# Occurrence and Interaction of Three Species of Colletotrichum on Alfalfa in the Mid-Atlantic United States

J. H. Graham, T. E. Devine, and C. H. Hanson

Research Plant Pathologist, Plant Stress Laboratory; Research Geneticist, Cell Culture and Nitrogen Fixation Laboratory; Staff Scientist (now retired), National Program Staff, respectively, Agricultural Research Service, U.S. Department of Agriculture, Beltsville, Maryland 20705.

Mention of commercial products throughout this paper is for identification and does not constitute endorsement by the U.S. Department of Agriculture.

Accepted for publication 16 October 1975.

ABSTRACT

GRAHAM, J. H., T. E. DEVINE, and C. H. HANSON. 1976. Occurrence and interaction of three species of Colletotrichum on alfalfa in the mid-Atlantic United States. Phytopathology 66: 538-541.

*Colletotrichum trifolii* is the principal causal agent of anthracnose of alfalfa in the mid-Atlantic United States. Local isolates of *C. destructivum* and *C. dematium* f. *truncata* were weakly pathogenic; however, one isolate of *C. destructivum* from Canada was moderately pathogenic to alfalfa. An isolate of *Colletotrichum* found in a greenhouse was classified in the composite group, *C. gloeosporioides*. It produced anthracnose-type stem lesions on experimental strains and cultivars of alfalfa resistant or susceptible to *C. trifolii*. Seedlings simultaneously inoculated with a mixture of *C. trifolii* and local isolates of *C. destructivum* or with *C. trifolii* and *C. dematium* f. *truncata* were significantly less damaged than those inoculated with *C. trifolii* alone. The two weakly pathogenic species produced many acervuli on stem lesions caused by *C. trifolii*. Similar results were obtained when plants were inoculated with *C. trifolii* 5 days after inoculation with the two weakly pathogenic *Colletotrichum* spp. When plants were inoculated with *C. trifolii* alone and with *C. trifolii* before inoculation with *C. destructivum* and *C. dematium* f. *truncata*, disease incidence (anthracnose) was similar on all plants.

Three species of *Colletotrichum* – namely, *C. trifolii* Bain, *C. destructivum* O'Gara, and *C. dematium* (Pers. ex Fr.) Grove f. *truncata* (Schw.) v. Arx. have been isolated from alfalfa grown in various areas of the United States (2, 5, 7, 9, 10) and in several other countries (4, 6). Researchers have recognized the moderate-to-severe damage caused by *C. trifolii* on alfalfa; however, the importance of the other two species has not been fully determined. We report here the occurrence, pathogenicity, and interaction of *C. trifolii, C. destructivum,* and *C. dematium* f. *truncata* on alfalfa in the mid-Atlantic United States.

## METHODS AND RESULTS

**Occurrence of Colletotrichum spp.**—Diseased specimens of alfalfa were collected from various areas in the mid-Atlantic United States during the growing seasons of 1973 and 1974.

*Colletotrichum trifolii* was first collected in central Maryland in early June from stem tissue near the crown. Setae were visible on the dead or dying stems, and numerous conidia developed after 2 days in a moist chamber. New stem lesions were not found until early July, with maximum occurrence in late summer.

*Colletotrichum destructivum* was regularly collected in early spring from overwintered stems and later (May) from early shoots of alfalfa that had been killed or were severely damaged from shading by the vigorous spring growth. *Colletotrichum destructivum* was also recovered from diseased leaves and from blackened stems of alfalfa in early June. In late summer, *C. destructivum* was sometimes isolated from stem lesions typical of *C. trifolii*.

*Colletotrichum dematium* f. *truncata* was found on overwintered alfalfa and on living plants at intervals through the season. The recognizable long black setae could not be associated with any particular type of stem lesions. However, it was often mixed with *C. trifolii* or near stem lesions caused by *C. trifolii*.

