Effects of Temperature and Light on Development of Anthracnose on Alfalfa

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ABSTRACT

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Anthracnose was more severe in susceptible plants incubated at 24 C than at 16 C. Differences in disease severity were not significant among susceptible plants incubated at light/dark temperatures ranging from 14/10 C to 30/26 C after a 3-day infection period at 16 C or 2 and 3 days at 24 C. The severity of anthracnose was similar in susceptible cultivars Team and Saranac infected at either 16 or 24 C. Anthracnose severity was similar in resistant cultivars Arc, Vangard, and Saranac AR when the temperature during infection was 16 C but was more severe in Saranac AR when the temperature during infection was 24 C. Disease severities of susceptible plants incubated in 1,600, 6,500, and 24,800 lux during the infection period were not statistically different.

Anthracnose, caused by *Colletotrichum trifolii*, limits the growth and persistence of alfalfa (*Medicago sativa* L.) in various areas of the United States (1,2,10). Generally, Flemish alfalfas are highly susceptible to the disease (16), but procedures for screening alfalfa populations for resistance have been developed (14,18) and resistant cultivars are available (6,11).

The effects of survival and dissemination of spores (2,12), age of host seedlings (14), and interaction of three species of *Colletotrichum* (8) on the development of the disease are known. Despite reports that this disease is severe in warm and humid areas (1,9) and is sometimes called summer decline, the effect of temperature on disease severity is not known.

We evaluated disease severity of susceptible and resistant alfalfa cultivars inoculated with *C. trifolii* incubated at various temperatures. We also examined the effect of various light intensities during and after the infection. The infection period is the time that inoculated seedlings were in the moisture chamber, and the postinfection period is the time between removing the plants from the mist and disease scoring. A portion of these results were reported previously (19).

MATERIALS AND METHODS

The culture of *C. trifolii* used in these studies, designated PA, was obtained from the U.S. Pasture Laboratory, University Park, Pennsylvania (5). The culture was stored at 3–4 C on potato-dextrose agar until use. Petri dishes of lima bean agar were flood-inoculated with the fungus and incubated for 7–10 days at 25 C. Spore suspensions were then made with 2 drops of Tween 20 (17) and 30 ml of orange juice per liter of distilled water (13) to stimulate spore germination. Spores were counted in a hemocytometer and spore suspensions adjusted to 1×10^6 spores per milliliter. Control plants sprayed with Tween 20 and orange juice without spores did not develop symptoms of anthracnose and were not used after the first experiment.

All studies were done in controlled-environment chambers and glasshouses (7) in the Southeastern Plant Environmental Laboratories at North Carolina State University, Raleigh. Alfalfa seeds were sown in 180-ml Styrofoam cups containing a standard potting mixture of one-third peat moss-perlite and two-thirds gravel. The cups were placed in a glasshouse in natural daylengths, and nights were interrupted for 3 hr (2300–0200 hr) by incandescent lamps to provide physiologically long days. The glasshouse was programmed for day temperatures of 22 C (0700–1900 hr) and night temperatures of 18 C (1900–0700 hr). Seedlings were thinned to five per cup and irrigated with a complete nutrient solution each morning and evening.

Plants (21-day-old) were inoculated by spraying them with 10 ml of spore suspension and then incubated 1–3 days. Moist conditions for infection were maintained by enclosing the plants and cup with a 1-L polyethylene bag or by placing the cups in a dew chamber. The infection period lasted 1, 2, or 3 days in experiments 1 and 2 and 3 days in experiments 2 and 3. The day/night temperatures were 16 and 24 C in experiments 1 and 2 and 14/10 and 26/22 C in experiment 3. After the infection period, plants were moved either to growth chambers with 15 hr of light and 9 hr of darkness or to the glasshouse with 15 hr of light at 22 C and 9 hr of darkness at 18 C.

