Blue Lupine as a Host for *Colletotrichum trifolii* from Alfalfa and for *C. fragariae* from Strawberry

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ABSTRACT

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Seedlings of blue lupine (*Lupinus angustifolius*) were inoculated in the greenhouse with race 1 or 2 of *Colletotrichum trifolii* or with one of three isolates of *C. fragariae* from North Carolina, Louisiana, or Florida. In a disease severity index (DSI) of 1–5, blue lupine was highly susceptible to race 1 or 2 of *C. trifolii* (DSI 4.3 and 4.9, respectively) and the three isolates of *C. fragariae* (DSI 4.5–4.9). Alfalfa (*Medicago sativa*) cultivars Saranac AR and Arc were also inoculated with the five isolates of *Colletotrichum*. The DSI of Saranac AR and Arc inoculated with *C. fragariae* ranged from 1.1 to 1.8. Saranac AR was resistant to races 1 and 2 of *C. trifolii* (DSI 2.3 and 3.4, respectively). Arc was resistant to race 1 (DSI 2.4) and susceptible to race 2 (DSI 4.9) of *C. trifolii*. The host range of *C. trifolii* and *C. fragariae* was extended to include blue lupine.

The type disease anthracnose is caused by numerous species of *Colletotrichum* on a wide range of plant species and is especially destructive in warm, humid climates (2). When the perfect stage is found, it is usually a species of *Glomerella* (12).

Blue lupine (*Lupinus angustifolius* L.) is an annual winter forage grown in the southeastern United States for soil improvement, spring grazing, or silage (5). Because it is susceptible to anthracnose (*Glomerella cingulata* Stonem. & Schr.) (13), blue lupine can serve as an overwintering host for *G. cingulata* and be a source of inoculum for serious outbreaks of anthracnose in peaches (7).

In cross-inoculation studies with *Colletotrichum acutatum* Simmds. f. sp. *pinea* (4), primary hosts include blue lupine, tree lupine (*L. arboreus* L.), and four species of pine (*Pinus radiata* D. Dom., *P. contorta* Dougl., *P. elliottii* Engelm., and *P. pinaster* Ait.). The list of hosts for this species includes strawberry (*Fragaria* × *ananassa*). *C. acutatum* induces a serious fruit rot of strawberry in Australia (8,11).

This article describes the results of inoculating blue lupine seedlings with race 1 or 2 of *C. trifolii* Bain from alfalfa and three isolates of *C. fragariae* Brooks from strawberry.

MATERIALS AND METHODS

Plant material. Scarified seeds were planted in 10-cm-diameter pots containing Metromix-200 (Florist Products, Inc., 780 W. Oakton Street, Des Plains, IL 60018). Plants were watered and fertilized to maintain vigorous growth in a greenhouse at 18–30 C and thinned to 10 seedlings per pot 7–10 days after seeding. Seedlings of *Lupinus angustifolius, Medicago sativa* L. cv. Saranac AR, and *M. sativa* cv. Arc were inoculated when the first true leaf on the alfalfa plants was almost fully expanded (about 3 wk old).

Inoculum. Isolates of *C. trifolii* were cultured from diseased alfalfa. The sources of race 1 (isolate PA, ATCC 42874) and race 2 (isolate NC-4, ATCC 42041) were described previously (15). Isolates of *C. fragariae* (CF 1, Louisiana; CF-4, North Carolina; and CF-7, Florida) were cultured from diseased strawberry plants.

All isolates were maintained on potato-dextrose agar at 3–4 C. Inoculum was increased on lima bean agar incubated 5–7 days at room temperature. Conidia of both races of *C. trifolii* for both tests were harvested and suspended in distilled water supplemented with two drops of Tween 20 and 30 ml of freshly squeezed, filtered orange juice and adjusted to 1×10^6 conidia per milliliter. Because *C. fragariae* (isolates CF-4 and CF-7) grew poorly and had limited sporulation in the first test, inoculum from these isolates was prepared by comminuting one petri dish culture of each isolate with 500 ml of sterile distilled water in a blender and filtering through two layers of cheesecloth. The filtrates were used as inoculum. In test 2, the three isolates of *C. fragariae* grew well and had abundant sporulation; inoculum was prepared as described for *C. trifolii*. Control plants in test 1 were sprayed with Tween 20 and orange juice without conidia or with comminuted lima bean agar without mycelium. Control plants in test 2 were sprayed with Tween 20 and orange juice without conidia.

