A New Race of *Colletotrichum trifolii* on Alfalfa in Oklahoma

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ABSTRACT

Allen, S. J., Barnes, G. L, and Caddel, J. L. 1982. A new race of *Colletotrichum trifolii* on alfalfa in Oklahoma. Plant Disease 66:922-924.

Pathogenicity of five Oklahoma isolates of *Colletotrichum trifolii* (OK1–OK5) and one Kansas isolate (KS1) were compared with Pennsylvania isolate PA1 (race 1) on alfalfa (*Medicago sativa*) in a greenhouse. Pathogenicity was determined by differential percentage of seedling survival of 10 alfalfa cultivars. Cultivars highly resistant to PA1 also had resistance to Oklahoma and Kansas isolates. One Oklahoma isolate (OK3) and the Kansas isolate (KS1) appeared to be race 1. Four cultivars were more resistant to four of the Oklahoma isolates than to PA1. No Oklahoma isolate was pathogenic on Arc, so none was considered to be race 2. Three Oklahoma isolates (OK1, OK2, and OK4) produced similiar reactions different from race 1. Race 3 is proposed to designate this group. The fifth Oklahoma isolate (OK5) produced resistant reactions on all cultivars tested, but it appears to be discrete.

Anthracnose (caused by *Colletotrichum trifolii* Bain & Essary) is a serious disease of alfalfa (*Medicago sativa* L.) in many areas of the world (7,10,11,19). The disease may kill plants during the growing season or may predispose them to winter injury (9).

Production of four alfalfa strains resistant to *C. trifolii* by Devine et al (6) showed the importance of anthracnose to stand longevity. When four strains of alfalfa resistant to the pathogen and their parent cultivars (Beltsville 1-An4 and Glacier; Beltsville 2-An4 and Saranac; Beltsville 3-An4 and Vernal; and Arc and Team) were grown at Stillwater, a significant increase in annual dry forage yields and final stand density was observed for three of the four pairs. Glacier and Beltsville 1-An4, the lowest yielding pair, never differed significantly for yield or stand density (3). These results, along with observations of symptoms in several areas of Oklahoma, suggest that anthracnose is an important disease in the state.

Isolation in 1979 of a strain of *C. trifolii* (now designated race 2 [13]) in Maryland and North Carolina that is highly virulent to Arc indicated that physiologic specialization must now be taken into account in breeding cultivars resistant to *C. trifolii* (14,20). To our knowledge, no survey of *C. trifolii* isolates has been made outside of the mid-Atlantic states. The objective of this study was to collect isolates of *C. trifolii* from Oklahoma and compare their virulence with that of race 1 of the pathogen.

This research was supported in part by Oklahoma Agricultural Experiment Station and by grant 910-15-48 of the U.S. Department of Agriculture, Agricultural Research Service.

Approved for publication as Journal Article J-4040 of the Oklahoma Agricultural Experiment Station.

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Accepted for publication 3 February 1982.

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0191-2917/82/10092203/$03.00/0
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MATERIALS AND METHODS

Source of isolates. In September and October 1980, alfalfa samples were collected throughout Oklahoma. Plant material was collected for isolation regardless of symptom expression. *C. trifolii* was successfully isolated from alfalfa collected at Goodwell, Guymon, Haskell, Stillwater, and Lamont (designated as isolates OK1, OK2, OK3, OK4, and OK5, respectively). *C. trifolii* was not found at Lahoma, Mangum, Tipton, Chickasha, or Perkins. The typical symptoms of anthracnose—diamond-shaped lesions on lower stems and a blue black discoloration of crown tissue (9)—were observed only at Guymon and Goodwell. In addition to *C. trifolii, C. dematium* (Pers. ex Fr.) Grove f. sp. *truncata* (Schw.) von Arx was isolated at Stillwater, Chickasha, Haskell, Tipton, Mangum, and Lamont. *C. destructivum* O'Gara was isolated at Mangum. The *C. dematium* f. sp. *truncata* and *C. destructivum* isolates were only slightly pathogenic (seedling survival was greater than 80%). These results agree with those of Graham et al (8) and Raynal (15).