**Pathogenicity of isolates of Colletotrichum spp.**—Alfalfa seedlings were grown in flats of a commercial mixture of shredded peat moss and vermiculite with nutrients added. The medium was covered with 0.6 cm of fine white river sand. The following alfalfa cultivars and strains were used to test pathogenicity of the isolates and to identify possible races of the pathogens: Glacier, Glacier-AN4, Saranac, Saranac-AN4, Vernal, Vernal-AN4, Team, and Arc. The suffix "AN4" indicates three cycles of selection for resistance to *C. trifolii* (3). Arc was derived from Team germplasm by three cycles of selection. Plants were grown in a growth chamber at 21-24 C with a 16-hour daylength and 17.2 klux light intensity. Three tests, each with three replications, were conducted. A replication consisted of a single flat of eight two-row plots of 30 seeds per plot.

Eight isolates of *C. trifolii,* three of *C. destructivum,* and three of *C. dematium* f. *truncata* collected from alfalfa in the mid-Atlantic United States, were grown in petri plates for 7-8 days on V8 juice agar and on potato-dextrose agar at 22-24 C. Seedlings were inoculated 2

weeks after seeding with comminuted cultures of the *Colletotrichum* spp. Approximately one plate was used for each flat of seedlings. Plants were sprayed with the inoculum until run-off and held in a translucent polyethylene moist chamber for 72 hours at 26-28 C. A constant moisture-saturated atmosphere was provided by humidifiers programmed for intermittent operation. Plants were scored individually for disease severity 2 weeks after inoculation. A 1-to-5 scale of severity was used: 1 = (highly resistant) no stem lesions or only few small water-soaked or black spots; 2 = (moderately resistant) stems with elongated black lesions but without acervuli; 3 = (intermediate in susceptibility) stems with long, wide, but non-girdling lesions, with acervuli present; 4 = (susceptible) large, coalescing and sporulating lesions which girdle and kill upper part of seedling; 5 = (highly susceptible) seedling dead (8). From these scores, a disease severity index (DSI) was calculated by the formula:

$$\text{DSI} = \frac{(\text{seedlings in class 1}) \times 1 + (\text{in class 2}) \times 2 + \ldots}{\text{total no. of seedlings}}$$

$$\frac{(\text{in class 5}) \times 5}{\text{total no. of seedlings}}$$

An additional rating was taken for *C. destructivum* (petiole blackening) and two more for *C. dematium* f. *truncata* (petiole and petiolule blackening).

The results of one of three similar pathogenicity tests are shown in Table 1. Only four of the alfalfa cultivars and breeding lines are included since Glacier and Glacier-AN4, and Vernal and Vernal-AN4 reacted similarily to Saranac and Saranac-AN4. Data for cultivars Team and Arc are presented because of their distinct reactions.

The eight isolates of *C. trifolii* varied slightly in pathogenicity. That there was no significant isolate × alfalfa strain interaction indicated an absence of races of the fungus. Isolate 23, a reisolate from plants inoculated with the Beltsville dry inoculum mix (8), was a prolific sporulator and highly pathogenic.

The three isolates of *C. destructivum* caused only slight to moderate superficial blackening of stems of seedlings under conditions that were optimum for infection by *C. trifolii* and, in auxiliary experiments at higher and lower temperatures. On the scale used for *C. trifolii,* the DSI ranged from 1.3 to 1.5 (Table 1). Petioles and leaves also were attacked; petioles blackened, collapsed or both; and round, brown lesions (with light center) formed on leaves. Infection by the three isolates (and additional isolates in earlier tests) was low on all alfalfa strains. The alfalfa strains did not differ significantly in response to the isolates.

Isolates of *C. dematium* f. *truncata* blackened stems, petioles, and petiolules, but were only mildly pathogenic. Sporulation occurred on any part of the plant affected, often unassociated with visible lesions. In fact, acervuli of *C. destructivum* or *C. dematium* f. *truncata* seldom formed on the blackened stems.