Inoculation. Seedlings in both tests were sprayed with inoculum until they were wet and dripping. Plants were placed in a mist chamber maintained at 19–20 C for 3 days; the foliage was kept wet by intermittent misting. Plants were returned to the greenhouse bench and observed at 2- to 3-day intervals and symptoms were recorded 10 and 20 days after inoculation. The tests were arranged on the greenhouse bench in a randomized complete block with five replicates. An analysis of variance and means separation using Duncan's new multiple range were performed (10) on the disease severities.

Disease severity. Alfalfa seedlings were rated for disease on a severity scale of 1–5 (3,6), where 1 = no lesions or only hypersensitive flecking; 2 = small nonsporulating lesions; 3 = typical diamond-shaped lesions not girdling the stem, but with sporulation and setae in the acervuli; 4 = stem-girdling lesions with sporulation, but with new shoots originating from lower axillary buds; and 5 = dead plant. Blue lupine seedlings were rated for disease on a slightly modified severity scale, where 1 = no lesions, hypersensitive flecking on the stem or slight necrosis on cotyledonary scar; 2 = small nonsporulating lesions, necrosis on the cotyledonary scar; 3 = elongating lesions not girdling the stem, but sporulating; 4 = stem-girdling lesions with sporulation, collapsing stems; and 5 = dead plant. The description of each disease class was slightly different because of growth characteristics of the host; however, scales with the same number were equivalent. The disease severity index (DSI) is the calculated

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average of the disease severity scores. Plants resistant to each isolate were evaluated on the basis of the percentage of total plants rated 1 or 2 from the total inoculated.

Measurements of conidia. Three stems of blue lupine with disease symptoms (classes 3–5) from each of four replicates were collected from the inoculated plants (total 12 stems per isolate). Stems were surface-sterilized with 1% sodium hypochlorite for 1 min, rinsed with sterile distilled water, and incubated 48–72 hr at room temperature on sterile filter paper kept moist with sterile distilled water. Conidia were mounted in distilled water and the length and width of 100 spores per stem section were measured at ×160. When sporulation was adequate, 1,200 conidia from each isolate used for inoculation were measured.

RESULTS AND DISCUSSION

Necrosis developed 10 days after inoculation, and blue lupine and alfalfa seedlings could be separated into distinct disease classes (Table 1). Necrosis continued and diseased plants were easily separated on the scale of 1–5 by 20 days after inoculation. The disease was most severe in the second test for seedlings scored 20 days after inoculation. In this test, blue lupine was highly susceptible to race 1 or 2 of *C. trifolii* (DSI 4.3 and 4.9, respectively) and to the three isolates of *C. fragariae* (DSI 4.5–4.9). Necrosis developed in some of the seedlings of alfalfa cultivar Saranac AR and Arc inoculated with *C. fragariae*; susceptible seedlings died and the DSI ranged from 1.1 to 1.8. Saranac AR was resistant to both races of *C. trifolii* (DSI 2.3 and 3.4 for races 1 and 2, respectively); Arc was resistant to race 1 (DSI 2.4) and susceptible to race 2 (DSI 4.9). The reaction of Saranac AR and Arc to both

