Dissemination and Survival of Colletotrichum trifolii Under Field Conditions

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Contribution No. 697, Department of Plant Pathology, The Pennsylvania Agricultural Experiment Station. Authorized for publication 31 October 1972 as Journal Series Paper No. 4325.

The author wishes to acknowledge the technical assistance of Mrs. R. G. Levine and Mr. B. R. Miehle.

Accepted for publication 14 June 1973.

ABSTRACT

Colletotrichum trifolii can persist from one harvesting season to the next in debris on the surfaces of protected alfalfa harvesting equipment. The fungus was able to survive in alfalfa stems for only 100 days under field conditions. This suggests that under Pennslyvania conditions, infected plants can be important sources of secondary inoculum, but may not be important as a source of primary inoculum. Anthracnose was shown to be important in predisposing the infected plants to winter injury.

Phytopathology 64:57-59

Additional key words: alfalfa, anthracnose, winter injury.

Anthracnose of alfalfa (*Medicago sativa* L.) caused by *Colletotrichum trifolii* Bains and Essary is severe in the alfalfa stands of Maryland (1), but usually is not a problem in the north central United States and southern Canada (6). Lopez-Matos (3), studied the ability of *C. trifolii* to overwinter in New York, and concluded that the geographical limitation may result from the inability of large numbers of propagules of the fungus to overwinter in the northern areas. His conclusion is supported by the statement by Barnes et al. (1) about the severity of the disease in Maryland: "In stands with a history of anthracnose, the pathogen is usually present in the crown and on the old stubble in infected plants" which suggests overwintering of the pathogens in the field.

Lopez-Matos (3) showed that *C. trifolii* could be recovered only from infected areas of living tissue at the base of a stem and not from dead plants or buried plant material left outdoors from November to March. Monteith (4) reported that the fungus remains viable for a long period in dried clover tissue stored under varying conditions. However, he reported that attempts to overwinter the fungus on clover stems in flasks stored outside at Madison, Wisconsin, were unsuccessful. Ostazeski et al. (5) showed that *C. trifolii* survived in dry alfalfa stems for 10 mo stored at −20 C and was nearly avirulent at the end of 4 mo when stored at 21 C.

The research of Lopez-Matos (3) and Barnes et al. (1) suggest that overwintering of *C. trifolii* occurs in the field in the stubble of infected alfalfa plants. This mode of overwintering, and the fact that the gelatinous matrix containing the conidia is splash-dispersed, usually is indicative of a disease that spreads in an increasingly larger circle, with the original site being the center. However, personal observations in alfalfa fields with plants infected with anthracnose usually do not support this. Instead, one commonly observes in the early part of an anthracnose season a random distribution of single infected plants. Then the number of infected plants increases around these sites, while new single-plant infection sites appear at other areas. This distribution, while not negating the theory that infected plants are important sources of causal overwintering, certainly suggests that other factors may be involved.

Anthracnose is becoming a serious problem in the alfalfa-growing areas of central and southern Pennsylvania. This increase in incidence suggests either a change in the pathogen, or shifting environmental conditions which allow increased overwinter survival of the fungus. Field investigations in several areas of Pennsylvania and laboratory investigations were carried out to determine possible reasons for this increase of the disease.

MATERIALS AND METHODS.—*Isolation studies.*— On April 10 the ability of *C. trifolii* to overwinter in diseased plants was determined by randomly selecting 100 plants from a three-year-old alfalfa field, cultivar 'Cardinal', that had had a high incidence of anthracnose the previous year. Based upon the presence of anthracnose lesions in the stubble, and using the bluish-black tissue (2) as a criteria for the presence of the disease in the crown, 21 plants were selected from the original collection. These were washed with water, then with 1% Clorox for one minute, rinsed with distilled water and cut into ca. 2-mm^2 pieces. This treatment usually removed the *C. trifolii* spores and matted the setae, allowing the observer to distinguish between the preformed structures and those formed after the treatment. Sections were placed on potato-dextrose agar and acidified potato-dextrose agar, pH 5.5. Observations of the microflora were made periodically until the plates were overgrown with fungi.

The fungal survival experiment showed that *C. trifolii* lesions were nearly always contaminated with *Fusarium* spp., which rapidly grew over the acervuli of *C. trifolii* when it was present. This problem was partially remedied by following the sterilization techniques and then placing the samples on sterile moist filter paper in moist chambers. Direct observations of samples for the presence of spore masses were made using a dissecting scope. Identification was confirmed by making and observing spore mounts.