Toward the end of the study, an alfalfa disease specimen was received from eastern Ontario. *Colletotrichum destructivum* was recovered from the blackened stems. In two inoculation tests, the isolates from this specimen were slightly to moderately pathogenic to the strains and cultivars of alfalfa used earlier. Cultivars Arc and Team were slightly more susceptible. Stems of inoculated plants were blackened over large areas or had smaller, sunken, oval lesions. Often the discoloration was superficial; however, some lesions caused the seedlings to collapse. Several days of incubation in a moist chamber was required for the fungus to fruit abundantly on diseased stem segments.

In 1973 a different *Colletotrichum* sp. was isolated from infected alfalfa plants growing in a greenhouse at Beltsville, Maryland. Pink spore masses were evident on stems and leaves. Setae were rarely formed. Conidia developed on seedlings were intermediate in size between those of the other two straight-spored species. The ratio of average width to average length of conidia of eight isolates of *C. trifolii* ranged from 0.36 to 0.60. The ratio for four isolates of *C. destructivum* ranged from 0.22 to 0.29. For three isolates from the one source of infected plants in the greenhouse, the ratio was 0.28 to 0.36. By von Arx' classification (1), the greenhouse isolate would most likely be placed in the composite group, *C. gloeosporioides* Penz. This fungus produced anthracnose-type lesions similar to those of *C. trifolii,* except that the stem lesions were much darker. The fungus was not as pathogenic as *C. trifolii;* however, the susceptible cultivars and resistant selections were all moderately susceptible. Susceptible cultivar Team and its resistant

TABLE 1. Pathogenicity (disease severity index) of *Colletotrichum* spp. on two susceptible (S) alfalfa cultivars and their anthracnose-resistant (R) derivatives

| | Disease severity index (DSI)[y] | | | |
|---|---|---|---|---|
| Fungus inoculum | Saranac (S) | Saranac-AN4 (R) | Team (S) | Arc (R) |
| *C. trifolii* | 4.1 a[z] | 1.7 c | 3.2 b | 1.5 c |
| *C. destructivum* | 1.4 c | 1.3 c | 1.4 c | 1.5 c |
| *C. dematium* f. *truncata* | 1.5 c | 1.3 c | 1.4 c | 1.3 c |

[y]$\text{DSI} = \dfrac{(\text{no. of seedlings in class 1}) \times 1 + (\text{in class 2}) \times 2 + \ldots (\text{in class 5}) \times 5}{\text{total no. of seedlings}}$

Class 1 = No disease or few small spots on stems; class 5 = plant dead.

[z]Indices followed by same letter are not significantly different ($P = 0.05$) by Duncan's multiple range test.

TABLE 2. Pathogenicity (disease severity index) of *Colletotrichum destructivum* (d), *C. dematium* f. *truncata* (dt), and *C. trifolii* (t) separately and in mixtures on anthracnose-resistant and -susceptible alfalfa seedlings

| | Disease severity index (DSI)[y] | | | |
|---|---|---|---|---|
| Fungus inoculum | Saranac | Saranac-AN4 | Team | Arc |
| d | 1.1 g[z] | 1.0 g | 1.1 g | 1.1 g |
| dt | 1.1 g | 1.1 g | 1.1 g | 1.1 g |
| t | 3.6 a | 1.3 fg | 3.0 b | 1.3 fg |
| d + t | 2.0 cd | 1.1 g | 1.8 de | 1.3 fg |
| dt + t | 2.3 c | 1.1 g | 1.6 def | 1.4 ef |

[y]Disease severity index (DSI).

$$\text{DSI} = \frac{(\text{no. of seedlings in class 1}) \times 1 + (\text{in class 2}) \times 2 + \ldots (\text{in class 5}) \times 5}{\text{total no. of seedlings}}$$

Class 1 = No disease or few small spots on stems; class 5 = plant dead.

[z]Indices followed by same letter are not significantly different ($P = 0.05$) by Duncan's multiple range test.