Three weeks after inoculation, the main stem of each plant in a cup was evaluated and the severity of disease scored on a 1–5 scale (6,14). The average score of the five plants in each cup was used to determine a disease severity index (DSI).

Similar designs were followed in the three experiments. Nine cups containing five plants each were used for each treatment combination in each experiment, and each experiment was repeated. Thus, the mean DSI for each treatment was the mean of 90 plants ($5 \times 9 \times 2$). The experimental designs involved blocking by runs (time) and were variations of the split-plot designs. During the infection stage in all three experiments, plants were assigned to growth chambers providing the desired temperature or light conditions. After the infection period, either plants were reallocated among the chambers to provide the desired combination of conditions during the postinfection periods (experiments 1 and 3) or all plants were moved into a single glasshouse in a randomized complete block design (experiment 2). In experiments 1 and 3, the assignments of plants first to the infection period treatments and subsequently to the postinfection period treatment generated a factorial set of treatments in the variation of the split-plot design referred to as the split-plot with "sub-unit treatments in strips" (3). The two treatment factors being investigated corresponding to the whole plot and subplot factors are conditions during the infection and postinfection periods. The interaction with each of the treatment factors with runs serves as the experimental error for testing each of the respective factors. Errors of the split-plot analysis that were not appreciably different were pooled for testing of treatment factors.

To determine how temperature during infection and postinfection periods affected DSI, we inoculated seedlings of the anthracnose-susceptible cultivar Team and incubated them in a dew chamber without light for 1, 2, or 3 days at 16 or 24 C, then moved them to growth chambers at light/dark temperatures of 30/26, 26/22, 22/18, 18/14, or 14/10 C to

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complete the 21-day postinfection period (15-hr light at 30,000 lux and 9-hr darkness). The treatment set for this experiment consisted of all combinations of the three factors: length of time for the infection period, temperature during the infection period, and temperature during the postinfection period. The split-plot analysis for this experiment gave no indication of the error terms being heterogeneous, and a single pooled interaction with runs was used for testing.

To compare anthracnose on several cultivars, we inoculated anthracnose-resistant cultivars Arc, Saranac AR, and Vangard and anthracnose-susceptible cultivars Team and Saranac and incubated them in a dew chamber for 1, 2, or 3 days at 16 or 24 C. After the infection period, the plants were moved to a glasshouse at 22/18 C (day/night) to complete the 21-day postinfection period. The experimental factors included two temperatures and three incubation times for the infection period and five cultivars. The five cultivars in this experiment were the subplot treatment factor.

To compare the effects of light and temperature during the infection and postinfection periods on the DSI, we inoculated Team seedlings, covered them with plastic bags, and placed them in growth chambers with 24,800, 6,500, or 1,600 lux and light/dark temperatures of 26/22 or 14/10 C. Each growth chamber provided 15 hr of light and 9 hr of darkness. After a 3-day infection period, we removed the bags and incubated the plants in these chambers at all combinations of light intensities and temperatures, to complete the 21-day incubation. This factorial experiment included three light levels and two temperatures during the infection period and three light levels and two temperatures during the postinfection period, a 3 × 2 × 3 × 2 factorial set of treatments.

RESULTS

The DSI of plants from susceptible cultivar Team tended to increase with the length of the infection period (DSI 3.5, 4.3, and 4.5 for 1, 2, and 3 days, respectively, LSD 0.01 = 0.24) and with the temperature of the infection period (DSI 3.9 and 4.3 for 16 and 24 C, respectively, LSD 0.01 = 0.2). These two factors, however, showed an interaction. Likewise, temperature during the infection period interacted with the temperature during the postinfection period, and the three-factor interaction, ie, length of time for the infection period, temperature during the infection period, and temperature during the postinfection period, was significant. The individual treatment means are presented for comparison (Table 1).