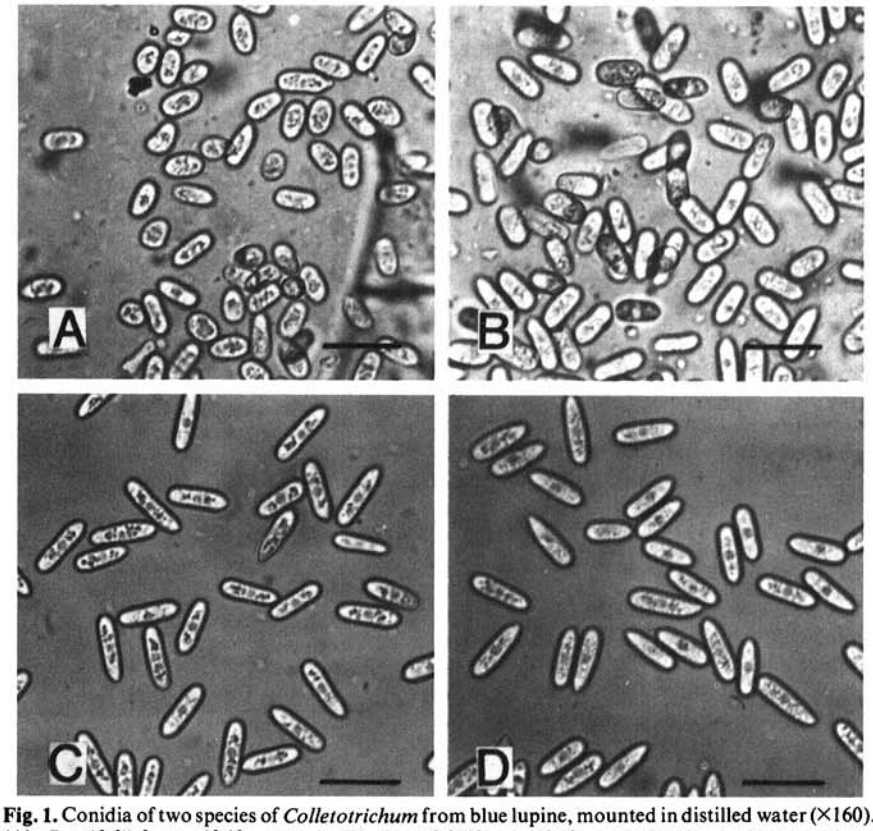

Fig. 1. Conidia of two species of *Colletotrichum* from blue lupine, mounted in distilled water (×160). **(A)** *C. trifolii* from alfalfa, race 1; **(B)** *C. trifolii* from alfalfa, race 2; **(C)** *C. fragariae* from strawberry, North Carolina; and **(D)** *C. fragariae* from strawberry, Florida. Scale bars = 10 μm.

Table 1. Response of seedlings of blue lupine and two cultivars of alfalfa to inoculation in the greenhouse with race 1 (PA) or race 2 (NC-4) of *Colletotrichum trifolii* or one of three isolates of *C. fragariae* from strawberry[a]

	Test 1						Test 2					
			Alfalfa						Alfalfa			
	Blue Lupine		Saranac AR		Arc		Blue Lupine		Saranac AR		Arc	
Incubation time and inoculum treatment	DSI[b]	Resistant[c] (%)	DSI	Resistant (%)	DSI	Resistant (%)	DSI	Resistant (%)	DSI	Resistant (%)	DSI	Resistant (%)
10 Days after inoculation												
Control (water)	1.0 a[d]	100	1.0 a	100	1.0 a	100	1.2 a	94	1.0 a	100	1.0 a	100
C. trifolii (race 1)	1.9 b	76	2.9 b	50	1.7 c	84	3.3 b	30	2.1 b	64	2.0 b	72
C. trifolii (race 2)	1.9 b	76	2.4 b	54	4.2 d	4	4.2 c	4	3.0 c	44	4.9 c	0
Control (agar)	1.0 a	100	1.0 a	100	1.0 a	100	...	...	...	...	...	...
C. fragariae (CF-7)[e]	2.8 c	36	1.1 a	98	1.4 b	94	4.4 cd	2	1.2 a	96	1.5 a	88
C. fragariae (CF-4)	1.9 b	70	1.0 a	100	1.4 b	90	4.6 cd	8	1.2 a	96	1.6 a	82
C. fragariae (CF-1)	...	...	...	...	...	...	4.9 d	0	1.7 ab	82	1.7 b	82
20 Days after inoculation												
Control (water)	1.1 a	100	1.0 a	100	1.0 a	100	1.3 a	91	1.0 a	100	1.1 a	96
C. trifolii (race 1)	3.1 c	18	3.1 b	52	2.0 c	84	4.3 b	14	2.3 c	62	2.4 b	62
C. trifolii (race 2)	3.5 c	20	2.8 b	48	4.7 d	4	4.9 b	2	3.4 d	34	4.9 c	2
Control (agar)	1.0 a	100	1.0 a	100	1.0 a	100	...	...	...	...	...	...
C. fragariae (CF-7)	3.4 c	33	1.3 a	90	1.4 b	92	4.5 bc	4	1.2 a	94	1.4 a	90
C. fragariae (CF-4)	2.3 b	70	1.0 a	100	1.6 b	82	4.6 bc	10	1.1 a	98	1.6 a	84
C. fragariae (CF-1)	...	...	...	...	...	...	4.9 c	0	1.7 b	82	1.8 ab	78

