STUDIES ON SOUTHERN ANTHRACNOSE OF ALFALFA IN OKLAHOMA

By

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CHAPTER I

INTRODUCTION

Since its introduction to Oklahoma around 1900, alfalfa (*Medicago sativa* L.) has become a major crop in the State. Alfalfa has become known as "the Queen of the Forages" as the species contains the highest feed value of all common hay crops (38, 41). On an annual average basis, 200,000 hectares of alfalfa are grown for hay and seed production in Oklahoma. The value of this cash crop to the State exceeds $120 million each year (90).

Since 1950, several potentially devastating disease and insect pest problems have developed, and become distributed throughout Oklahoma. The disease problems include *Phytophthora* root rot, *Fusarium* wilt and root rot, *Rhizoctonia* root rot, and southern anthracnose caused by *Colletotrichum trifolii* Bain and Essary (24). The full significance of southern anthracnose was not realized until Devine et al (36) developed resistant strains to *C. trifolii* from each of four commonly grown alfalfa cultivars. When these strains were grown at Stillwater, a large increase in yield during the fourth and fifth year was observed for resistant strains over the susceptible parents in three of the four pairs (22).

Colletotrichum trifolii, along with Phytophthora megasperma Drechs f. sp medicaginis (Erwin & Kuan), Rhizoctonia solani Kuehn, and Fusarium spp., is a crown and root rotting pathogen. These fungi are especially serious alfalfa pathogens, as they may kill plants and cause stand decline (50). After a few years of such decline, the alfalfa stand becomes weedy and unproductive (33).

Host resistance is the most practical means to control alfalfa diseases (71). Resistance to C. trifolii is available and resistant cultivars have been developed by recurrent phenotypic selection (36).

A need exists to integrate pest management practices and procedures for control of plant pathogens, insects and weeds in alfalfa (112). A review by Altman and Campbell (3) discusses several studies reporting both increases and decreases in diseases in several crops as a result of herbicide application. Insecticide-disease interactions have not been studied extensively, but have been reported (106). Cultural practices also affect occurrence and degree of plant diseases (37). Development of multiple pest resistant cultivars is a basic step in integrating pest management procedures. Successful production and use of multiple-pest resistant cultivars will result in improved crop performance as well as a cleaner environment due to reduced pesticide applications (55).

The overall objective of the research reported in this thesis is to collect information regarding the importance of

southern anthracnose of alfalfa in Oklahoma, and to lay the foundation for integrating resistance to C. trifolii into the alfalfa breeding program at the Oklahoma Agricultural Experiment Station. The individual objectives were to: 1) determine the pathogenic characteristics of Oklahoma isolates of C. trifolii; 2) determine the importance of C. destructivum O'Gara and C. dematium (Pers. ex Fr.) Grove f. sp. truncata (Schw.) v. Arx as alfalfa pathogens in Oklahoma; 3) determine when symptoms or southern anthracnose occur in the field; 4) determine the applicability of recurrent phenotypic selection as a procedure for use in breeding for resistance; 5) screen and evaluate selected strains, lines, and cultivars of alfalfa for resistance to C. trifolii; and 6) compare possible components of adult plant resistance to C. trifolii with greenhouse measurements of seedling resistance to the pathogen.

CHAPTER II

LITERATURE REVIEW

Historical. The causal agent of southern anthracnose, Colletotrichum trifolii was first described in Tennessee on red clover (Trifolium pratense L.) by Bain and Essary in 1905. Colletotrichum trifolii was first isolated from alfalfa (Medicago sativa L.) the same year by Westgate in Virginia (9). By 1906, the disease had also been reported in West Virginia, Kentucky, Arkansas, Ohio, and Delaware (86). Preston (99) reported C. trifolii as a pathogen on alfalfa in Oklahoma in 1945. Within North America, C. trifolii has also been reported in New York, Washington, Minnesota, and Quebec (44, 80, 102, 103). The pathogen is distributed worldwide, as southern anthracnose has been reported in France, Japan, India, Argentina, and Australia (46, 63, 97, 98, 120).

Varying assessments of the importance of southern anthracnose have been made since the disease was first reported. Bain and Essary (9) described the devastation C. trifolii caused in red clover fields as "remarkable". Henderson and Smith (60) in 1948 suggested that southern anthracnose was a more important component of "summer killing" of alfalfa stands than previously thought. By

contrast, Hanson and Allison (54) in 1950 reported that C. trifolii was responsible for only a minor part of stand decline. Roberts et al (103) in 1959 did not consider C. trifolii a threat to New York alfalfa production unless a strain of the fungus adapted to lower temperatures in NY were to develop.

The development of four alfalfa strains resistant to C. trifolii by Devine et al (36) established the importance of southern anthracnose as a major limiting factor to alfalfa production. When these strains and their parent cultivars (the bioindicator pairs: Beltsville 1-An4 and Glacier; Beltsville 2-An4 and Saranac; Beltsville 3-An4 and Vernal; and Arc and Team) were grown at 24 locations across the United States, a significant increase in average annual forage yields of resistant strains over their parental cultivars was observed (40). At Stillwater, a significant increase in annual dry forage yields and final stand density was observed for three of the four bioindicator pairs. Glacier and Beltsville 1-An4, the lowest yielding pair, never differed significantly for yield or stand density (22). This result may have been due to the dormancy of Glacier (21).

Symptomatology. The most distinctive symptom of southern anthracnose is the occurrence of diamond shaped lesions on the stems of susceptible plants. Stems may be infected without the presence of this symptom, however. Other symptoms include a blue-black discoloration of

infected crown tissue. The formation of straw colored shepherds crooks when wilted stems die suddenly is often associated with southern anthracnose. Other diseases also cause formation of shepherds crooks, so care must be taken in associating this symptom with southern anthracnose (50, 112).

Taxonomy and Morphology. The form-genus Colletotrichum is classified in the form-order Melanconiales (containing only the Melanconiaceae) of the Fungi Imperfecti. The production of dark setae within the acervulus is the distinguishing characteristic of Colletotrichum (1). This character is variable, however, as Frost (45) found that C. linicola produced setose acervuli only at relative humidities below 95%. The hyaline conidia of C. trifolii are relatively short and broad (12-18 X 4.5-6 u). No perfect stage has been reported (118). Physiological specialization within C. trifolii will be discussed in Chapter III.

Epidemiology. Several means of overwintering by C. trifolii have been reported. In southern areas, the fungus may overwinter in infected crown tissue (50, 112). Lukezic (78) found that C. trifolii could survive no more than 100 days during winter conditions in Pennsylvania. Since this length of time is not sufficient to insure survival to the next growing season, survival in the field is probably not the source of primary inoculum in Pennsylvania. Carroll et

al (26) had no trouble re-isolating C. trifolii from the field after 136 winter days in Delaware. The difference in the reported results was probably due to the milder climatic conditions in Delaware.

Two other means of overwintering by C. trifolii have been reported. Watkins et al (121) have reported that C. trifolii can overwinter in hay stacks. Lukezic (78) found that C. trifolii survived in crop debris on harvesting equipment stored in a non-heated shed in Pennsylvania. The fungus did not survive in debris on exposed harvesting equipment.

Several workers have discussed the possibility that C. trifolii may be seed-borne. Several species of Colletotrichum are seed transmitted (75). Carroll et al (26) were not able to isolate C. trifolii directly from seed. Montieth (86) suggested that inoculum was carried with the seed, on fragments of diseased stems or petioles.

The primary means of spread of C. trifolii to new alfalfa stands is probably the use of infested harvesting equipment (79). When old stands are harvested before new stands, equipment should be cleaned to prevent inoculum spread (50). Wahab and Chamblee (119) reported the spread of C. trifolii in irrigation water during the late summer.

Secondary inoculum spreads quickly during warm wet-humid weather (112). Welty and Rawlings (124) found in greenhouse and growth chamber tests that southern anthracnose develops in alfalfa at 10-30 C. The disease is

limited by high temperatures, as conidial germination and appresorium formation are reduced above 27 C (84). Rain, dew, and water from overhead sprinklers spread conidia from sporulating acervuli to noninfected tissue (112). Tu (115) found that conidia of C. lindemuthianum on white bean (Phaseolus vulgaris L., cultivar Fleetwood) could spread a short distance by splashing rain. Long distance spread of 3-4 m required wind-driven raindrops. New alfalfa plantings adjacent to old stands have been reported to be infected by rain-disseminated inoculum (112). Tu (115) reported that white bean anthracnose spread from an infection focus in the same direction as the prevailing winds. Severity of the disease declined from the infection focus outward.

Data from Barnes et al (12) indicate that a dense canopy is beneficial to spread of C. trifolii. In their study, the cultivar Iroquois had the highest yielding plots in August followed by the highest level of disease in September, 1967.

Conidia produced by members of the form-genus Colletotrichum are borne in a water soluble mucilaginous matrix (110). Nicholson and Moraes (89) have found that the matrix is essential for survival of conidia of C. graminicola. Spore masses placed in relative humidities as low as 45% turned dry and powdery within 2 weeks. The conidia remained viable in this condition for 4 weeks. Washed spores (matrix removed) lost viability after 24 hr at

90% relative humidity. An acid invertase is the probable agent responsible for survival. A partially-purified invertase preparation was found to protect washed spores from a loss of viability (15).

Damage caused by Colletotrichum trifolii. The most obvious damage caused by C. trifolii is the immediate death of infected stems and plants during the growing season. Affected plants result in reduced yield of the next cutting. The pathogen can grow down the stem into crown tissue and cause the less obvious but more damaging crown rot phase of southern anthracnose (50). Crown tissue may be predisposed to infection by C. trifolii after mechanical injury due to livestock, harvesting or insect feeding (127).

Many crown-rotting pathogens play a role in progressive loss in stand density. C. trifolii has been implicated as a major cause of stand decline, especially in areas of high relative humidities (51, 64).

The crown rot phase of southern anthracnose may be responsible for winter injury and the lack of vigor in spring regrowth. Barnes et al (12), Jones et al (70) and Devine (32) have all found that resistance to C. trifolii was significantly related to winter hardiness and frost tolerance. Three other root and crown-rotting pathogens, Rhizoctonia solani Kuehn, Fusarium spp. and Phytophthora megasperma Drechs f. sp. medicaginis also predispose alfalfa to winter injury (127). Barnes et al (12) have suggested that the lethal effect of C. trifolii on underground stems

and buds was responsible for predisposing infected plants to winter kill. However, Devine (32) found no such relationship and concluded that increased winter survival of resistant cultivars was due to a greater number of surviving plants and a greater amount of regrowth. The effect of crown rot on winter hardiness under field conditions is probably underestimated in experimental plots, as plants are not exposed to excessive injury from harvesting equipment (127).

Stand decline leads to serious weed problems (33). Subsequent harvests are of reduced yield and forage quality (24). Devine and McMurtrey (34) found that the four strains selected for resistance to C. trifolii from cultivars Team, Glacier, Saranac and Vernal (bioindicator pairs) were significantly more competitive against weeds than their parent cultivars. The resistant strains showed a higher percentage of ground cover and a higher number of plants per unit area as a result of lower mortality due to disease (35).