Isolates KS1 (obtained from D. L. Stuteville, Kansas State University, Manhattan) and a Pennsylvania isolate, PA1 (obtained from R. E. Welty, USDA Forage Research Laboratory, Oxford, NC), were also included in pathogenicity tests.

Isolation of the pathogen. The lower 6 cm of stems was cut into 2-cm sections, and chips of crown tissue (approximately 0.5 × 0.5 × 1.0 cm) were surface-disinfested in a 1.31% solution of sodium hypochlorite for 2 min. Tissue segments were placed on water agar amended with tetracycline (25 μg/ml) and streptomycin (50 μg/ml) and incubated at 3 C for 2 days followed by 20 C for 5 days (S. A. Ostazeski, *personal communication*). Tissue segments were observed under a dissecting microscope, and streaks were made from sporulating acervuli (14) onto Proteose Peptone No. 2 (Difco) dextrose agar (PP2DA) (1). Isolates were stored at 21 C under sterile mineral oil.

Pathogenicity determinations. The procedure used to test isolates for pathogenicity was modeled after the technique of Ostazeski et al (12). Wooden flats (56 × 13 × 8 cm) were filled with a 3:1 mixture of sterilized mortar sand and perlite containing 25 g of 14-14-14 Osmocote (Sierra Chemical Co., Milpitas, CA 95035) fertilizer per 20 L of potting mix. One row each of the following cultivars was planted in each flat: Arc, Buffalo, Cimarron, Riley, WL318, Vangard, Saranac AR, Liberty, Baker, and Oklahoma Common (Kohler). Sufficient seeds (counted with an electronic counter [The Old Mill Company, Savage, MD 20863]) of each cultivar were planted to ensure 40 ± 5 seedlings per row. After planting, seeds were covered with 5–10 mm of vermiculite.

Ten-day-old seedlings in 12 flats were

inoculated at one time; six flats served as a standard and were inoculated with PA1 (an isolate of race 1). Plants in the remaining six flats were inoculated with conidia from either OK1, OK2, OK3, OK4, OK5, or KS1 isolates of *C. trifolii*. Each isolate was inoculated twice onto plants in two sets of flats.

Inoculum was prepared by flooding petri plates containing 10-day-old colonies of *C. trifolii* on PP2DA with distilled water; conidia were dislodged with a rubber policeman. The resulting conidial suspension was standardized with a hemacytometer to $10^6 \pm 10^5$ conidia per milliliter. Inoculum was sprayed onto 10-day-old plants with a hand-held sprayer. Plants were incubated for 72 hr in a mist chamber (100% RH) with daily high temperatures of 20–28 C and mean night temperatures of 18–20 C (16-hr photoperiod). Coverage of foliage by free water is necessary for infection (2). Two humidifiers (Standard Engineering Works, Inc., Pawtucket, RI 02860) supplied enough moisture for the mist chamber, which measured 117 × 262 × 117 cm and was covered with clear polyethylene. After incubation, plants were returned to the greenhouse (18–21 C night, 25–29 C day).

Initial plant counts were made 9 days after seeding, and the surviving plants were counted 14 days after inoculation. Because 10-day-old seedlings were used, all but the plants most resistant to *C. trifolii* (and rare escapes caused by late germination) were killed (12). Pathogenicity was based on percentage of initial plants that survived until the second count was made. Anthracnose was more severe on cultivars susceptible to the pathogen at higher incubation temperatures. Similar results have been observed by Welty and Rawlings (21). This effect did not significantly alter results of the experiment because a standard isolate of *C. trifolii* (PA1) was included in each run.

Results from analysis of variance indicated that data from each isolate were homogeneous. Therefore, data from all flats of plants inoculated with a given isolate were pooled.