Survival of C. trifolii under different environmental conditions.—Artificially inoculated alfalfa stems from greenhouse-grown plants, that had been killed by anthracnose within the last 5 days were harvested, the lesion areas removed and grouped into eight lots. Each lot was placed in a small cheesecloth bag. To determine how long the organism would survive in the field under environments present in the crown, in stems covered by the leaf canopy, and stems within the canopy, one bag was placed in each of the following areas: (i) at ground level shaded by nearby

TABLE 1. Effect of storage conditions on persistence of *Colletotrichum trifolii* in alfalfa stems

Days after harvest (11 June)	Storage conditions: Outside			Storage conditions: Constant temperature				
	Ground level	5 cm above	60 cm above	−15C	5C	25C	32C	Lab
33	100[a]	50	50	62	70	62	50	32
55	50	25	75	38	50	38	44	32
100	25	25	25	62	62	44	38	62
142	0	0	0	62	50	44	8	8
257	—	0	0	38	50	31	0	0
494	—	—	—	19	25	0	0	0
853	—	—	—	15	10	0	0	0

[a] Percent of 16 samples with viable *C. trifolii*.

plants; (ii) 5 cm above a; and (iii) 120 cm above a. The effect of temperature alone in the absence of periodical wetting and drying was determined by placing the rest of the lots at the following constant temperatures: d) −15 C; e) 5 C; f) 25 C; and g) 32 C. One lot was placed in an air-conditioned laboratory. No attempt to control or monitor humidity was made. The stems were placed in the experimental sites on 11 June.

At weekly intervals, 16 samples were removed from each site, surface-sterilized with 70% ethyl alcohol for 4 min and placed in a sterile moist chamber. Observations were made in the same manner as described in the isolation section. The sampling continued until the fungus could not be detected in the material.

To determine whether *C. trifolii* can survive when stored under conditions similar to those in which the harvesting equipment is stored, alfalfa stems with anthracnose lesions that remained on different surfaces of two alfalfa choppers after they had been stored 3-5 mo, after harvest, in the shed and one that had been stored outside (located near Nazareth, Pa.) were collected. This experiment was terminated at this time due to removal of the equipment for overhaul. In a separate experiment on 10 May, 128 stems with anthracnose lesions were collected from alfalfa debris on and around an alfalfa baler stored in an unheated barn located near Landisville, Pa. This baler was last used ca. 15 October, which means that the debris was 7 mo or possibly older when collected. In this area of Pennsylvania the average date of the first harvest is 15 May. The stems were surface-sterilized with the 1% Clorox solution and placed in moist chambers. The samples usually consisted of single stems which were laying on the surface of the equipment or nearby on the floor. However, one sample from the 3-mo period was a compacted mass of alfalfa believed to have been compressed by mechanical action of the machine.

TABLE 2. Persistence of *Colletotrichum trifolii* in alfalfa debris collected from stored harvesters A, B, and C at Nazareth, Pennsylvania

Days in Storage	Under cover[a]		Exposed[b]
	A	B	C
90	12.5[c]	20.0	25
150	5.5	7.2	0

[a] Stored in a closed, non-heated shed.
[b] Placed next to the above shed.
[c] Percent of 40 stems with viable C. *trifolii*.

The virulence the *C. trifolii* spores observed on the stems, stored 7 mo, was determined by placing nine of the stems with fresh acervuli in contact to stems of a greenhouse-grown alfalfa test plant (cultivar 'Saranac'). The plant was covered with a plastic bag for 48 hr and observed for anthracnose symptoms 14 days later.

Effect of anthracnose on survival of alfalfa plants.— Forty-six alfalfa plants, cultivar Saranac, with obvious anthracnose lesions near the crown area, were labeled in September. Companion plants which were clear of the lesions were also labeled at the same time. The plants, which were part of a 2-yr-old variety trial planted in southwest Pennsylvania, were observed in April, when the number of surviving plants was counted.

RESULTS.— Attempts to isolate *C. trifolii* from the diseased alfalfa crowns were not successful. All the samples yielded several Fusaria and bacteria.

On alfalfa stems exposed to natural wetting and drying, *C. trifolii* survived for 100 days, but not 142 days (Table 1). This was true regardless of the lesion position on the plant. In a dry protected area at room temperature, the pathogen survived for at least 142 days. At constant temperatures of 25 C it persisted for 257 days. At 5 C and at −15 C it was still present at low levels when the experiment was terminated after 853 days. Of the stems collected from the harvesters at Nazareth, viable samples of *C. trifolii* were obtained from all three of the harvesters at the end of three months (Table 2). At the end of 5 mo, however, the pathogen could not be recovered from samples taken from the exposed harvester. Eighteen of the 128 samples collected 7 mo after harvest produced new acervuli. Four of the nine positive samples tested caused typical anthracnose lesions on stems of the inoculated plant. *Colletotrichum trifolii* was not detected in the compacted sample although several lesioned stems were tested.

