Symptomatology and Ecology of Alfalfa Anthracnose in Oklahoma

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ABSTRACT

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A study was conducted in Oklahoma to determine if occurrence of alfalfa anthracnose symptoms are linked to varying yearly temperature and precipitation patterns and to determine when the causal agent (*Colletotrichum trifolii*) is present in stands of alfalfa (*Medicago sativa*). Data from five sites were compared with weather patterns over a 3-yr period. Yearly variation in amount and distribution of precipitation affected occurrence of symptom development. *C. trifolii* appears to be present in alfalfa stands throughout the growing season. Anthracnose damage occurs periodically in Oklahoma when weather patterns are favorable, and resistance to *C. trifolii* is an important consideration in cultivar development for the state.

Colletotrichum trifolii Bain was described in Tennessee on red clover (*Trifolium pratense* L.) by Bain and Essary (2) in 1905 and was isolated from alfalfa (*Medicago sativa* L.) in the same year by Westgate in Virginia (2). Since the disease was first reported, different assessments have been made of the importance of anthracnose as a limiting factor in alfalfa production. Bain and Essary (2) described the devastation *C. trifolii* caused in Tennessee red clover fields as "remarkable." Henderson and Smith (16) suggested in 1948 that anthracnose was a more important component of "summer killing" of Virginia alfalfa stands than previously thought. In contrast, Hanson and Allison (14) reported in 1950 that *C. trifolii* was responsible for only a small part of stand decline in North Carolina. Roberts et al (20), in 1959, did not consider *C. trifolii* a threat to New York alfalfa production unless a strain of the fungus adapted to the lower temperatures of that state were to develop.

The potential importance of *C. trifolii* as a major pest limiting alfalfa forage yields was realized after Devine et al (11) developed four alfalfa strains resistant to *C. trifolii*. When these resistant strains and their susceptible parent cultivars—bioindicator pairs Arc (resistant) and Team (moderately resistant), Beltsville 1-An5 (resistant) and Glacier (susceptible), Beltsville 2-An4 (resistant) and Saranac (susceptible), and Beltsville 3-An4 (resistant) and Vernal (susceptible)—were tested at 24 locations in the United States, a significant increase was observed in average annual forage yield of resistant strains over their parent cultivars (12). In Oklahoma, two resistant strains (Arc and Beltsville 3-An4), were less susceptible to stand decline (percent stand) and produced significantly greater forage yield than their susceptible parent cultivars (6). However, cultivars highly resistant to *C. trifolii* (such as Arc) do not yield as well as cultivars possessing no or low levels of resistance to *C. trifolii* in current yield evaluation trials (21).

Anthracnose is considered a warm weather disease of alfalfa. The most distinctive symptom is occurrence of diamond-shaped lesions on stems of susceptible plants. Other symptoms include blue-black discoloration of infected crown tissue and formation of straw-colored shepherd crooks when infected wilted stems die suddenly. Because other diseases also cause formation of shepherd crooks, care must be taken in associating this symptom with anthracnose (13).

In Oklahoma, the predominant anthracnose symptom is occurrence of shepherd crooks. Diamond-shaped lesions and blue-black crown discoloration have been observed infrequently at scattered locations throughout the state (1).

Symptoms of anthracnose on alfalfa appear sporadically in Oklahoma, possibly because of erratic growing-season weather patterns. The objectives of this study were to determine if occurrence of anthracnose symptoms are linked to temperature and precipitation patterns during the growing season (study 1) and to determine if *C. trifolii* is present in alfalfa stands throughout the growing season (study 2).

MATERIALS AND METHODS

The climate of north central Oklahoma is temperate with large season-to-season variation in precipitation and temperature. The alfalfa growing season in north central Oklahoma lasts about 246 days—from the last killing freeze (24 C) in the spring to the first killing freeze in the fall. The 48-yr mean date of the last freeze is 19 March and the mean date of the first fall freeze is 20 November (9,19,22).

In Stillwater, OK, the greatest amount of monthly precipitation typically occurs in May, followed by June. Thunderstorms are most frequent in June, followed by August, then May. Days with the greatest amount of measurable precipitation occur in July, followed by September,

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October, and May (10). Yearly mean rainfall is 830 mm, but variation from year to year is great. Ambient relative humidity is extremely variable and depends on several meteorological factors (19).