The treatments involving 3 days at 16 C and 2 and 3 days at 24 C during the infection period gave virtually identical DSI (4.4, 4.6, and 4.6, respectively), and this result was independent of the temperature during the postinfection period (average DSI = 4.6 for these 15 treatment combinations). The DSI for plants in treatments involving 1 and 2 days at 16 C and 1 day at 24 C was 3.2, 4.1, and 3.8, respectively (LSD 0.01 = 0.35). Two days of infection period at 16 C gave a lower DSI (3.7) at the highest postinfection temperature, but this was not significantly different (LSD 0.05 =

Table 1. Disease severity of anthracnose-susceptible alfalfa cultivar Team infected with *Colletotrichum trifolii*

Infection period		Disease severity index[a]				
		Postinfection period day/night temp.[b]				
Length (days)	Temp. (C)	14/10	18/14	22/18	26/22	30/26
1	16	3.8[c]	3.5	3.0	3.3	2.6
2	16	4.1	4.0	4.2	4.2	3.7
3	16	4.7	4.5	4.5	4.4	4.4
1	24	3.0	4.2	4.3	4.0	3.7
2	24	4.5	4.6	4.6	4.6	4.5
3	24	4.6	4.6	4.7	4.5	4.7

[a] The average disease severity index was computed for 90 plants per treatment from disease scores 1–5: 1 = no lesion or only hypersensitive flecking; 2 = coalescing lesions, without sporulation; 3 = typical diamond-shaped lesions not girdling the stem, with sporulation and setae in the acervuli; 4 = stem girdling lesion with sporulation, but new shoots originating from lower axillary buds; and 5 = dead plant.
[b] The infection and postinfection periods totaled 21 days.
[c] The LSD 0.05 for the time × temperature × postinfection temperature means is 0.57.

Table 2. Disease severity in alfalfa cultivars resistant and susceptible to anthracnose after infection with *Colletotrichum trifolii*

	Disease severity index[a]				
	Infection period temp. (C)		Infection period time (days)		
Cultivar[b]	16	24	1	2	3
Susceptible					
Team	4.3	4.5	3.9	4.5	4.7
Saranac	4.5	4.8	4.3	4.8	4.9
Resistant					
Arc	2.3	2.3	1.8	2.2	2.9
Saranac AR	2.1	3.0	1.6	2.8	3.4
Vangard	2.3	2.6	2.0	2.3	2.4

[a] The average disease severity index was computed for 90 plants per treatment from disease scores 1–5: 1 = no lesion or only hypersensitive flecking; 2 = coalescing lesions, without sporulation; 3 = typical diamond-shaped lesions not girdling the stem, with sporulation and setae in the acervuli; 4 = stem girdling lesion with sporulation, but new shoots originating from lower axillary buds; and 5 = dead plant.
[b] The LSD 0.05 for the temperature × cultivar means is 0.36 and the cultivar × time means is 0.44.

Table 3. Disease severity of anthracnose-susceptible alfalfa cultivar Team infected with *Colletotrichum trifolii* and incubated at varying temperatures and light intensities

Day/night temp. (C)		Light intensity (lux) during infection period	Disease severity index[a]		
			Light intensity (lux) during postinfection period		
Infection period[b]	Postinfection period[b]		1,600	6,500	24,800
14/10	14/10	1,600	2.2	2.3	2.4
		6,500	2.6	3.1	2.1
		24,800	2.2	2.2	2.2
	26/22	1,600	2.3	2.5	2.0
		6,500	2.9	2.8	1.9
		24,800	3.3	2.9	2.1
26/22	14/10	1,600	3.9	4.0	3.6
		6,500	4.1	4.3	4.0
		24,800	4.2	4.2	4.1
	26/22	1,600	4.2	4.5	3.8
		6,500	4.4	4.4	4.0
		24,800	4.3	4.3	3.9

[a] The average disease severity index was computed for 90 plants per treatment from disease scores 1–5: 1 = no lesion or only hypersensitive flecking; 2 = coalescing lesions, without sporulation; 3 = typical diamond-shaped lesions not girdling the stem, with sporulation and setae in the acervuli; 4 = stem girdling lesion with sporulation, but new shoots originating from lower axillary buds; and 5 = dead plant.
[b] Incubation time for the infection period was 3 days and for the postinfection period, 18 days.