RESULTS AND DISCUSSION

In culture, the Oklahoma and Kansas isolates appeared different from PA1 (Fig. 1). Isolates OK1, OK2, OK3, OK4, OK5, and KS1 produced white to orange colonies; PA1 produced dark brown colonies on PP2DA. Several of the Oklahoma isolates and KS1 produced aerial hyphae. Cultural variation in *C. trifolii* has been noted previously (22).

Reactions of 10 cultivars to seven *C. trifolii* isolates were recorded as percentage of survival (Table 1). The reaction of each cultivar to isolates KS1 and OK3 was not statistically different ($P = 0.05$) from the reaction of seedlings to PA1, implying that KS1 and OK3 are race 1. Cultivars of alfalfa both susceptible and resistant to PA1 have equal resistance to isolate OK5 of *C. trifolii*.

The cultivars Baker, Riley, and WL318 were significantly ($P = 0.05$) more resistant to isolates OK1, OK2, and OK4 than to isolate PA1. In addition, Buffalo was significantly ($P = 0.05$) more resistant to OK1 than to PA1; and the Oklahoma Common (Kohler strain) was significantly ($P = 0.05$) more resistant to OK2 than PA1 (Table 1). It is probably unwise to consider isolates OK1, OK2, and OK4 distinct races because of the similarity of their reactions. If one considers the $P = 0.10$ level of significance, then cultivars Baker, Buffalo, Riley, WL318, and the Oklahoma Common (Kohler strain) were all more resistant to isolates OK1, OK2, and OK4 of *C. trifolii*, thus forming a pattern.

Within these three inoculation groups, apparent survival of the cultivars resistant to *C. trifolii* (Arc, Cimarron, Liberty, Saranac AR, and Vangard) was not significantly different ($P = 0.05$) from their reaction to PA1 (Table 1).

The strain-cultivar interaction was significant ($P < 0.1$), suggesting that isolates OK1, OK2, and OK4 should be considered a distinct race that is less virulent than PA1 on cultivars that are susceptible to PA1 (race 1). According to

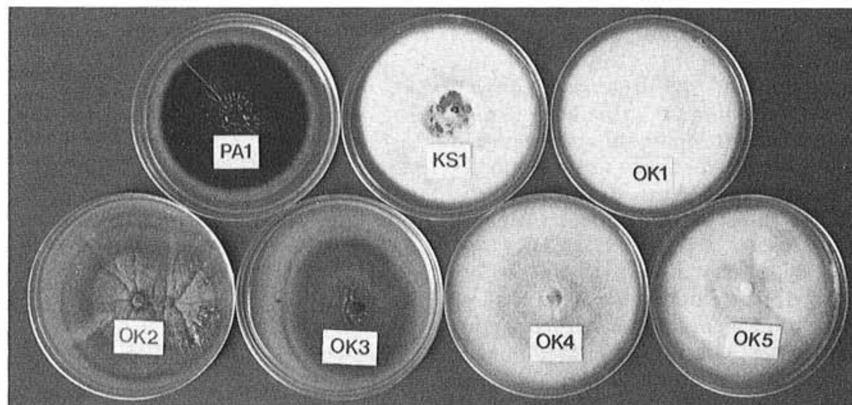

Fig. 1. Cultural variation of *Colletotrichum trifolii* isolates on Proteose Peptone No. 2-dextrose agar.