TABLE 3. Pathogenicity (disease severity index) of *Colletotrichum trifolii* (t) following infection of *C. destructivum* (d) and *C. dematium* f. *truncata* (dt) on alfalfa seedlings

| | Disease severity index (DSI)[y] | | | |
|---|---|---|---|---|
| Fungus inoculum | Saranac | Saranac-AN4 | Team | Arc |
| d | 1.5 efg[z] | 1.2 h | 1.4 fgh | 1.4 fgh |
| dt | 1.5 efg | 1.6 efg | 1.5 efg | 1.5 efg |
| t | 4.6 a | 1.7 ef | 4.0 b | 1.8 e |
| t after d | 4.0 b | 1.7 ef | 3.1 d | 1.8 e |
| t after dt | 3.6 c | 1.7 ef | 3.4 c | 1.7 ef |

[y]Disease severity index (DSI).

$$\text{DSI} = \frac{(\text{no. of seedlings in class 1}) \times 1 + (\text{in class 2}) \times 2 + \ldots (\text{in class 5}) \times 5}{\text{total no. of seedlings}}$$

Class 1 = No disease or few small spots on stems; class 5 = plant dead.

[z]Indices followed by same letter are not significantly different ($P = 0.05$) by Duncan's multiple range test.

counterpart Arc were significantly more susceptible than the other cultivars and breeding lines. This fungus type has never been found in the field.

**Interaction of Colletotrichum spp. on alfalfa seedlings.**—Field observations indicated an interaction of *C. trifolii* with *C. destructivum* and *C. dematium* f. *truncata*. One or more of the species often could be recovered from typical stem-girdling lesions caused by *C. trifolii*. Three experiments were run to determine this relationship. Resistant and susceptible alfalfa seedlings were inoculated with each of the three *Colletotrichum* spp. separately and in the mixtures *C. trifolii* plus *C. destructivum* and *C. trifolii* plus *C. dematium* f. *truncata*. Equal numbers of conidia of each isolate were used as inoculum in separate and mixed inoculations.

*Colletotrichum destructivum* and *C. dematium* f. *truncata* caused superficial stem blackening on all of the seedlings (Table 2). *Colletotrichum trifolii* caused stem blackening and anthracnose-type lesions; average disease severity rating index ranged from 1.3 to 1.5 on resistant lines, and from 3.0 to 3.6 on susceptible lines. Mixtures of inocula of *C. destructivum* and *C. trifolii* and of *C. dematium* f. *truncata* and *C. trifolii* caused slight blackening of stems and few anthracnose-type lesions on resistant lines. Damage on susceptible lines from the mixed inocula averaged significantly less than that from *C. trifolii* inoculum alone.

To study further the interaction of *Collectotrichum* spp., 2-week-old alfalfa seedlings were inoculated with *C. trifolii*. Five days later, the seedlings were segregated into three groups. One group was inoculated with *C. destructivum*, and one group was inoculated with *C. dematium* f. *truncata*, and the third group was left uninoculated. There were no significant differences in disease severity between the seedlings inoculated with *C. trifolii* alone and those inoculated with *C. trifolii* plus *C.*

*destructivum* or *C. trifolii* plus *C. dematium* f. *truncata*.

In a third experiment, one group of alfalfa seedlings was inoculated with *C. destructivum* and another group was inoculated with *C. dematium* f. *truncata*. A third group was left uninoculated. After 5 days, the seedlings were inoculated with *C. trifolii*. As a check, other groups of seedlings were inoculated with *C. destructivum* alone and with *C. dematium* f. *truncata* alone.

There were only slight differences in disease severity on seedlings of resistant strains inoculated with *C. trifolii* alone and *C. trifolii* following inoculation with the other two species (Table 3). However, the susceptible cultivars were damaged significantly more by *C. trifolii* alone than by *C. trifolii* sprayed onto plants previously infected with either of the two weakly pathogenic *Colletotrichum* spp. Maximum differences were observed in the rating classes 4 (stem girdled and top killed) and 5 (seedling killed). The inoculated seedlings were cut back, and the survivors were counted 2 weeks later. Of the susceptible lines, 16% survived after being inoculated with only *C. trifolii*. Of the seedlings previously inoculated with *C. destructivum* and with *C. dematium* f. *truncata* followed by *C. trifolii*, an average of 50% had survived.