0.57) from the DSI of plants at lower postinfection temperatures. On the other hand, the 1-day infection period at 16 C showed a significant linear decline (b = −0.07 DSI per degree) in DSI with increasing postinfection temperature. Also, at the 24 C infection temperature for 1 day, there was an apparent maximum disease expression at the 22/18 C postinfection temperature and the DSI was significantly lower at the higher and lowest postinfection temperatures (LSD 0.05 = 0.57). Except for the lowest 14/10 C development temperature, 1-day infection period at 24 C gave results similar to the 2-day infection period at 16 C.

When the reaction of the five cultivars to *C. trifolii* at the two temperatures and three infection periods were compared, temperature (DSI 3.1 and 3.4 at 16 and 24 C, respectively, LSD 0.05 = 0.16) and time (DSI 2.7, 3.3, and 3.8 for 1, 2, and 3 days, respectively, LSD 0.05 = 0.20) effects were significant, and both interacted significantly with cultivars but not with each other. The results can therefore be summarized in two 2-way tables of means. The interaction between temperature and cultivar (Table 2) seemed to be due primarily to the relatively greater response in disease development of Saranac AR to the increased infection period temperature of 24 C. The interaction between cultivar and time of exposure appeared to be due primarily to the fact that Team, Saranac, and Vangard had reached nearly maximum response in DSI by the second day of the infection period, whereas the DSI of Arc and Saranac AR increased with the third day. Among the resistant cultivars, Saranac AR showed the greatest response in disease development when the infection period was at the more favorable temperature (24 C) and length of time (3 days).

When alfalfa seedlings of the cultivar Team were incubated in all combinations of two temperatures and three light intensities during the infection period and two temperatures and three light intensities during the postinfection period (Table 3), DSI during the infection at 14/10 (DSI 2.5) was significantly (LSD 0.01 = 0.34) smaller than that at 26/22 C (DSI 4.1). In contrast, the DSI of plants at 14/10 and 26/22 during the post-infection period were not significantly different (3.2 and 3.4, respectively). Light intensity during the infection period did not appear to influence the DSI of plants incubated at 24,800, 6,500, or 1,600 lux; the DSI 3.3, 3.4, and 3.2, respectively, were not statistically different. During the postinfection periods, however, the DSI at 24,800 lux (3.0) was significantly less (LSD 0.01 = 0.34) than at either 6,500 lux (3.4) or 1,600 lux (3.4). There were no significant interactions among any of the factors in this experiment.

DISCUSSION

We found that temperatures during the infection period influence the DSI of anthracnose more than do temperatures during the postinfection period. Comparing cultivar reaction to *C. trifolii*, the disease index was similar when susceptible cultivars were incubated at either 16 or 24 C. Arc and Vangard were slightly more resistant to anthracnose at 24 C than was Saranac AR. The slightly larger DSI of Saranac AR, compared with that of Arc, was similar to the earlier reports on anthracnose studies involving these cultivars (6).

Although light affects the severity of other diseases (4,15), only during the postinfection period did the highest light intensity (24,800 lux) result in a significant decrease in the DSI for anthracnose on alfalfa.

We found that anthracnose develops in alfalfa seedlings at 10–30 C and that temperature is more critical during the infection period. We also found that light intensities with fourfold differences during the infection period did not critically affect disease development. An increase in the length of the infection period from 2 to 3 days did not affect the DSI of plants of susceptible cultivars Saranac and Team, but the DSI was larger for the plants in two (Saranac AR and Arc) of the three resistant cultivars when incubated for 3 rather than 2 days.

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