Pathogenesis and resistance. Before disease can develop, inoculum of the pathogen must come into contact with a susceptible host. Several environmental factors must also be favorable. Conidia of Colletotrichum spp. are borne in a water-soluble mucilaginous matrix. The matrix functions in spore survival as well as to bond the pathogen to the surface of the host (16, 88). Conidia are released from the matrix by water, such as splashing raindrops (129).

Free water is necessary for successful inoculation in winged water primrose [Jussiaea decurrens (Walt.) DC.] with C. gloeosporioides f. sp. jussiaea in growth chambers (18). Mercer et al (83) reported that germination of C. lindemuthianum conidia was poor in water or on bean hypocotyls. Germination was stimulated by nutritional sources such as orange extract, peptone, mixtures of glucose and casamino acids, and pollen diffusate. Several of these compounds inhibited formation of appresoria and subsequent lesion development. Spores which had germinated in water caused maximum lesion development. Miehle and Lukezic (84) found that Tween 20 (polyoxyethylene sorbitan monolaurate) increased germination of C. trifolii conidia (but not appressorial formation) at low rates (0.01 ml/liter). Germination was inhibited as rates increased. The two optimum pH values for germination of C. lagenarium conidia are pH 5.5 and 6.5 (128). High temperatures effect germination and appresorium formation in several species of Colletotrichum. Ishida and Akai (65) found conidia of C. lagenarium (Pass.) Ell. & Halst. would germinate at 20-30 C. Germination, although normal, at 32 C was not followed by appresorium formation. Miehle and Lukezic (84) found similar results with C. trifolii. Mycelia of C. lindemuthianum were able to survive heat treatments lasting five times as long as the duration required to destroy the pathogen's ability to produce germ tubes or appresoria

(100).

After formation of the appresorium, a penetration peg forms. Wheeler (126) suggests that enzymes may be bound to the tip of the penetration peg. Enzymatic activity is sharply localized during the penetration of corn by C. graminicola. Several pectolytic enzymes are produced by C. trifolii during disease development. The resulting degradation of primary cell walls and middle lamellae aid the movement of the pathogen within the host (52).

A toxin is also implicated in disease development. Johnson and Stuteville (67) isolated a high molecular weight compound which after purification was able to induce wilting and death in susceptible alfalfa seedlings. Frantzen et al (42) found the toxin to be a polysaccharide with units of galactose, glucose, mannose with a probable trace of uronic acid. Anderson (5) has isolated a toxic polysaccharide from C. trifolii cultures which caused browning and phytoalexin accumulation in bean.

Three outcomes are possible after successful establishment of C. trifolii within its host. Infections may be localized, latent, or systemic. Localized infections result in a canker, severely weakening the stem (29).

Latent infections by C. trifolii have not been previously reported, but were observed during the course of this study. Latent infections by colletotricha which infect fruits and vegetables resulting in storage rot is a common problem (117). According to Tiffany (113), seed-borne

inoculum of C. dematium f. sp. truncata in soybean (Glycine max L.) result in establishment of internal mycelium. Under favorable conditions, the fungus then sporulates on pods at maturity. Latent infections were not found to be a significant inoculum source in tomato anthracnose epidemics caused by C. coccodes (76).

Systemically infected seedlings wilt and die resulting in a "seedling blight". Colletotrichum trifolii grows rapidly in vascular tissue of susceptible plants (111). In older plants, C. trifolii may grow down to the crown starting the crown rot phase of the disease (50). Davis et al (31) have found that shoots of alfalfa infected with C. trifolii produce more ethylene than did healthy shoots. Ethylene evolution coincided with maximum sporulation of C. trifolii and symptom production. Epinasty can be induced by ethylene (111).

The resistant reaction of alfalfa in the presence of C. trifolii is hypersensitivity. Germination and appresorium formation is normal, but penetration does not occur. Cortical cells beneath the appressorium die and collapse resulting in a dark fleck (71).

Phytoalexins have been implicated in the resistance of alfalfa to C. trifolii. Ostazeski and Elgin (94) speculate that race 1 of C. trifolii can induce phytoalexin accumulation in resistant cultivars (to race 1), but race 2 does not. When seedlings of the cultivar Arc (resistant to

race 1; susceptible to race 2) were inoculated with race 1, the seedlings were protected against race 2 within 60 min (92).

A second type of resistance mechanism may be involved in the resistance of alfalfa to C. trifolii. Kuc and his coworkers (27, 57, 66, 74) have described a systemic resistance to C. lagenarium in cucurbits such as watermelon (Citrullus vulgaris L.) and cucumber (Cucumis sativus L.). A question exists if this type of resistance is due to a phytoalexin, as these antifungal compounds do not move appreciably from their point of synthesis (4). When watermelon and muskmelon were inoculated with C. lagenarium, the plants were systemically protected from subsequent inoculations (27). Both numbers and size of lesions were reduced when cucumber was inoculated after a cross protection inoculation with C. lagenarium. The systemic protection was evident in the second true leaf 96 hrs after inoculation of the first true leaf (79). This system is neither pathogen nor cultivar specific as protection against C. lagenarium in cucumber could be induced by either C. lagenarium or Tobacco Necrosis Virus. Protection could be transferred by grafting material from the protected cucumber to pumpkin or squash (66). Subsequent inhibition of penetration associated with the host epidermal cell wall is due to lignification directly under appressoria. Mycelia of the pathogen is also lignified. A precursor of lignin, coniferyl alcohol, is toxic to C. lagenarium, and if it is

present in the host in sufficient quantities, may function as a phytoalexin (57).

Control of southern anthracnose. Losses due to southern anthracnose can be reduced by cultural measures such as mowing young stands before old stands, sanitation of harvesting equipment, and crop rotation. The most practical means of control is the use of resistant cultivars. Although resistant cultivars are available, they are not adapted to all areas of the country where southern anthracnose is a problem (19, 50, 112). By use of appropriate breeding techniques, locally-adapted cultivars can be developed (48).

Bain started to breed red clover lines for resistance to C. trifolii in 1906 when resistant plants were observed in diseased populations (110). In 1950, Jones (68) found differing susceptibilities of alfalfa lines to C. trifolii during an unusual outbreak of southern anthracnose in a Wisconsin greenhouse.

Prior to 1925, the main goal of alfalfa breeding was to increase winter hardiness. After 1925, a second goal was added: that of breeding lines resistant to Corynebacterium insidiosum (McCull.) H. L. Jens., the causal agent of bacterial wilt. When the need for multiple-pest resistant alfalfa cultivars was realized about 1955, several germplasm sources were added to the traditional pools. Flemish germplasm was introduced into the United States about 1960

(11). This germplasm has proven to be very susceptible to C. trifolii (50). The development of four alfalfa strains resistant to C. trifolii by Devine et al (36; discussed previously) has shown that recurrent phenotypic selection can be utilized to increase resistance to the pathogen in susceptible populations.

Screening of alfalfa populations for plants resistant to C. trifolii has been reported successful under both greenhouse and field (natural epidemic) conditions (12, 95). Greenhouse screening has been utilized widely during development of resistant cultivars, as favorable conditions for the pathogen can be assured. A large amount of resistant material can be obtained in a short time (95). The basic greenhouse screening procedure is described in Chapter III. Smith (108) selected surviving plants from the cultivar DuPuits after a natural epidemic in Virginia. Experimental strains from the polycrossed seed were found superior in resistance to C. trifolii and in stand persistence to the check cultivar, Williamsburg. The resulting experimental strains did not show resistance in greenhouse tests. Two laboratory techniques for screening for resistance to C. trifolii using agar plates and plastic shoeboxes have been described, but not widely utilized (49, 87). Ostazeski and Elgin (93) have devised a method which involves the injection of a spore suspension into the stem. Maxon et al (81) have developed a tissue culture system for selection on a cellular basis.

CHAPTER III

PATHOGENICITY OF OKLAHOMA ISOLATES OF *COLLETOTRICHUM TRIFOLII* TO ALFALFA

INTRODUCTION

The isolation, in 1979, of a strain of *Colletotrichum trifolii* [now designated race 2 (91)] in Maryland and North Carolina that is highly virulent on alfalfa cultivars Arc and Liberty indicated that physiological specialization must now be taken into account in breeding resistant cultivars (96, 123). Races 1 and 2 can be differentiated on the basis of their reactions on Arc, Saranac and Saranac AR (91). Race 1 is pathogenic only on Saranac; race 2 is pathogenic on both Arc and Saranac, but not Saranac AR. Welty (122) has reported that races 1 and 2 are also able to differentially attack several species of forage legumes, including *Trifolium pratense* L., *Melilotus alba* Desr. and *Vicia villosa* Roth.

The only race identification from outside of the mid-Atlantic states has been the classification of two Minnesota isolates collected in 1978 and 1979 as race 1 (44). Race 1 appears to predominate worldwide, as the four bioindicator

pairs developed by Devine et al (36, described in Chapter II) have been tested in Argentina (97), France (46) and at 24 locations in the United States (40). At each location, Arc was not seriously debilitated in comparison to Beltsville 2-An4 (from which Saranac AR was derived).

Before adequate resistance to C. trifolii can be incorporated into germplasm adapted to Oklahoma, knowledge on the pathogenicity characteristics of the pathogen is necessary. The objective of this study was to collect isolates of C. trifolii from Oklahoma and compare their virulence to race 1 of the pathogen.

MATERIALS AND METHODS

Sources 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 (Fig. 1). The typical symptoms of anthracnose, diamond shaped lesions on lower stems, and a blue-black discoloration of crown tissue (50), were observed only at Guymon and Goodwell.