Table 1. Percentage of seedling survival of 10 alfalfa cultivars 14 days after inoculation with seven isolates of *Colletotrichum trifolii*

	Inoculation group[a]											
	A		B		C		D		E		F	
Cultivar	**PA1**	**KS1**	**PA1**	**OK1**	**PA1**	**OK2**	**PA1**	**OK3**	**PA1**	**OK4**	**PA1**	**OK5**
Arc	79.5	72.8	68.1	68.0	70.2	60.9	79.7	76.3	67.5	68.3	69.7	72.7
Baker	1.5	5.4	10.7	29.4[b]	1.9	25.4	4.1	11.2	1.2	16.9	10.2	69.6
Buffalo	2.9	6.4	11.1	28.1	3.1	16.1[c]	2.2	6.5	0.8	12.4	10.9	69.8
Cimarron	44.2	38.7	38.7	46.2	40.5	37.1	48.2	51.7	35.6	40.9	39.7	75.2
OK Common (Kohler)	4.8	6.0	18.2	31.6	3.2	23.1	4.2	15.6	2.0	13.8	19.3	73.6
Liberty	48.8	52.8	57.0	51.9	57.3	49.1	56.2	60.6	48.1	57.2	49.7	70.4
Riley	5.3	5.8	10.1	33.9	5.8	34.3	6.9	16.5	1.4	18.6	22.1	71.3
Saranac AR	72.3	66.5	67.9	63.9	73.0	65.0	71.8	67.9	60.8	61.1	71.6	77.1
Vangard	62.6	57.6	55.4	64.5	53.1	57.9	68.4	63.6	53.4	56.5	54.1	72.0
WL318	7.1	2.6	11.4	30.3	4.8	24.0	2.5	9.0	0.8	18.4	22.5	70.8

[a] During each inoculation, 40–45 ten-day-old plants of each cultivar in six flats were inoculated with PA1 and those in six flats were inoculated with KS1, OK1, OK2, OK3, OK4, or OK5. Means are the average of two inoculations (12 flats).

[b] Pairs of means underlined twice are significantly different at $P = 0.05$ (LSD = 14.0).

[c] Pairs of means underlined once are significantly different at $P = 0.10$ (LSD = 11.6).

this set of differential cultivars, these three isolates belong to the same race but are different from race 1 as exemplified by reactions of isolates PA1, KS1, and OK3 in this test. Because none of these isolates attacks the cultivar Arc, none can be race 2. We propose to designate these isolates as race 3. Because OK5 is the result of isolation from only one infected stem at one location and no other similar isolates were found, it cannot be assumed that this culture is representative of a population (18). Isolate OK5 appears to be discrete but should not be given a race designation until other, similar isolates are collected.

Resistance to race 1 of *C. trifolii* in alfalfa is conditioned by one dominant gene inherited in a tetrasomic manner (4). The cultivars resistant to *C. trifolii* included in this experiment had similar levels of resistance to all isolates of *C. trifolii* tested, indicating that the same host gene is probably operating. Some reports suggest that other genes influence the resistant reaction of the host to *C. trifolii*. The cultivar Riley is reported to have resistance to *C. trifolii* under field conditions (17). Devine et al (6) reported that the response of Glacier under recurrent phenotypic selection was lower than that of Vernal using the same selection criteria. Collins (5) has suggested that one or more modifying genes in addition to the major gene were responsible for conditioning resistance to *C. trifolii*. The decrease in susceptibility of cultivars Baker, Buffalo, Riley, WL318, and the Oklahoma Common strain Kohler to isolates OK1, OK2, and OK4 suggests that race 3 of *C. trifolii* lacks virulence on one or more of these secondary genes. The heterozygous nature of alfalfa, and presumably heterokaryotic nature of *C. trifolii*, will make genetic studies of this host-pathogen system difficult.

Resistance in alfalfa to race 1 of *C. trifolii* should work well in Oklahoma for the foreseeable future. However, as acreages of cultivars resistant to *C. trifolii* increase, effectiveness of this resistance to the pathogen may decrease. The variability within the host should make new sources of resistance to *C. trifolii* relatively easy to identify and incorporate into adapted germ plasm.

Race 3 appears to be common in Oklahoma. In-depth surveys will be required to determine which race of *C. trifolii*, if any, predominates in the region. If further studies show race 3 to be widespread, race 1 rare, and race 2 nonexistent, this may explain why cultivars resistant to *C. trifolii* do not show as much yield advantage in Oklahoma as in other areas (16).

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