Study 1. During 1980–1982, five alfalfa stands varying in age from 1 to 2 yr (1980) and located within 15 km of Stillwater were visited once a week during the growing season. Presence and types of symptoms were recorded. When shepherd crooks (the only symptom identifiable with anthracnose in these studies) were present, samples were taken. Within each location, 25–50 stems were collected in each of the following classes: 1) no symptoms; 2) stems showing symptoms of other diseases; 3) stems girdled by the three-cornered alfalfa hopper (*Spissistilus festinus* (Say)), producing symptoms similar to anthracnose; 4) shepherd crooks without girdles or lesions; and 5) girdled stems with shepherd crooks. In the laboratory, the lower 6 cm of each stem was cut into three 2-cm segments, which were disinfested in a 1.3% NaOCl solution for 2 min and placed onto water agar amended with 25 μg/ml of tetracycline and 50 μg/ml of streptomycin. Stem segments were incubated for 2 days at 3 C, then for 5 days at 21 C (1). After incubation stem segments were observed under a dissecting microscope, and the presence of *C. trifolii* and other fungi was recorded. Acervuli of *C. trifolii* were observed on an average of 5% of symptomless stems, 3% of stems showing symptoms of other diseases, 8% of girdled stems, 87% of ungirdled shepherd crooks, and 96% of girdled shepherd crooks. On the basis of these results, shepherd crooks were considered a reliable symptom of anthracnose.

Study 2. During the 1980 and 1981 growing seasons, three to five alfalfa stands were surveyed for *C. trifolii* at approximately 2-wk intervals. Each stand was within 15 km of Stillwater, was 1–2 yr old (1980), and was planted to cultivars Cody, Riley, or Baker. Surveys included both irrigated and dryland alfalfa stands. To detect *C. trifolii*, stems were collected at random along a W-shaped path in each stand (17), and stem sections were incubated as described.

RESULTS

Study 1. Symptoms of anthracnose appeared 1–2 wk after a large amount of rainfall and during a rise in average daily high temperature (Figs. 1–3). According to this pattern, symptoms also should have appeared on several other occasions, but these occurred during harvest or before sufficient time had elapsed for regrowth to form a canopy. These results are similar to those of Hartung et al (15), who found a 2-wk delay of symptom expression of blueberry anthracnose after conidial spread of *C. gloeosporides* Penz. When shepherd crooks were present, about 2% of alfalfa stems were affected. Harvesting alfalfa stands appeared to be a means of removing both inoculum and susceptible host tissue. Thus, time available for disease buildup during the growing season was between cuts, rarely more than 6 wk.

Study 2. Although anthracnose symptoms were not always present, recovery of *C. trifolii* from alfalfa tissue was possible throughout the 1981 growing season at most sites (Table 1). Abnormally hot, dry weather during most of the 1980 growing season was probably the reason *C. trifolii* was not recovered until September. In most stands, percent recovery of *C. trifolii* was greater early in the season. This may have been due to different pathogens competing for the stem surface. *Phoma medicaginis* Malbr. & Roum. var. *medicaginis* Fekl. was the predominant pathogen observed during June and July, and *Cercospora medicaginis* Ell. & Ev. along with *Leptosphaerulina briosana* (Poll.) Graham & Luttrell predominated from August through October.

DISCUSSION

Formation of shepherd crooks (after spread of inoculum during rainfall) appears to be a result of hot weather (Figs. 1–3). Infected stems often die prematurely, thus precluding spread of infection to the crown and to surrounding plants. Death of infected stems and plants is probably beneficial for prolonged stand life. Dead tissue observed during