Isolate KS1 (obtained from D. L. Stuteville, Kansas State University, Manhattan, KS) and a Pennsylvania isolate, PA1, (obtained from R. E. Welty, USDA Forage Research

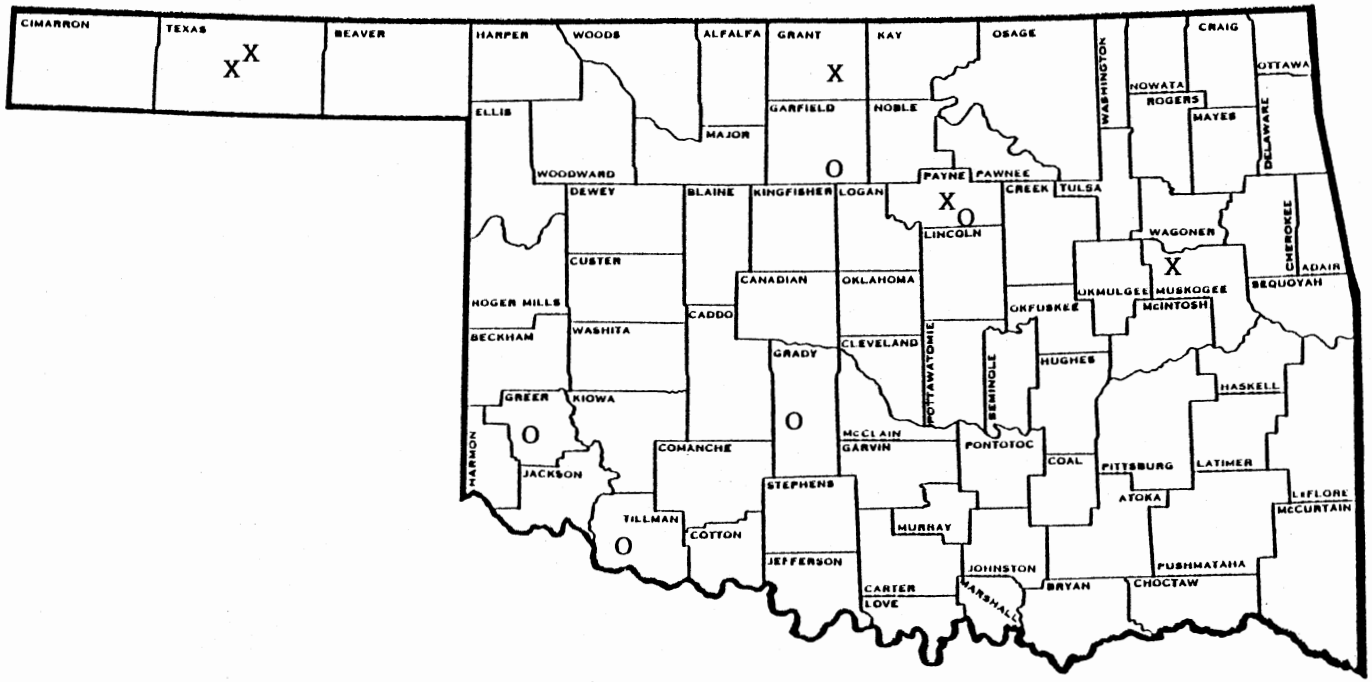

Fig. 1. Location of alfalfa fields surveyed for presence of Colletotrichum trifolii. An "X" indicates C. trifolii was isolated, and an "O" indicates the pathogen was not found.

Laboratory, Oxford, NC) were also included in pathogenicity tests.

Isolation of the pathogen. The lower 6 cm of stems were cut into 2-cm sections, and chips of crown tissue (approximately 0.5 X 0.5 X 1.0 cm) were surface-disinfested in a 1.31% NaOCl solution for 2 min. Tissue segments were placed on water agar amended with tetracycline (25 ug/ml) and streptomycin (50 ug/ml) (WTSA) and incubated at 3 C for 2 days followed by 20 C for 5 days (S. A. Ostazeski, personal communication). C. trifolii, C. dematium f. sp. truncata and C. destructivum were differentiated microscopically (Figs. 2 and 3). Tissue segments were observed under a dissecting microscope, and streaks were made from sporulating acervuli (Fig. 4) (96) onto Proteose Peptone No. 2 (Difco) - dextrose agar (PP2DA) (13). Isolates were stored at 21 C under sterile mineral oil.

Pathogenicity determination. The procedure used to test isolates for pathogenicity was modeled after the technique of Ostazeski et al (95). Wooden flats (56 X 13 X 8 cm) were filled with a 3:1 mixture of sterilized mortar sand and perlite containing 75 g of 14-14-14 Osmocote (Sierra Chemical Co., Milpitas, CA 95035) fertilizer per 20 liters of potting mix. Seeds were disinfested in 15% H_2O_2 for 10-20 min and air dried before counting (2). 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

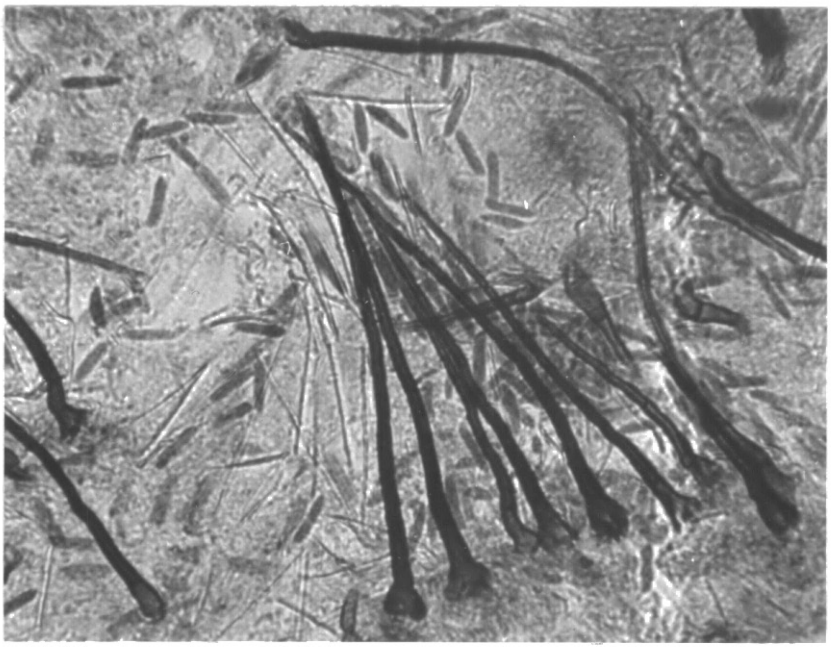

Fig. 2. Setae and spores of *Colletotrichum trifolii* on alfalfa tissue.

Fig. 3. Acervuli and spores of Colletotrichum dematium f. sp. truncata on alfalfa tissue.

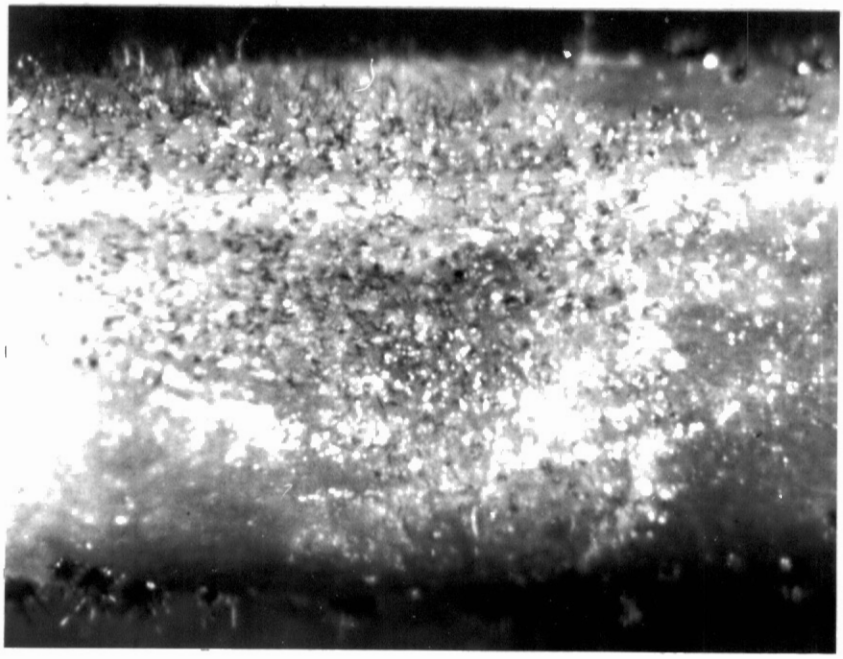

Fig. 4. Sporulating acervuli of Colletotrichum trifolii on an alfalfa stem.

seeds, [counted with an Electronic Counter (The Old Mill Company, Savage, MD 20863)] of each cultivar were planted to insure 40 to 45 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 to 900,000 - 1,000,000 conidia per ml with a hemacytometer. Two drops of Tween 20 (polyoxyethylene sorbitan monolaurate) were added per liter of suspension. 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 20-38 C and mean night temperatures of 18-20 C (16 hr photoperiod). Two humidifiers (Standard Engineering Works, Inc., Pawtucket, RI 02860) supplied sufficient moisture for the 117 X 262 X 117 cm mist chamber (covered with clear polyethylene; Fig. 5). 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

Fig. 5. Mist chamber used for the pathogenicity studies.

(Fig. 6); and surviving plants were counted 14 days after inoculation (Fig. 7). Because 10-day-old seedlings were used, all but the most resistant plants to C. trifolii (and rare escapes caused by late germination) were killed (95). Pathogenicity was based on percentage of initial plants that survived until the second count was made.

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 of C. trifolii appeared different from PA1 (Fig. 8). 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 (130).

Reactions of 10 cultivars to several C. trifolii isolates are presented in percent survival (Table 1). The reaction of each cultivar to isolates KS1 and OK3 were not significantly 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

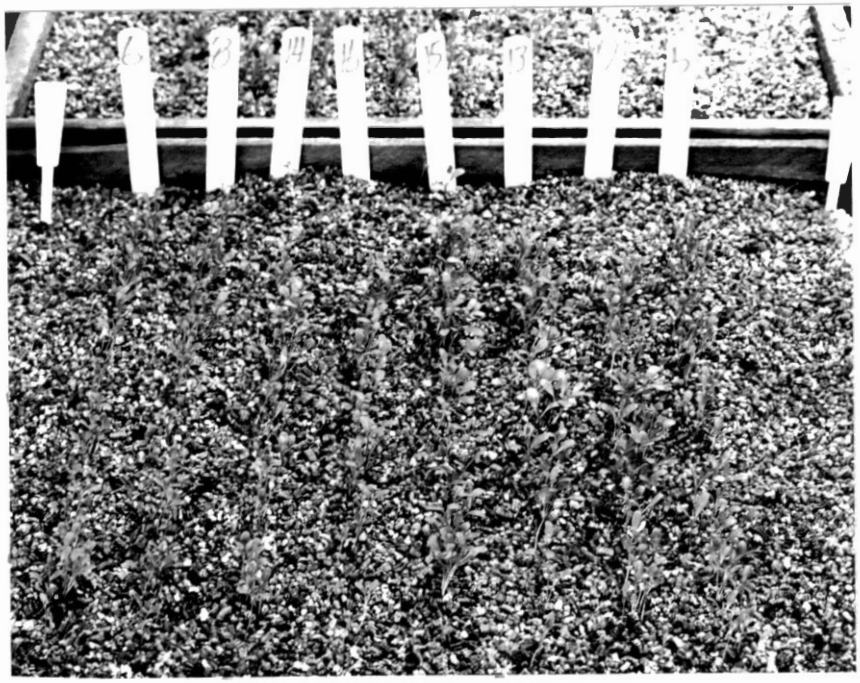

Fig. 6. Appearance of a typical flat of alfalfa prior to inoculation.