[a] Values are the means of five replicates of 10 plants each.

[b] DSI = disease severity index, which is the calculated average of the disease severity scores. Alfalfa: 1 = no lesions, or only hypersensitive flecking; 2 = small nonsporulating lesions; 3 = lesions enlarging but not girdling the stems, with sporulation and setae in the acervulii; 4 = stem-girdling lesions with sporulation; and 5 = dead plant. Blue lupine: 1 = no lesions, hypersensitive flecking on the stem or slight necrosis on cotyledonary scar; 2 = small nonsporulating lesions, necrosis on the cotyledonary scar; 3 = elongating lesions not girdling the stem, but sporulating; 4 = stem-girdling lesions with sporulation, collapsing stems; and 5 = dead plant. Scales with the same number were equivalent.

[c] Percentage of all plants with disease severity scores of 1 or 2.

[d] Means in columns followed by different letters differ significantly ($P = 0.05$) according to Duncan's multiple range test within a test and inoculation time.

[e] *C. fragariae* CF-7 = Florida, CF-4 = North Carolina, and CF-1 = Louisiana.

races was consistent with earlier findings (14,15). Because alfalfa is an autotetraploid and is cross-pollinated, cultivars are mixtures of individuals whose disease reactions range from susceptible to highly resistant. It is not uncommon to have individual plants in a cultivar die and for the DSI of the cultivar to be less than 2.

Conidia of *C. trifolii* (Fig. 1A,B) from stem lesions in blue lupine were hyaline, aseptate, elliptical, smooth, and blunt at the ends. The shortest-(average)-longest length and width of 1,200 conidia of race 1 of *C. trifolii* were 6-(11.6)-14 × 3-(5.5)-7 μm, respectively, and for race 2 of *C. trifolii* (1,200 conidia), they were 9-(13.3)-22 × 4-(5.4)-7 μm. The average size and shape of conidia were typical for *C. trifolii* (12).

Conidia of *C. fragariae* (Fig. 1C,D) from blue lupine lesions were hyaline, aseptate, straight, smooth, cylindrical, and attenuated or pointed on one end. The shortest-(average)-longest length and width for 830 conidia of isolate CF-4 (North Carolina) were 8-(14.5)-30 × 3-(4.7)-7 μm, respectively, and for 1,078 conidia of isolate CF-7 (Florida), they were 8-(16)-24 × 3-(5.2)-7 μm, respectively. The length of these conidia was more variable than that described by Brooks (1), but the average was similar. Brook's description (1) of the conidia of *C. fragariae* (spindle- to boat-shaped with rounded ends) was not consistent with these observations.

The shape of the conidia of *C. fragariae*, which induces anthracnose of strawberry, is slightly different from the shape of conidia of *C. acutatum*, which induces fruit rot of strawberry. Spores of *C. acutatum* also are much smaller (range 8.3–14.4 μm, 2.5–4.0 μm, and average 11.1 × 3.1 μm) than those of *C. fragariae* (9).

It is concluded from these results that blue lupine seedlings grown in the greenhouse are susceptible to *C. trifolii* and *C. fragariae*. This host may serve as a source or reservoir of inoculum for natural outbreaks of anthracnose in alfalfa or strawberry.

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