## DISCUSSION

By the reaction of the four resistant and four susceptible cultivars of alfalfa, we could not detect races of *C. trifolii* among the field isolates tested. However, overall virulences of the isolates differed.

One isolate of *Colletotrichum* somewhat like *C. trifolii* was mildly pathogenic to Glacier, Saranac, and Vernal and their resistant derivatives, and significantly more pathogenic to cultivars Team and Arc. The source of the isolate was alfalfa plants held for several months in a greenhouse. This fungus, *C. gloeosporioides*, may have migrated from other host genera held in the greenhouse. It has never been observed in the field and does not significantly threaten cultivars resistant to *C. trifolii*.

*Colletotrichum destructivum* isolates from the mid-Atlantic United States, although only weakly pathogenic, were quite prevalent and readily overwintered on alfalfa. In attempting isolations of *C. trifolii* from typical anthracnose-type lesions in late summer and fall, we recovered only *C. destructivum* in many instances. In inoculations with mixtures of *C. trifolii* and *C. destructivum* and with these species in sequence, *C. destructivum* rarely sporulated on the blackened stem lesions that it caused, but formed acervuli often on the *C. trifolii* lesions and more often at the margins of the *C. trifolii* lesions. Thus, all isolates of *C. destructivum* from the mid-Atlantic United States, although mildly pathogenic on petioles and leaves, were primarily secondary invaders in stem lesions caused by *C. trifolii*.

The one isolate of *C. destructivum* from Canada differed from mid-Atlantic isolates in being slightly to moderately pathogenic to all of the alfalfa strains and cultivars used in the study.

*Colletotrichum dematium* f. *truncata* was not as prevalent in the mid-Atlantic United States as *C. destructivum*, but it acted also as an invader of *C. trifolii* lesions.

The results of this study indicate that prior infection with *C. destructivum* and *C. dematium* f. *truncata* reduced the subsequent disease severity of *C. trifolii*.

## LITERATURE CITED

1. ARX, J. A. VON. 1957. Die Arten Der Guttung Colletotrichum Cda. Phytopathol. Z. 29:413-468.
2. BARNES, D. K., S. A. OSTAZESKI, J. A. SCHILLINGER, and C. H. HANSON. 1969. Effect of anthracnose (Colletotrichum trifolii) on yield, stand, and vigor of alfalfa. Crop Sci. 9:344-346.
3. DEVINE, T. E., C. H. HANSON, S. A. OSTAZESKI, and T. A. CAMPBELL. 1971. Selection for resistance to anthracnose (Colletotrichum trifolii) in four alfalfa populations. Crop Sci. 11:854-855.
4. GHAFOOR, A., and S. A. JAMIL KHAM. 1966. Record of Colletotrichum truncatum on Medicago sativa in Pakistan. Agric. Pakist. 17:299-300.
5. JONES, F. R., and J. L. WEIMER. 1940. Three anthracnoses of alfalfa. Plant Dis. Rep. 24:30-31.
6. LAVIOLA, C. 1963. Osservazioni sull' antracnosi dell'erba medica (Medicago sativa L.) in Puglia. Ann. Fac. Agric. Univ. Bari (Italy) 17:337-345.
7. LÓPEZ-MATOX, L. 1961. Studies on certain species of Colletotrichum on Medicago sativa, Trifolium pratense, and Lotus corniculatus in New York State, Ph.D. Thesis, Cornell University. 84 p.
8. OSTAZESKI, S. A., D. K. BARNES, and C. H. HANSON. 1969. Laboratory selection of alfalfa for resistance to anthracnose, Colletotrichum trifolii. Crop Sci. 9:351-354.
9. ROBERTS, D. A., R. E. FORD, C. H. WARD, and D. T. SMITH. 1959. Colletotrichum anthracnose of alfalfa in New York. Plant Dis. Rep. 43:352.
10. TIFFANY, L. H., and J. C. GILMAN. 1954. Species of Colletotrichum from legumes. Mycologia 46:52-75.