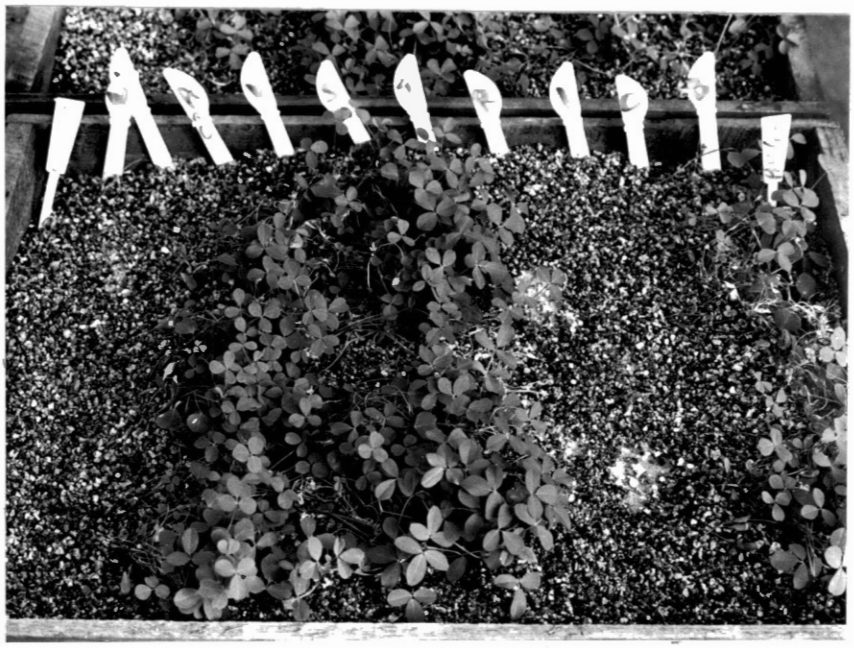

Fig. 7. Appearance of a typical flat of alfalfa 14 days after inoculation.

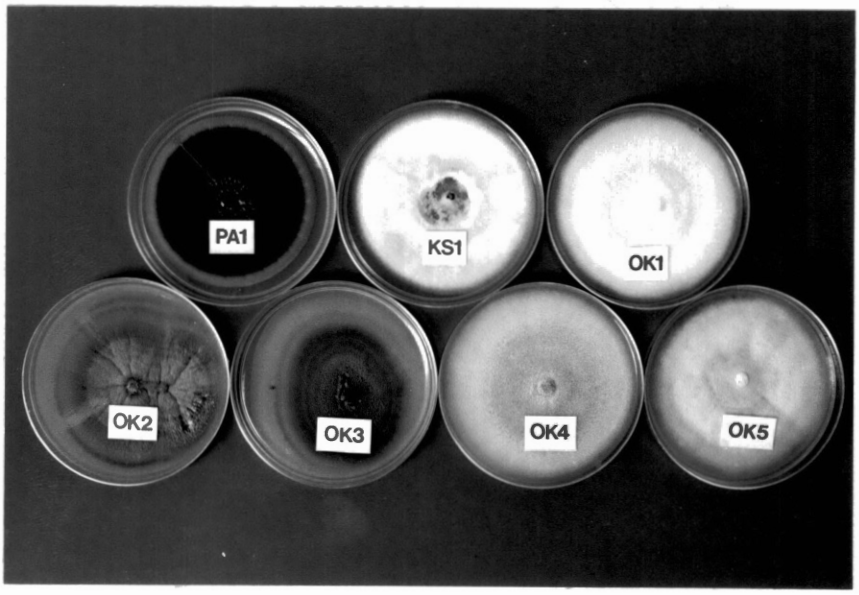

Fig. 8. Cultural variation of <u>Colletotrichum trifolii</u> isolates on Proteose Peptone Number 2 - dextrose agar.

Table 1. Percent 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	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[c]	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 to 45-10 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 (Fishers Protected LSD=14.0).

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

(P=Ø.Ø5) more resistant to isolates OK1, OK2 and OK4 than to isolate PA1. In addition, Buffalo was significantly (P=Ø.Ø5) more resistant to OK1 than to PA1; and Oklahoma Common (Kohler) was significantly (P=Ø.Ø5) more resistant to OK2 than PA1 (Table 1). It is probably unwise to consider isolates OK1, OK2 and OK4 distinct races due to the similarity of their reactions. Considering the P=Ø.1Ø level of significance, cultivars Baker, Buffalo, Riley, WL318 and Oklahoma Common (Kohler) were all significantly 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=Ø.Ø5) from their reaction to PA1 (Table 1).

The strain-cultivar interaction was significant (P=Ø.Ø5), 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 this set of differential cultivars, these three isolates belong to the same race but are different from race 1 was exemplified by reactions of isolates PA1, KS1 and OK3 in this test. Since none of these isolates attack cultivar Arc or Liberty, none can be race 2. Race 3 is proposed to designate the population represented by isolates OK1, OK2

and OK4. Since 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 (116). 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 (25). 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 of C. trifolii. The cultivar Riley is reported to have resistance to C. trifolii under field conditions (109). Devine et al (36) reported that the response of Glacier under recurrent phenotypic selection was lower than Vernal using the same selection criteria. Welty et al (125) have suggested that the reactions of the plants included in each cycle of selection during the production of resistant cultivars influences the general or specific nature of resistance to C. trifolii. Collins (28) has suggested that one or more modifying genes in addition to a major gene were responsible for conditioning resistance to C. trifolii. The decrease in susceptibility of cultivars Baker, Buffalo, Riley, WL318 and Oklahoma Common (Kohler) to 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, would 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 and other species of Medicago should make new sources of resistance to C. trifolii relatively easy to identify and incorporate into adapted germplasm (19, 39).

Race 3 appears to be common in Oklahoma. In-depth surveys would be required to determine which races of C. trifolii, if any, predominate in the region. If further studies show race 3 to be widespread, race 1 rare, 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 (104).

CHAPTER IV

PATHOGENICITY OF OKLAHOMA ISOLATES OF *COLLETOTRICHUM DEMATIUM* F. SP. *TRUNCATA* AND *COLLETOTRICHUM DESTRUCTIVUM* TO ALFALFA

INTRODUCTION

In addition to *Colletotrichum trifolii*, two other species of *Colletotrichum* are often reported to occur on alfalfa. In 1940, Jones and Weimer (69) found that *C. trifolii*, *C. destructivum* O'Gara and *C. graminicola* (Ces.) Wilson occurred on alfalfa in Georgia. Subsequent workers have reported both *C. destructivum* and *C. dematium* (Pers. ex Fr.) Grove f. sp. *truncata* (Schw.) v. Arx as secondary colletotricha on alfalfa (47, 114). Spore shapes of *C. graminicola* and *C. dematium* f. sp. *truncata* are somewhat similar (118) which could have confused Jones and Weimer (69).

Reports on the pathogenicity of *C. dematium* f. sp. *truncata* and *C. destructivum* to alfalfa vary. Cox (30) in North Carolina found that *C. dematium* f. sp. *truncata* caused development of lesions during greenhouse inoculations which increased in size, girdled stems, and resulted in an "aerial damping off effect". Tiffany and Gilman (114) stated that

C. destructivum was pathogenic in Iowa on a number of legume species including alfalfa. Graham et al (47) reported that isolates of C. dematium f. sp. truncata and C. destructivum from Maryland were weakly virulent to alfalfa. One isolate used in their study from Canada was described as moderately virulent. Colletotrichumm dematium f. sp. truncata is reported to be a serious pathogen of soybean and lima bean (6, 8, 107). Physiological specialization in C. dematium f. sp. truncata among host species has been described (62).

Colletotrichum dematium f. sp. truncata was frequently isolated from alfalfa tissue in Oklahoma during 1980. Colletotrichum destructivum was isolated only from Mangum (Table 2).

Since C. dematium f. sp. truncata was 1) frequently encountered in Oklahoma, 2) has been reported to be an important pathogen on alfalfa and other crops, and 3) no studies on the pathogenicity of the fungus has been made in the Southwest, this study was conducted to determine the importance of C. dematium f. sp. truncata and C. destructivum to alfalfa in Oklahoma.

MATERIALS AND METHODS

Source of isolates. The isolates used in this study were collected throughout Oklahoma during 1980 (Table 2). Isolation procedures were as described in Chapter III.

Pathogenicity determinations. Due to the large number of C. dematium f. sp. truncata isolates collected, isolates

Table 2. Isolates of *Colletotrichum dematium* f. sp. *truncata* and *Colletotrichum destructivum* collected in Oklahoma during 1980

Isolate number	Location collected	Date	Included in CDT inoculation group
CDT 1	Stillwater	6-30-80	CDT-A
CDT 2	El Reno	7-9-80	CDT-A
CDT 3	Ripley	6-27-80	CDT-A
CDT 4	Stillwater	8-24-80	---[a]
CDT 5	Stillwater	9-5-80	---[a]
CDT 6	Chickasha	9-3-80	CDT-B
CDT 7	Haskell	9-23-80	CDT-C
CDT 8	Tipton	9-30-80	CDT-B
CDT 9	Mangum	9-30-80	CDT-B
CDT 10	Grant Co.	10-20-80	CDT-C
CDT 11	Stillwater	9-23-80	CDT-C
CD 1	Mangum	9-30-80	CD 1

[a]Not included in the inoculations.

were pooled as to geographic location in three groups. During inoculum preparation, conidia were collected from an equal amount of surface area from each isolate. Pathogenicity of the C. dematium f. sp. truncata and C. destructivum isolates were compared to race 1 (PA1) of C. trifolii. All other procedures were described in Chapter III.

RESULTS AND DISCUSSION

Reactions of 10 cultivars to C. dematium f. sp. truncata and C. destructivum isolates are presented in percent survival (Tables 3 and 4). In most instances resistance to C. dematium f. sp. truncata and C. destructivum was significantly greater than to C. trifolii (P=0.05). Where this was not true (C. dematium f. sp. truncata group A to Saranac AR and C. dematium f. sp. truncata groups B and C to Arc) resistance to both fungi was very high. C. dematium f. sp. truncata group B appeared to be less virulent than groups A or C, but this was not significant. The overall results agree with those of Graham et al (47) and Raynal (101) who found that most isolates of C. destructivum and C. dematium f. sp. truncata were weakly virulent.

The prevalence of C. dematium f. sp. truncata on alfalfa in Oklahoma may be due to its status as a secondary invader. Acervuli of C. dematium f. sp. truncata were often observed on wounded areas and next to the ends of stem

Table 3. Percent seedling survival of 10 alfalfa cultivars 14 days after inoculation with *Colletotrichum trifolii* and three groups of *C. dematium f. sp. truncata* isolates

	Inoculation group[a]					
Cultivar	**PA1**	**CDT-A**	**PA1**	**CDT-B**	**PA1**	**CDT-C**
Arc	78.5	100.0[b]	76.3	84.2	78.3	89.0
Baker	0.6	81.7	3.4	66.6	0.8	90.0
Buffalo	8.6	86.7	5.1	65.0	1.6	88.6
Cimarron	53.7	86.7	42.9	72.8	44.2	90.0
Kohler	0.9	86.8	6.8	61.5	0.5	91.9
Liberty	63.8	81.5	61.5	76.2	62.7	92.4
Riley	0.8	89.1	5.1	70.0	1.1	89.7
Saranac AR	74.5	90.1	68.6	87.0	73.0	90.3
Vangard	61.5	83.3	64.0	82.5	63.6	97.6
WL318	1.9	86.6	6.3	65.3	1.5	92.1

[a]During each inoculation, 40 to 45 - ten day old plants of each cultivar in six flats were inoculated with PA1, and those in six flats were inoculated with CDT group A, B, or C. Means are the average of two inoculations (12 flats).
[b]Pairs of means underlined twice are significantly different at P=0.05 (Fisher's protected LSD=15.8).