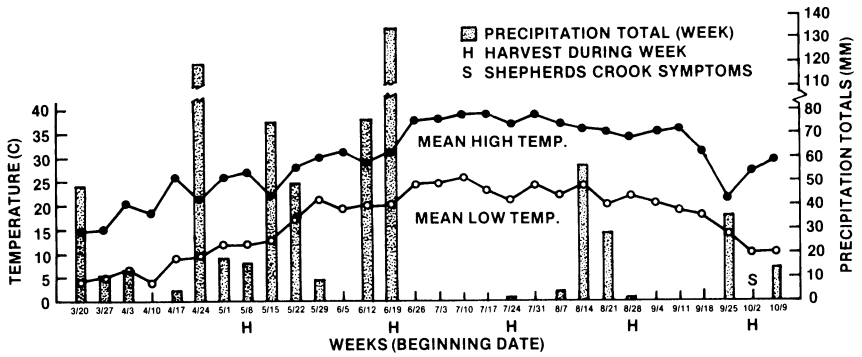

Fig. 1. Weekly precipitation totals, mean weekly high and low temperatures, harvests, and occurrence of alfalfa anthracnose symptoms during the 1980 growing season.

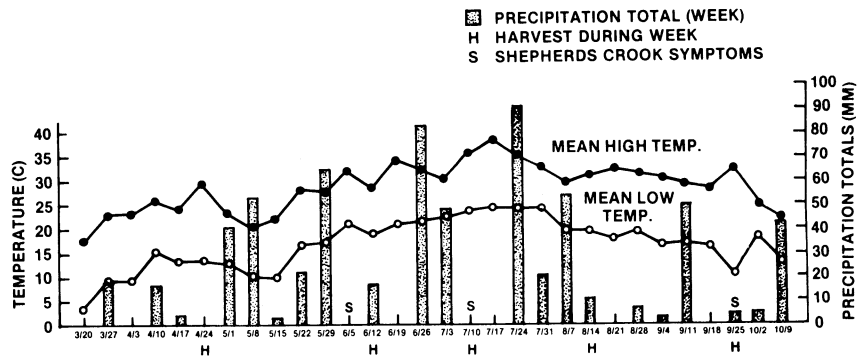

Fig. 2. Weekly precipitation totals, mean weekly high and low temperatures, harvests, and occurrence of alfalfa anthracnose symptoms during the 1981 growing season.

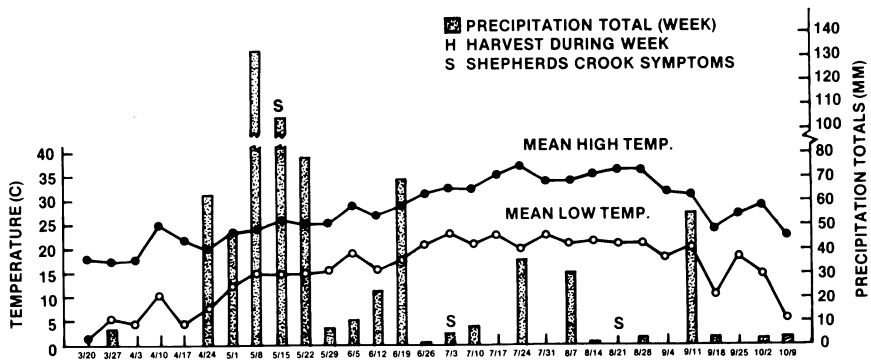

Fig. 3. Weekly precipitation totals, mean weekly high and low temperatures, harvests, and occurrence of alfalfa anthracnose symptoms during the 1982 growing season.

this study was quickly overrun by saprophytic fungi that suppressed sporulation by *C. trifolii*. Therefore, the pathogen is less likely to spread from this material. If a shepherd crook does not form after infection, the pathogen is free to grow within the host, possibly inciting the crown rot phase of anthracnose (13).

We did not attempt to isolate *C. trifolii* during the winter, but reports from other states indicate the pathogen should have no trouble surviving Oklahoma winters. Lukezic (18) reported that *C. trifolii* could survive as long as 100 days under winter conditions in Pennsylvania. Carroll et al (8) had no trouble reisolating *C. trifolii* from the field after 136 winter days in Delaware, probably because of milder climatic conditions in that state. *C. trifolii* has been reported to overwinter in haystacks, in crop debris, and on harvesting equipment stored in unheated sheds (13,18).

