered susceptible to *C. trifolii*, those with 6–14% had low resistance, 15–30% had moderate resistance, 31–50% were considered resistant, and those with 51% and above seedling survival had high resistance to *C. trifolii*. Using this scheme for experiment 3, all species except *Coronilla varia* cv. Chemung, *Melilotus alba* cv. Floranna, and *Medicago sativa* cv. Arc were resistant or highly resistant to both races of *C. trifolii*.

In every instance, when foliage was either cultured or incubated, conidia and setae typical of *C. trifolii* (12) developed from each legume species inoculated. *C. trifolii* was not obtained from tissue of

Table 1. Foliar symptoms and survival of 3-wk-old forage legumes inoculated with race 1 or 2 of *Colletotrichum trifolii*

Species	Race 1		Race 2	
	Foliar symptoms[a]	Survival (%)[b]	Foliar symptoms	Survival (%)
Medicago sativa cv. Saranac	H, LL, SL, SC, D	5	H, LL, SL, SC, D	10
M. sativa cv. Arc	H, LL, SL, SC, D	75	H, LL, SL, SC, D	15
M. sativa cv. Saranac AR	H, LL, SL, SC, D	60	H, LL, SL, SC, D	65
Melilotus alba cv. Floranna	H, LL, SL, SC, D	10	H, LL, SL, SC, D	5
Coronilla varia cv. Chemung	H, LL, SL, SC, D	25	H, LL, SL, SC, D	10
Vicia villosa	H, LL, SL, SC, D	50	H, LL, SL, SC, D	50
Lespedeza cuneata	H, LL, SL, SC, D	85	H, LL, SL, SC, D	55
Trifolium incarnatum	H, LL, SL, SC, D	50	H, LL, SL, SC, D	65
T. subterraneum cv. Mt. Barker	H, LL, SL, SC, D	70	H, LL, SL, SC, D	35
T. pratensis cv. Kenland	H. LL, SL, SC, D	95	H, LL, SL, SC, D	65
T. dubium	H, LL, SL, SC, D	85	H, LL, SL, SC, D	85
T. hybridum	H, LL, SL, SC, D	85	H, LL, SL, SC, D	85
T. vesiculosum cv. Yuchi	H, LL	100	H, LL	100
T. repens cv. Tillman	H, LL	100	H, LL	100
T. resupinatum cv. Abon	H, LL	100	H, LL	100
Lotus corniculatus cv. Viking	H	100	H	100

[a] H = hypersensitive flecking, LL = leaf lesions with sporulation, SL = diamond-shaped stem lesions with sporulation, SC = stem collapse, D = seedling death.
[b] Based on inoculation of 10 plants in each of two replicates of each cultivar.

Table 2. Survival of 3-wk-old forage legumes inoculated with race 1 or 2 of *Colletotrichum trifolii*

Species[a]	Race 1		Race 2		Uninoculated	
	No. survived/ inoculated	Survival (%)[b]	No. survived/ inoculated	Survival (%)	No. survived/ inoculated	Survival (%)
Medicago sativa cv. Arc	108/141	76 cd	3/141	2 e	132/137	96
M. sativa cv. Saranac AR	96/146	66 de	80/146	55 d	146/146	100
Melilotus alba cv. Floranna	3/142	2 f	7/147	5 e	139/143	97
Coronilla varia cv. Chemung	10/144	8 f	1/132	1 e	124/125	98
Lespedeza cuneata	137/139	99 a	114/143	79 bc	132/137	96
Trifolium incarnatum	70/109	64 de	62/122	54 d	113/114	99
T. subterranean cv. Mt. Barker	41/74	58 de	53/74	73 c	64/64	100
T. pratensis cv. Kenland	130/149	87 abc	126/150	84 abc	150/150	100
T. dubium	122/147	83 bc	136/150	91 ab	146/146	100
T. hybridum	134/143	94 ab	139/148	94 a	148/149	99
T. resupinatum cv. Abon	132/144	92 ab	133/149	89 ab	145/147	99

[a] Six replicates, 25 seeds planted per replicate; survival determined 4 wk after inoculation.
[b] Entry numbers within a column followed by the same letters are not significantly different ($P = 0.05$) using Duncan's new multiple range test.

uninoculated controls.

This study extends the host range of *C. trifolii* to include *Coronilla varia* cv. Chemung, confirms other species as hosts (6,7), and provides quantitative evidence of the number of susceptible seedlings in populations of *T. incarnatum, Melilotus alba* cv. Floranna, and *T. subterraneum* cv. Mt. Barker. Also, increasing resistance to *C. trifolii* in *Coronilla varia* cv. Chemung and *Melilotus alba* cv. Floranna by recurrent selection may be possible. The importance of seedling blight on these hosts in the field is not known; however, *C. trifolii* may not only thin stands but infect volunteers from seed of these hosts, providing enough inoculum for natural outbreaks of the disease in *Medicago sativa* cvs. Saranac, Arc, and Saranac AR, *Melilotus alba* cv. Floranna, *T. pratensis* cv. Kenland, or *Coronilla varia* cv. Chemung.

Several species contained different percentages of plants susceptible to either race 1 or 2, but this is not considered support for the preliminary suggestion (13) that physiologic specialization may exist in *C. trifolii* for legume species. It seems more logical to conclude that race 2 developed or increased in response to resistance to *C. trifolii* in Arc and was not already present as a virulent pathogen on one or more of the other legume species evaluated in this study.

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