Table 4. Percent seedling survival of 10 alfalfa cultivars 14 days after inoculation with *Colletotrichum trifolii* (PA1) and *C. destructivum* (CD1)

	Isolate[a]	
Cultivar	**PA1**	**CD1**
Arc	75.4	99.7
Baker	6.3	95.1
Buffalo	6.2	92.1
Cimarron	51.9	94.2
Kohler	12.1	99.9
Liberty	62.9	93.9
Riley	13.1	96.6
Saranac AR	71.1	94.2
Vangard	69.5	100.0
WL318	9.3	90.6

[a]During each isolation, 40 to 45 - ten day old plants of each cultivar in six flats were inoculated with PA1, and those in six flats were inoculated with CD1. Means are the average of two inoculations (12 flats).

[b]Pairs of means underlined twice are significantly different at P=0.05 (Fisher's protected LSD = 15.8).

segments. Descriptions of anthracnose symptoms on alfalfa often mention the presence of large black fruiting bodies (50, 112). However, the acervuli of C. trifolii are small compared to C. dematium f. sp. truncata, and are not macroscopically visible (Figs 2 and 3). Colletotrichum dematium f. sp. truncata has been reported to be the only fungus that can be isolated from older anthracnose lesions (47).

Colletotrichum dematium f. sp. truncata and C. destructivum are not important pathogens on alfalfa in Oklahoma. These species may be, in fact, beneficial, as Graham et al (47) has reported the ability of C. dematium f. sp. truncata and C. destructivum to cross protect against subsequent infection by C. trifolii.

CHAPTER V

SEASONAL OCCURRENCE OF *COLLETOTRICHUM TRIFOLII* AND SOUTHERN ANTHRACNOSE SYMPTOMS

INTRODUCTION

Southern anthracnose (caused by *Colletotrichum trifolii*) is usually considered a warm weather disease of alfalfa (85). Symptoms are usually observed in September to early October (50). The occurrence of shepherds crooks and diamond shaped stem lesions has been associated with dryer areas within alfalfa stands (88). However, conidia of *C. trifolii* require periods of high humidity and free water on plant surfaces to spread and infect (112).

Plants may be infected with *C. trifolii* without expressing symptoms (112). *Colletotrichum trifolii* was isolated from several alfalfa stands which appeared healthy during 1980 in Oklahoma (Chapter III). The records of Oklahoma State University Plant Disease Diagnostic Laboratory for the past 5 years show that *C. trifolii* has been isolated from alfalfa tissue collected in the state from May to November. The majority of the isolations were made during June. This information indicated that *C.*

trifolii is present in alfalfa stands throughout the growing season.

Symptoms of southern anthracnose appear sporadically, possibly due to environmental influences. Knowledge of when *C. trifolii* can be isolated from alfalfa stands and when symptoms appear in relation to temperature and precipitation patterns would be helpful in possible future epidemiological studies. The objective of this study was to determine whether the isolation of *C. trifolii* from alfalfa tissue and the presence of symptoms could be linked to temperature and precipitation patterns during the growing season.

MATERIALS AND METHODS

From 28 May through 23 October 1981, five alfalfa stands were surveyed for presence of *C. trifolii* and southern anthracnose symptoms at 1 to 2 week intervals (Table 5). The only symptom observed during 1981 were "shepherds crooks" (Fig. 9). During each visit to a stand, presence or absence of shepherds crooks was recorded. Also, 1Ø stems were collected at random along a "W" shaped path in each stand (77). In the laboratory, the lower 6 cm of each stem was cut into three 2 cm segments. Segments were disinfected in a 1.31% NaOCl solution for 2 min and placed onto WTSA (described in Chapter III). Plates were incubated 2 days at 3 C and 5 days at 21 C. After incubation, segments were observed under a dissecting miscroscope and the presence of *C. trifolii* and other fungi were recorded.

Table 5. Location, age, size, and composition of alfalfa stands surveyed during June - October 1981 for presence of *Colletotrichum trifolii* and southern anthracnose symptoms

Location	Planting Date	Plot Size	Composition	Irrigated?
Stillwater	9/80	$50m^2$	Baker/Riley mix	Yes
Lake Carl		$330m^2$	Riley	No
Blackwell	9/78	$430m^2$	Cody	No
Perkins	9/79	$46m^2$	Cody	No
Perkins	9/79	$125m^2$	Riley	Yes
	9/79	$125m^2$	Baker	Yes

Fig. 9. A straw colored shepherds crook (a symptom of southern anthracnose).

RESULTS AND DISCUSSION

Results from the surveys from the Lake Carl Blackwell and Stillwater areas are presented in Fig. 10, and the results from Perkins in Fig. 11. At all locations, symptoms appeared 1 or 2 weeks after a relatively large amount of rainfall and during a rise in average high temperature. By this pattern, symptoms would have also been expected during week 4 (8-16 June), but this coincided with the second cutting date. Recovery of *C. trifolii* from alfalfa tissue was possible throughout the season. The percent recovery was somewhat variable, but did seem to increase during rainy periods (Figs. 10 and 11). These results are similar to those of Hartung et al (58) who found a 2 week delay of symptom expression of blueberry anthracnose after conidal spread of *C. gloeosporides*. Except at Lake Carl Blackwell plots, percent recovery was greater early in the season. This may have been due to the difference in other pathogens competing for the stem surfaces. *Phoma medicaginis* Malbr. & Roum. var *medicaginis* Fekl. was the predominant pathogen observed during June and July, *Cercospora medicaginis* Ell. & Ev. and *Leptosphaerulina briosana* (Poll.) Graham and Lutterell predominated during August to October. The increase in percent recovery at Lake Carl Blackwell in the Cody stand late in the season was probably due to the decrease in plant density as a result of poor stand maintenance (105).

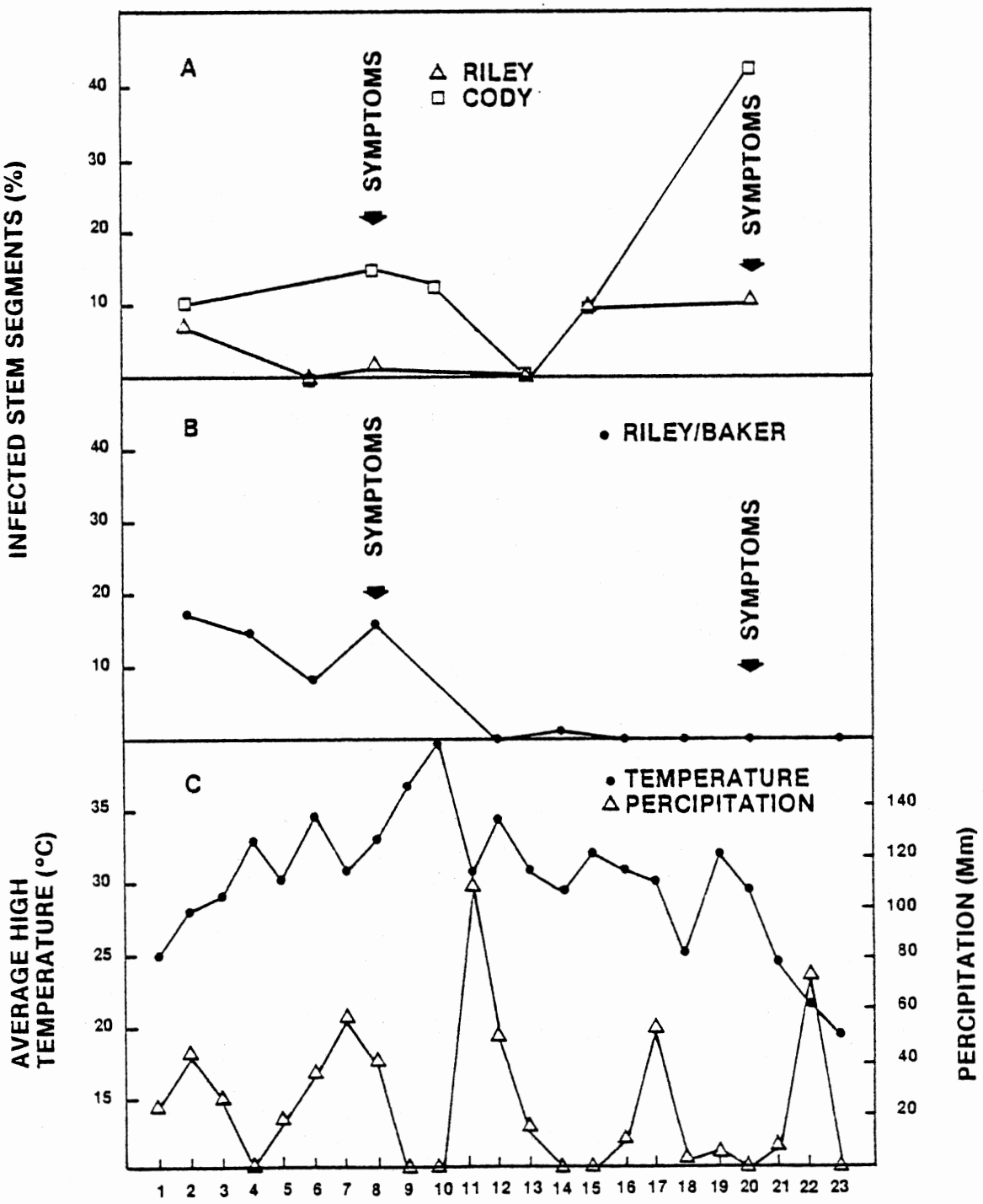

Fig. 10. Results of surveys at Lake Carl Blackwell and Stillwater for presence of Colletotrichum trifolii and southern anthracnose symptoms. **(A).** Percent infected stem segments at Lake Carl Blackwell. **(B).** Percent infected stem segments at Stillwater. **(C).** Temperature and precipitation patterns during the summer of 1981 at Stillwater.