Secondary inoculum spreads quickly during warm, wet weather (13). Welty and Rawlings (26) found in greenhouse and growth chamber tests that anthracnose develops in alfalfa at 10–30 C. The disease is limited by high temperatures because conidial germination and appressorium formation are suppressed above 27 C. Tu (24), working with white bean (*Phaseolus vulgaris* L.) and *Colletotrichum lindemuthianum* (Sacc. & Magn.) Briosi & Cav. in southern Ontario, however, found that lower nocturnal temperatures permitted infection to occur. Prolonged high temperatures were found to suppress symptom development. Tu (23) found that conidia of *C. lindemuthianum* on white bean could be spread a short distance by splashing rain drops. Long-distance spread of 3–4 m required wind-driven rain. White bean anthracnose spreads from an infection focus in the same direction as the prevailing winds, with severity of the disease declining from the focus outward.

Data from Barnes et al (3) indicate that a dense canopy is beneficial to spread of *C. trifolii* because it creates a moist chamber effect. In their study, cultivar Iroquois produced the highest yields in August and had the highest level of disease in September 1967. An increase in canopy density may have several effects on disease development. Increased host density will result in a shorter distance for spores to travel and more targets to intercept inoculum. Increased host density will also cause environmental changes within the canopy that will favor disease, such as a change in the day-night temperature differential, an increase in relative humidity, and changes in wind velocity (5). Tu (25) reported that alternating wet-dry cycles are detrimental to survival of *C. lindemuthianum*.

In Oklahoma, spring appears to be the most favorable time for anthracnose development. Sporulation of *C. trifolii* is facilitated by cool-temperature (3 C) incubation (1). Temperatures become warmer in March and April, and May is normally the wettest month of the year. Precipitation in May does not depend on scattered heavy thunderstorms to make up the total (19). Yields are greatest during the spring, so canopies are also dense at this time (4). After mid-June, daytime temperatures are generally inhibitory to *C. trifolii*, precipitation becomes scattered, and yield is reduced, resulting in a less dense canopy (4,19,20). During the fall, precipitation increases and temperatures fall, allowing surviving *C. trifolii* inoculum to build up before winter (18).

The best evidence of when anthracnose caused significant damage in Oklahoma was data from a long-term study in which four alfalfa anthracnose bioindicator pairs were grown at Stillwater (6). Differences in forage yield between resistant and susceptible strains were not significant during 1974. During 1975 and subsequent years, differences between resistant strains Arc and Beltsville 2-An4 and their susceptible parent strains Team and Saranac for percent stand were significant ($P = 0.05$) (*unpublished*). In that study (6), the initial significant differences among resistant and susceptible strains coincided with an unusually large amount of precipitation during the fall of 1973 and of 1974 (19). During this time, in cultivar evaluation trials conducted throughout Oklahoma, cultivars that are highly resistant to *C. trifolii*, such as Arc, ranked well in forage yield (7). During subsequent years, maximum precipitation occurred in the spring, and long periods of drought were common during the summer. No prolonged periods favorable for *C. trifolii* inoculum increase could be identified. In alfalfa cultivar evaluation trials conducted throughout the state, cultivars resistant to *C. trifolii* (such as Arc) did not continue to show a yield advantage (21).

Resistance to *C. trifolii* does not appear necessary during typical years in Oklahoma. Although occurrence of anthracnose symptoms is common when weather patterns are favorable and *C. trifolii* appears to be present throughout the growing season in alfalfa stands, frequent harvests prevent inoculum buildup. Precipitation patterns occasionally change, however, and conditions favorable to anthracnose development do occur. If these conditions occur as frequently as every 5 yr (average stand life), resistance to *C. trifolii* becomes an asset to alfalfa production in the state.

Table 1. Percent recovery of *Colletotrichum trifolii* from alfalfa stem samples at five sites during the 1981 growing season

Date	Percent recovery at site				
	1	2	3	4	5
22 May	7	10	19	7	8
5 June	NC[a]	NC	15	15	18
19 June	0	NC	9	5	5
3 July	2	12	18	40	40
24 July	NC	11	0	9	9
7 August	0	0	2	18	0
28 August	10	10	0	NC	NC
11 September	NC	NC	0	NC	NC
25 September	11	NC	0	0	0
16 October	NC	40	0	8	2
30 October	NC	NC	NC	0	0

[a] No collection.

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