Twenty-one of the 46 plants infected by anthracnose did not overwinter while only four of the control plants were dead. Six of the surviving anthracnose-infected plants had only one or two live shoots compared to at least four-to-six shoots which were present on the controls.

DISCUSSION.— Apparently *Colletotrichum trifolii* can persist in exposed alfalfa stems long enough to serve as an inoculum source for primary infection, as was shown

by Lopez-Matos' work (3). However, in the research reported here, if it survived through the winter in the fields, it did so at a very low level. This is contrary to the observation of Barnes et al. (1) who inferred that the pathogen survives in the field in Maryland. Although Barnes presented no data to support this observation, it may be true because of the difference in climatic conditions; Maryland having milder conditions than Pennsylvania.

The data presented here strongly support the theory that a source of primary inoculum is dry alfalfa debris on equipment stored in sheds. Another source could be debris from baled hay which would be cleaned out in the summer before a new harvest was placed in the storage. This is suggested by the data presented here; the work of Ostazeski et al. (5), who showed persistency of *C. trifolii* stored at 5.5 C for 10 mo; and that of Monteith (4) who reported that *C. trifolii* survived for several mo on herbarium specimens of dried clover tissue. The failure to obtain *C. trifolii* from the tissue that was compressed and from samples of stems from the exposed harvester after 90 days suggests that the fungus cannot survive conditions where the humidity may be high enough to allow other microorganisms to grow. This is supported by the negative results in attempts to isolate *C. trifolii* from overwintered tissue from 21 crowns and from personal observations that anthracnose lesions are rapidly overgrown by *Fusarium* spp. once they are formed.

The observation that the fungus was able to persist in infected stems for at least 100 days in the field, suggests that infected plants are important sources of secondary inoculum. However, its inability to survive longer than 100 days suggests that this material may not be important as a source of primary inoculum.

Apparently the location of the lesion on the plant; i.e., on the crown, beneath the canopy, or in the canopy, does not affect the survival of the organism. The fungus did not survive for more than 100 days at either site.

The data of Table 1 and of Ostazeski et al. (5) show that the fungus survives at temperature below freezing (−20 to −15 C). The data could be interpreted as supporting a hypothesis that the fungus should survive as well in the colder climates as in the milder climates. However, this is not realistic, because in both cases the temperature and probably humidity were constant.

The production of viable *C. trifolii* spores on debris collected from harvesting equipment, strongly suggests that the material of this nature clinging to equipment could be a major means of long-distance spread. To prevent this possibility, cleaning of equipment before long-distance moving is recommended. Circumstantial evidence supporting this suggestion was gained by personal observation of the occurrence of anthracnose in an experimental field in northern Pennsylvania. That field had been harvested with an experimental harvester previously used on a field with infected plants located more than 100 miles away. A survey of fields of alfalfa within the vicinity of the experimental field failed to detect plants with anthracnose. Attempts to isolate the fungus from the seed used also failed. The fact that 21 of the 46 plants having anthracnose failed to overwinter supports Barnes' et al. (1) suggestion that anthracnose predisposes plants to winter injury.

LITERATURE CITED

1. BARNES, D. K., S. A. OSTAZESKI, J. A. SCHILLINGER, and C. H. HANSON. 1969. Effect of anthracnose (Colletotrichum trifolii) infection on yield, stand, and vigor of alfalfa. Crop Sci. 9:344-346.
2. KREITLOW, K. W., J. H. GRAHAM, and R. J. GARBER. 1953. Diseases of forage grasses and legumes in the Northeastern states. Pa. Agric. Exp. Stn. Bull. 573:42 pp.
3. LOPEZ-MATOS, L. 1961. Studies on certain species of Colletotrichum on Medicago sativa, Trifolium pratense, and Lotus corniculatus in New York State. Ph.D. Thesis. Cornell Univ. 84 pp.
4. MONTEITH, J., JR. 1928. Clover anthracnose caused by Colletotrichum trifolii. U.S. Dep. Agric. Tech. Bull. 28. 26 pp.
5. OSTAZESKI, S. A., T. A. CAMPBELL, T. E. DEVINE, and C. H. HANSON. 1971. Low temperature maintains virulence of dry Colletotrichum trifolii inoculum. Crop Sci. 11:935.
6. 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.