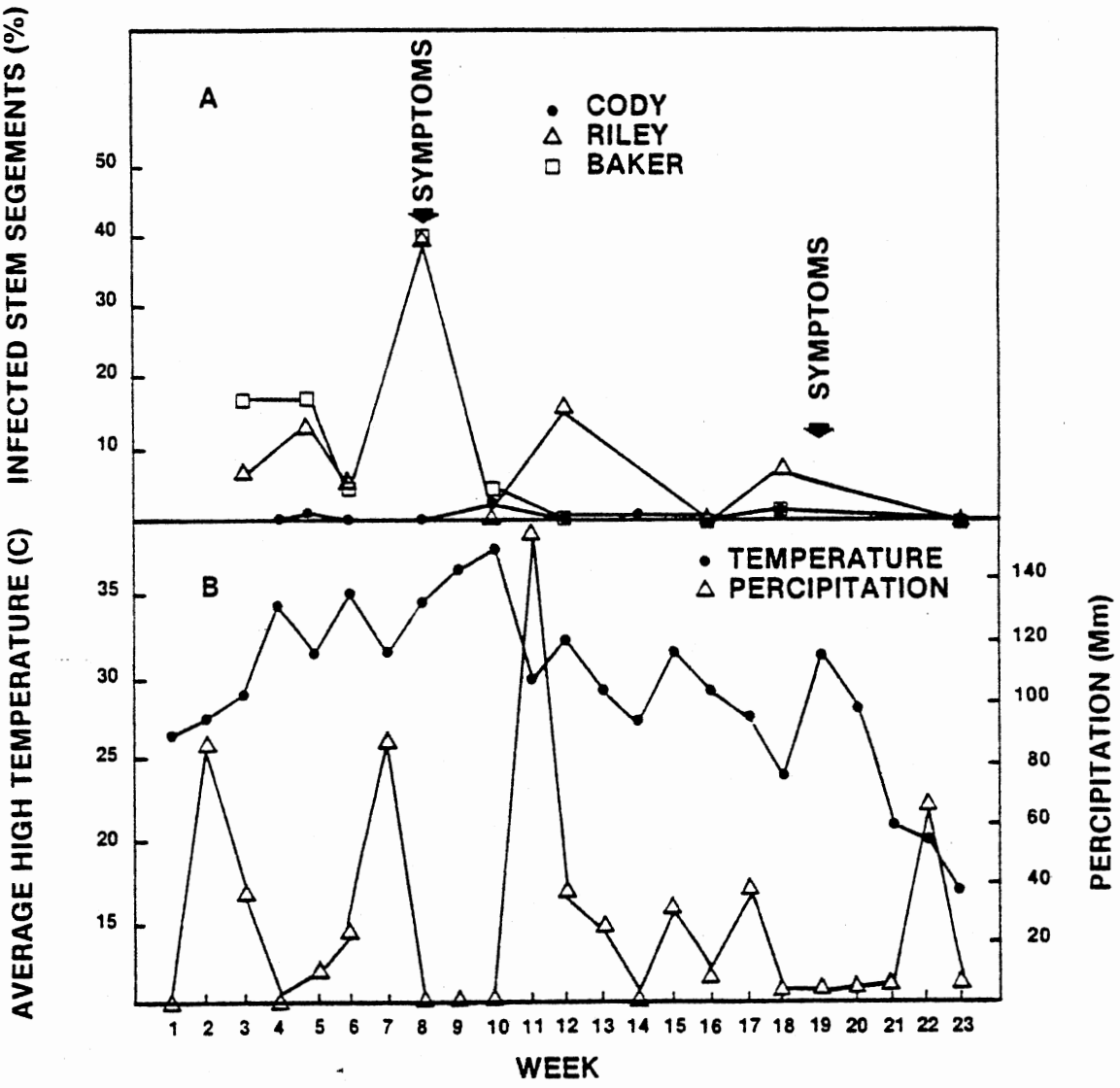

Fig. 11. Result of the survey at Perkins for presence of Colletotrichum trifolii and southern anthracnose symptoms at Stillwater. **(A).** Percent infected stem segments. **(B).** Temperature and precipitation patterns during the summer of 1981 at Perkins.

Formation of shepherds crooks (following spread of the pathogen during rainfall) is a result of hot weather. The resulting death of infected stems and plants is probably beneficial. Dead tissue observed in this study was quickly overrun by saprophytes which suppressed *C. trifolii*. The pathogen is less likely to spread from this material. If a shepherds crook does not form after infection, the pathogen is free to grow within the host, resulting in crown rot (50). Possibly the reason *C. trifolii* does not cause debilitating losses in Oklahoma is that weather patterns are variable, and hot periods often follow rainy periods. As a result infected stems often die (forming shepherds crooks), thus precluding spread to the crown and surrounding plants from infection.

CHAPTER VI

DEVELOPMENT AND EVALUATION OF SOURCES OF RESISTANT TO ALFALFA TO *COLLETOTRICHUM TRIFOLII*

INTRODUCTION

Importance of multiple-pest resistant cultivars. The successful production and use of multiple-pest resistant cultivars will result in improved crop performance as well as a cleaner environment due to reduced pesticide applications (55). Realization of the need for multiple-pest resistance in alfalfa did not come until such disease and insect pests as southern anthracnose, *Phytophthora* root rot [caused by *P. megasperma* Drechs f. sp. *medicaginis* (Erwin & Kuan)], spotted alfalfa aphid [*Therioaphis maculata* (Buckton)], and alfalfa weevil [*Hypera postica* (Gyllenhal)] were identified as major limiting factors to alfalfa production (10, 53). Successful development and use of the multiple pest resistant cultivar Arc which is resistant to *C. trifolii*, *Corynebacterium insidiosum* (McCull.) H. L. Jens., pea aphid [*Acyrthosiphon pisum* (Harris)] and tolerant to alfalfa weevil feeding should result in increased stand persistence in areas where it is adapted (7). However,

these cultivars are susceptible to the spotted alfalfa aphid and P. megasperma f. sp. medicaginis, two major pests limiting alfalfa production in Oklahoma (10, 14, 23). Genetic resistance to P. megasperma f. sp. medicaginis and T. maculata is available, and the successful combination of resistance to these two pests along with resistance to C. trifolii, C. insidiosum, A. pisum along with tolerance to H. positca feeding will be an important step in integrated pest management for alfalfa in Oklahoma.

Development of multiple pest resistance. Improvement through selection is the predominant means of developing alfalfa cultivars (19). Recurrent phenotypic selection, a form of mass selection, is based on the phenotypes of both male and female parents (no progeny test) followed by intermating of selected plants. This process results in a rapid increase in frequency of resistant gene(s) in intermating populations. Frequency of the favorable gene(s) is increased by repeated recombination, resulting in new genotypes (71). Success is due mostly to typically high heritability of disease and insect resistance. An added benefit of recurrent phenotypic selection is that genetic variability of unselected characters should be preserved (19). However, negative correlation between desirable characters has been reported such as reduction of resistance to C. trifolii within the breeding population after selection for resistance to Leptosphaerulina briosiana (Poll.) Graham & Luttrell or the spotted alfalfa aphid (61,

73).

Hanson et al (56) have reported success using recurrent phenotypic selection to develop multiple pest resistant populations. Large parental populations were subjected to successive cycles of selection for the following pests: Uromyces striatus Schroet var. medicagins (Pasa.), C. insidiosum, potato leafhopper [Empoasca fabae (Harris)], T. maculata, C. trifolii, and Psuedopeziza medicaginis (Lib.) Sacc. After 18 cycles of selection, new genotypes with multiple pest resistance could be isolated.

Sources of resistance. A good source of resistance to C. trifolii and other disease and insect pests for developing cultivars adapted to Oklahoma are Oklahoma common alfalfa strains. Oklahoma commons are naturalized strains of alfalfa which have been grown in the state for many generations. These strains were introduced into Oklahoma after 1900 from Kansas and Colorado. Oklahoma commons are preferred by growers due to their reliability. These strains do not contain appreciable resistance to any one pest (21). By developing pest resistant lines from Oklahoma commons, important agronomic characters such as dormancy and winter-hardiness should be left unaffected (56).

The first objective in this study was to intercross C. trifolii resistant selections to determine if the frequency of resistance gene(s) could be increased. The second objective was to evaluate experimental germplasms currently

under development in the alfalfa breeding program at the Oklahoma Agricultural Experiment Station for resistance to C. trifolii. The final objective was to evaluate several cultivars and experimental strains currently included in Oklahoma Agricultural Experiment Station alfalfa cultivar evaluation tests for resistance to C. trifolii.

MATERIALS AND METHODS

Screening procedure. The procedure used to screen alfalfa populations is described in Chapter III. The resistant selections crossed were obtained from the plant material utilized in Chapter III. Resistant plants were transplanted into containers and held in the greenhouse until they could be transplanted to the field.

Polycross procedure. Resistant selections from the cultivars Buffalo, Baker, Riley and Oklahoma common (Kohler) were transplanted to Cow Creek Bottom, Agronomy Research Station, Stillwater on 5 May 1981. Plants which did not survive were replaced on 22 May. A total of 115 plants (24 from Baker, 25 from Buffalo, 31 from Riley and 35 from Kohler) survived to be intercrossed making up the experimental strain OK12. On 17 July the crossing block was enclosed with a metal frame cage (3.7 x 7.4 x 2.5 m) which was covered with a fine mesh screen to prevent contamination by foreign sources of pollen (Fig. 12). A beehive containing 10,000 - 20,000 honey bees [Aphis mellifera (L.)] was placed in the cage on 18 July and removed on 5

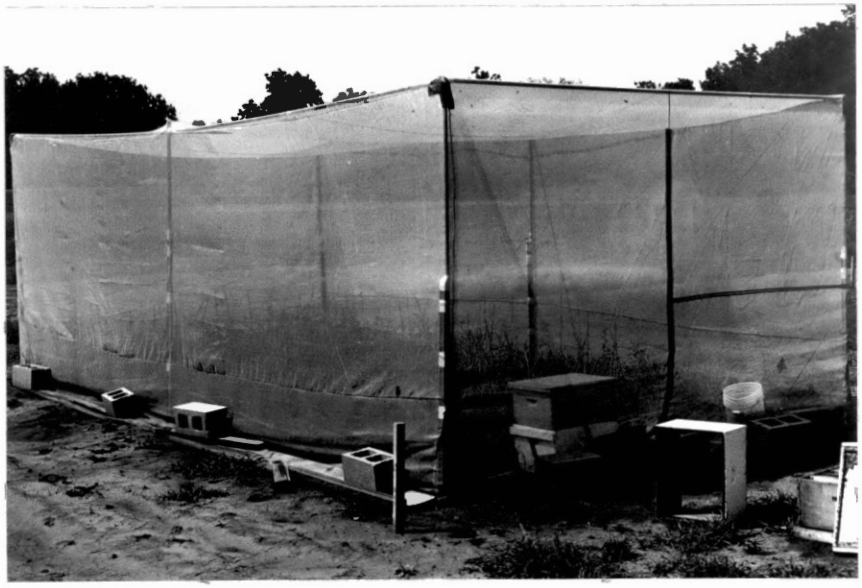

Fig. 12. Metal frame cage covered with a fine mesh screen utilized to prevent foreign sources of pollen from entering the alfalfa crossing block.

September. Plants in the crossing block were irrigated by flooding throughout the period. On 6 September the cage was removed and the plants sprayed with a desiccant (20 ml dinitrophenol per liter diesel oil). Plants were harvested on 8 September. After drying, the plants were hammermilled, and the seed cleaned.

Seed from OK12, its parent cultivars, as well as several cultivars and experimental strains were then evaluated for resistance in the greenhouse, as described in Chapter III. Means for all the cultivars and strains were compared from a matrix of t-test observed significance level values generated by the General Linear Models procedure of the Statistical Analysis System (59).

RESULTS AND DISCUSSION

Oklahoma experimental strains. A significant increase in resistant plants in OK12 over its four parental lines was observed (Table 6). This significant increase in resistance indicates that one cycle of mass selection can be utilized for developing strains of alfalfa resistant to C. trifolii. This response to one cycle of selection is good, but not unexpected as Hanson et al (56) observed 43 and 22% plants resistant to C. trifolii after one cycle of intermating in alfalfa pools MSA and MSB respectively.

The 15 experimental Oklahoma alfalfa strains contained 10.3 to 65.6% resistant plants (Table 7). OK11, the strain with the greatest percentage of resistant plants, was

Table 6. Percent seedling survival of OK12 and its parent cultivars 14 days after inoculation with race 1 of *Colletotrichum trifolii*

Cultivar or Strain	Percent survival
Arc[x]	73.5a[y]
OK12	45.1b
OK Common (Kohler)	17.3c
Baker	16.7c
Riley	16.5c
Buffalo	7.5d

[x]Resistant check.
[y]Means followed by the same letter are not significantly different at P=Ø.Ø5 (t-test).

Table 7. Parentage, resistance to pest selected for, and percent seedlilng survival of 15 experimental srains 14 days after inoculation with race 1 of Colletotrichum trifolii

Strain designation	Parentage	Selected for resistance to	Percent survival after inoculation with C. trifolii
Arc[s]	Team	Colletotrichum trifolii Corynebacterium insidiosum Hypera postica (tolerance) Acyrthosiphon pisum	73.5a[t]
OKll	Liberty	Root rot (in field)	65.6ab
OKlØ	Beltsville-6[u]	Therioaphis maculata	58.7bc
OKl	Arc	T. maculata	58.2bc
OK2	Arc	T. maculata	56.7bc
OK8	Arc	T. maculata	53.4c
OKl5[v]	OKl, OK3, OK4, OK5, OK6, Arc, Arc polycross[w]	Phytophthora megasperma f. sp. medicaginis	51.6cd
OKl2	Buffalo, Baker, Riley, OK common	C. trifolii	45.ld
OK6	OK commons, APC-76, NCMP Lines[x]	P. megasperma f. sp. medicaginis	37.4de
OK7	OK commons	C. insidiosum	36.7de
OK5	Several cultivars	C. trifolii	32.le
OKl4[vy]	OKl, OK3, OK4, OK5, OK6, Arc, Arc Polycross[w]	Fusarium oxysporum	25.5

Table 7. Continued

Strain designation	Parentage	Selected for resistance to	Percent survival after inoculation with *C. trifolii*
OK9	OK commons	*T. maculata* (antibiosis and nonpreference)	20.1f
OK13	OK commons	*T. maculata* (tolerance)	19.9fg
OK3	Team	*T. maculata*	19.2fg
OK4	OK commons	*P. megasperma* f. sp. *medicaginis*	10.3g
Buffalo[z]	Kansas common	*C. insidiosum*	7.5g

[s]Resistant check.

[t]Means followed by the same letter are not significantly different at P=0.05 (t-test).

[u]Experimental germplasm.

[v]Crossed in the greenhouse by hand.

[w]Arc polycross represents seed harvested from selected plants in old yield trials.

[x]North Carolina Multiple-pest resistant lines.

[y]Average of two replicates (instead of the normal 12).

[z]Susceptible check.

selected from Liberty for root rot resistance under field conditions (J. L. Caddel, personal communication). The cultivar Liberty contains about 55% plants resistant to C. trifolii (Table 1). These data indicate the C. trifolii may have been one of the pathogens responsible for the natural selection of strain OK11.

Experimental strain OK10 was selected from experimental germplasm Beltsville-6; OK1, OK2, and OK8 were selected from Arc; and OK3 from Team for resistance to the spotted alfalfa aphid (10). Both Beltsville-6 and Arc are highly resistant to C. trifolii (82, Table 1). A significant decrease in resistance to C. trifolii was observed in OK1, OK2, OK8 and OK10 in relationship to Arc. Reduction in resistance to C. trifolii after selection for resistance to T. maculata has also been observed by Knipe et al (73). This negative correlation between traits is unfortunate, as both pests can cause serious losses in alfalfa production. Resistance in OK3 was also reduced in relation to Team, which contains approximately 22% resistant plants (24).

Experimental strains OK9 and OK13 were selected for resistance to the spotted alfalfa aphid from Oklahoma commons by the alfalfa insect project at the Oklahoma Agricultural Experiment Station. OK9 consisted of clones exhibiting either antibiosis or non-preference as mechanisms of resistance. OK13 consisted of clones tolerant to the aphid. Non-preference and antibiosis mechanisms affect either the behavior or metabolic processes of the insect.

Tolerant clones are able to survive even when supporting a normally lethal number of insects (72). Presumably these mechanisms are under different genetic control. The similarity in the percent resistant plants to C. trifolii in both OK9 and OK13 is interesting in light of the evidence of negative correlation between resistance to C. trifolii and T. maculata (Table 7).

Strains OK14 and OK15 were selected from several sources, most of which contain resistance to C. trifolii. OK15, selected at the University of Minnesota for resistance to P. megasperma f. sp. medicaginis, maintained its resistance to C. trifolii. OK14, selected (at the University of Minnesota) for resistance to Fusarium oxysporum Schlect f. sp. medicaginis (Weimer) Snyd & Hans. appears to have lost resistance. This may have been a result of only two replicates being evaluated; therefore firm conclusions cannot be drawn. According to Frosheiser and Barnes (43), resistance to C. trifolii and F. oxysporum f. sp. medicaginis are not related.

OK4 and OK6 were both selected for resistance at the University of Minnesota to P. megasperma f. sp. medicaginis. OK4 was composed entirely of Oklahoma commons, and it has low resistance to C. trifolii. OK6 was composed of 45% commons and 55% North Carolina multiple-pest resistant and weevil-tolerant lines. OK6 contained approximately the expected amount of resistance, 37.4% (Table 7).

The experimental strain OK7 was selected for resistance to C. insidiosum at the University of Minnesota from Oklahoma commons. Resistance to C. trifolii has no relation to resistance to C. insidiosum (43). The high amount of resistance in OK7 in comparison to OK4 is surprising. This may be the result of natural selection in the field during the screening process or during crossing.

Strains OK12 and OK5 have both gone through one cycle of selection and intermating for resistance to C. trifolii. The mean number of resistant plants in each strain was significantly different (Table 7). OK12 and OK5 were selected by different workers. A difference in selection pressure may be responsible for the significant difference.

Evaluation of sources of resistance. None of the Oklahoma commons tested contained a high level of resistance to C. trifolii (Table 8). Resistance to C. trifolii is present in each strain. Experimental strains resistant to C. trifolii derived from Oklahoma commons can be developed by use of recurrent mass selection.

Of the seven public cultivars tested for resistance to C. trifolii during this study, only Arc (the resistant standard) was highly resistant (Table 9). Four of the cultivars had very low resistance and two were susceptible.

Advantage and Trident, produced by North American Plant Breeders, were the only two of 11 private cultivars which contained a significant amount of resistance to C. trifolii (Table 10). Two of Northrup-King's experimental lines,

Table 8. Percent seedling survival of 10 Oklahoma common alfalfa strains 14 days after inoculation with race 1 of Colletotrichum trifolii

Strain	Percent survival
Arc[x]	73.5a[y]
Kohler	17.3b
OCPX	15.1b
Wright	13.8b
Elsner	12.1b
Graham	11.5b
Schroder	11.3b
Spradlin	11.1b
Gastin	9.9b
Givens	9.1b
Buffalo[z]	7.4b

[x]Resistant check.
[y]Means followed by the same letter are not significantly different at P=0.05 (t-test).
[z]Susceptible check.

Table 9. Percent seedling survival of seven public cultivars 14 days after inoculation with race 1 of Colletotrichum trifolii

Cultivar	Percent survival
Arc[x]	73.5a[y]
Teason	9.6b
Buffalo[z]	7.5b
Kanza	6.9b
Cody	5.9b
Dawson	3.4b
Washoe	1.6b

[x]Resistant check.
[y]Means followed by the same letter are not significantly different at P=Ø.Ø5 (t-test).
[z]Susceptible check.

Table 10. Percent seedling survival of 11 commercial cultivars 14 days after inoculation with race 1 of Colletotrichum trifolii

Cultivar	Releasing Organization	Percent Survival
Arc[x]	USDA	73.5a[y]
Advantage	North American Plant Breeders	39.4b
Trident	North American Plant Breeders	29.2c
531	Pioneer	15.8d
Classic	FFR	15.5d
WL314	Waterton-Loomis	13.7d
Apollo	North American Plant Breeders	13.4d
555	Pioneer	9.9d
Hi-phy	FFR	8.1d
WL312	Waterton-Loomis	7.9d
Buffalo[z]	USDA, Kansas AES	7.5d
530	Pioneer	5.4d
Aztec	Northrup-King	4.2d

[x]Resistant check.
[y]Means followed by the same letter are not significantly different at P=0.05 (t-test).
[z]Susceptible check.

K7-29 and NK78Ø1Ø contain high levels of resistance (Table 11). The remaining commercial cultivars contained a low to moderate amount of resistance, none of which appears high enough to indicate that selection for resistance to C. trifolii has been conducted.

Maintaining the integrity of resistance during seed production of an alfalfa cultivar may present a problem if susceptible cultivars are grown nearby. To look at this problem, resistance contained in the original seed of the four bioindicator pairs developed by Devine et al (36) was compared to resistance in open-pollinated polycrossed seed [harvested from the original study (22)] by J. L. Caddel (personal communication) from the same four pairs. The expected decrease in resistance in the resistant strains and increase in resistance in the susceptible strains occurred only in four of eight comparisons (Table 12). The increase in resistance observed in open-pollinated polycross seed of Beltsville 1An4 and Beltsville 3An4 may be due to effects of natural selection or pollinator preference. Apparently outcrossing between contiguous plots is not a great threat to integrity of resistance. These results agree with Brown et al (17), who did not find outcrossing to cause significant reduction in resistance to C. trifolii along borders of certified seed fields.

From the data presented in this chapter it is evident that cultivars adapted to Oklahoma with resistance to C.

Table 11. Percent seedling survival of 10 experimental strains 14 days after inoculation with race 1 of *Colletotrichum trifolii*

Experimental strain	Originating organization	Percent survival
Arc[x]	USDA	73.5a[y]
APC-76	Oklahoma AES	55.7b
K7-29	Northrup-King	47.5c
NK 7810	Northrup-King	42.6d
WASH SN1	Washington AES	20.3d
G 7730	North American Plant Breeders	6.0de
MSF_6CLS_4	Nevada AES	11.3de
74-5T9	Waterton-Loomis	10.0e
Buffalo[z]	USDA and Kansas AES	7.5e
PCC77-122	Northrup-King	6.9e
UC176	California AES	3.5e
SYN XX	USDA	3.3E

[x]Resistant check.
[y]Means followed by the same letter are not significantly different at P=0.05 (t-test).
[z]Susceptible check.

Table 12. Percent seedling survival of orginal and open pollinated polycrossed seed of four pairs of cultivars 14 days after inoculation with race 1 of Colletotrichum trifolii

Cultivar	Percent survival		Percent resistant plants as reported by Devine et al (36)
	Original seed	Polycrossed seed	
Arc	76.5	65.6	89.Ø
Team	22.4	22.1	21.Ø
Saranac AR[a]	7Ø.7	65.Ø	78.Ø
Saranac	2.8	12.Ø	6.Ø
Beltsville 1An4	69.8	8Ø.2	85.Ø
Glacier	3.5	14.9	6.Ø
Beltsville 3An4	5Ø.6	67.Ø	7Ø.Ø
Vernal	8.3	18.2	1.Ø

[a]Seed of Beltsville 2An4 was not used due to poor germination.

[b]Pairs of means which are underlined are significantly different at P=Ø.Ø5 (t-test).

trifolii can be developed. By use of recurrent mass selection, resistance in interbreeding populations of Oklahoma commons can be increased. In the development of multiple-pest resistant cultivars, public cultivars or experimental strains which possess high levels of resistance to one pest, i.e. the spotted alfalfa aphid will also contain at least a small amount of resistance to C. trifolii, can be utilized. By use of recurrent mass selection, a moderate amount of resistance to both pests should be obtainable.

Several adapted cultivars with high resistance are available for use during the time cultivars with resistance to C. trifolii are being bred and developed for Oklahoma. These cultivars include Arc, Liberty, Cimarron, Vanguard, Advantage, and Trident (Tables 1 and 10). However, care must be taken in selection of a cultivar, as several listed here are susceptible to other serious pests of alfalfa in Oklahoma such as Phytophthora rot rot and the spotted alfalfa aphid (23).

CHAPTER VII

COMPARISON OF GREENHOUSE AND FIELD MEASUREMENTS OF RESISTANCE TO *COLLETOTRICHUM TRIFOLII* IN ALFALFA

INTRODUCTION

Resistance in alfalfa to *Colletotrichum trifolii* is usually developed with greenhouse screening procedures. When 10 day old seedlings are inoculated in a pathogen conducive environment, all but the most resistant plants die (95). Resistant cultivars developed in the greenhouse perform well in the field under pressure from the pathogen (34). In Oklahoma, resistance to *C. trifolii* has been shown to be beneficial to yielding ability and stand persistence (21, 22). However, many cultivars that contain low levels of resistance do not show a corresponding yield reduction in the field.

Several reports in the literature suggest that other factors which are not recognized during greenhouse screening procedures are capable of influencing resistance in the field. Smith (108) selected two experimental strains for stand persistence under field conditions from the cultivar

DuPuits. After one cycle of selection, persistence and yield after 2 years of the two experimental strains and the check cultivar Williamsburg were significantly greater than DuPuits. In the greenhouse, only small differences (non-significant) in resistance between DuPuits and Williamsburg was observed. The cultivar Riley has been selected for resistance to C. trifolii under field conditions (109). However, Riley is not resistant in the greenhouse (Table 1).

The objective of this study was to first compare measurements which could indicate resistance in the field (yield, stem density, and shepherds crooks per plot). The second objective was to compare resistance as measured in the greenhouse (percent seedling survival) to the mean number of shepherds crooks for each cultivar.

MATERIALS AND METHODS

Greenhouse seedling survival data was the same as presented in chapters III and VI (Tables 1, 7, 8, 9, 10, and 11). Yield data (total and fifth (final) cut) on Department of Agronomy alfalfa cultivar evaluation trials 804, 812, 901, 941, and 021 was supplied by Caddel and Rupp (20). Each trial contained 16-25 alfalfa cultivars or strains arranged in a randomized complete block design with six replications. Trials 804 and 812 were planted in Cow Creek Bottom, Agronomy Research Station, Stillwater, during September 1978. Trial 901 was also planted in Cow Creek Bottom, during September 1979. Trial 941 was planted at

North Central Research Station, Lahoma during September 1979. Trial 021 was planted at Agronomy Research Station, Perkins during September 1980.

Stem counts in trials 804 and 812 were made on 23 September 1981 and 22 March 1982. Stems were counted in three linear 30 cm segments of row in each plot. The mean of the three subsamples was then transformed to stems per m^2.

Shepherds crooks were counted in each plot at Stillwater on 28 September, at Lahoma on 24 September, and at Perkins on 25 September 1981. Within each trial, 25-50 stems were collected showing each of the following symptoms: no symptoms, shepherd crook, symptoms associated with other diseases, three cornered alfalfa hopper [Stirtocephala festina (Say)] girdle, and girdled shepherds crooks. A segment from each stem was incubated to indicate presence of C. trifolii as described in Chapter III.

Correlation coefficients among the field observations were generated by the MANOVA procedure of the Statistical Analysis System (59). To compare greenhouse seedling survival data and number of shepherds crooks, both values were plotted side by side for each cultivar within each trial.

RESULTS AND DISCUSSION

Reliability of shepherds crooks as a disease measurement. Acervuli of C. trifolii were observed on an average of 6% of the symptomless stems, 8% of the stems girdled by the three cornered alfalfa hopper, 3% of the stems exhibiting other symptoms, 96% of the girdled shepherds crooks, and 87% of the ungirdled shepherds crooks. On the basis of these results, shepherds crooks were concluded to be a reliable measure of disease.

Relationship between field measurements. Trial 804 consisted mostly of alfalfa cultivars, and trial 812 consisted mostly of Oklahoma commons. Total and fifth cut yield for 1981, number of shepherds crooks per plot, fall and spring stem count, and the stem count difference for trials 804 and 812 are presented in Tables 13 and 14 respectively. No significant differences among cultivars were observed among any of the measurements taken on trial 804 except for yield (total and fifth cut; P=0.05). In trial 812, significant differences (P=0.05) were apparent among cultivars for yield (fifth cut and total), number of stems in the fall, and stem count difference. Since no significant differences were observed among cultivars for shepherds crooks, nor did a cultivar-shepherds crook interaction exist, data was then compared on a plot-by-plot basis (across cultivars). In trial 804, only fall and spring stem counts were significantly correlated (Table 15).

Table 13. Yield (fifth cut and total), numbers of shepherds crooks, fall stem count, spring stem count and the difference between stem counts for each cultivar in experiment 804

Cultivars	Yield[x]		Numbers of shepherd's Crooks/Plot[y]	Fall stem Count	Spring Stem Count	Stem Count Difference
	Fifth cut	Total				
Buffalo	3.67a[z]	19.37a	17.0ab	441ab	540ab	99a
Cimarron	2.93cdefg	18.79ab	12.0ab	380b	496bc	115a
Aztec	3.20bc	18.25abc	17.8ab	371b	535abc	164a
Cody	3.35b	17.97abc	17.7ab	430ab	496abc	66a
Apollo	3.12bcd	17.91abc	15.0ab	476a	582ab	106a
Givens	3.06bcdef	17.27bcd	10.8ab	408ab	577ab	168a
531	3.08bcde	17.25bcd	12.0ab	380b	583ab	204a
Liberty	2.90cdefgh	17.22bcd	12.7ab	370b	537ab	167a
Kanza	3.08bcde	17.12bcd	15.7ab	447ab	647a	200a
Arc	2.62hi	17.07cde	10.0b	374b	448c	73a
WL318	2.99cdefgh	17.02cde	15.0ab	438b	583ab	145a
APC '76	2.74efghi	16.64cde	9.7b	401ab	601ab	199a
Vangard	2.69ghi	16.63cde	11.3ab	388ab	541abc	153a
Riley	2.80efghi	16.01de	19.5a	423ab	554abc	131a
530	2.72ghi	15.99de	17.3ab	368b	484abc	115a
Baker	2.53i	15.42e	11.3ab	432ab	553abc	121a

[x]Metric tons of cry matter/hectare.

[y]Plots were 5 m^2.

[z]Means followed by the smae letter are not significantly different at P=0.05 (Duncan's Multiple Range Test).

Table 14. Yield (fifth cut and total), numbers of shepherds crooks, fall stem count, spring stem count and the difference between stem counts for each cultivar and Oklahoma common strain in experiment 812

Cultivars	Yield[x]		Numbers of shepherd's Crooks/Plot[y]	Fall stem Count	Spring Stem Count	Stem Count Difference
	Fifth cut	Total				
Graham	2.0abc[z]	19.53a	21.2a	522bc	646a	124ab
Cody	3.31a	19.71ab	27.0a	606ab	608abc	2bcd
Givens	3.25a	19.09ab	21.5a	547bc	525abc	28abcd
Schroder	3.13ab	18.93abc	17.2ab	540bc	640ab	94ab
Dugan	3.12ab	18.89abcd	17.5ab	499bcd	587abc	88abc
Wright	2.94abc	18.47abcd	23.3a	426de	597abc	170ab
OCPX	3.01abc	18.43abcd	20.7a	456bcd	588abc	131ab
Berends	2.99abc	18.76abcd	21.8a	563bc	633ab	70abcd
Riley	2.71bcd	18.02bcde	18.2ab	484bcd	606abc	122ab
Gastin	3.07ab	17.99bcde	17.5ab	374d	581abc	207a
Kamza	2.90abc	17.96bcde	23.2a	704a	602abc	-102cd
Elsener	2.94abc	17.76bcde	20.2ab	508bcd	575abc	66abcd
Percy	2.93abc	17.55cde	20.0ab	576abc	643a	67abcd
Kohler	2.70bcd	17.53cde	17.5ab	535bc	536abc	2bcd
Dawson	2.62cd	17.39de	76.5a	600ab	492c	-107d
Arc	2.38d	16.65e	10.0b	480bcd	502bc	21abcd

[x]Metric tons of dry matter/hectare.

[y]Plots were 5 m^2.

[z]Means followed by the same letter are not significantly different at P=0.05 (Duncan's Multiple Range Test).

Table 15. Correlation coefficients between observations taken on experiment 804

	Fall stem count	Spring stem count	Number of shepherds crooks	Fifth cut yield[a]	Total yield[a]
Spring stem count	0.246[b]	----	-0.149	0.058	-0.040
Numbers of shepherds crooks	0.047	-0.149	----	0.032	-0.030
Fifth cut yield	0.045	0.058	0.083	----	----
Total yield	0.051	-0.040	-0.030	----	----

[a]Metric tons per hectare.
[b]Significant at P=0.01 (F-test).

In trial 812, total yield and shepherds crooks were positively correlated (Table 16). A positive correlation implies the greater the yield, the greater the shepherds crook count. A similar observation has been made by Barnes et al (12). These workers concluded that higher yielding plots also had a denser canopy. The canopy would create a moist chamber effect, creating favorable conditions for infection and spread of C. trifolii.

Trial 901 consisted of both cultivars and experimental strains. Significant differences (P=0.05) in total and fifth cut yield and number of shepherds crooks existed among cultivars and strains (Table 17). Shepherds crooks and total yield were positively correlated (significant at P=0.05; r=0.229).

Trial 941 consisted of the same cultivars and experimental lines as in Trial 901. Yield (fifth cut and total) were significantly different (P=0.05) among cultivars and strains (Table 18). No correlation existed between shepherds crooks and yield. Since the shepherds crook count in each plot was very low, the amount of infection present was evidently not high enough to cause observable differences.

Trial 021 consisted mostly of experimental alfalfa strains. Significant differences (P=0.05) were apparent only for yield (Table 19). Shepherds crooks and total yield were negatively correlated (r=0.304; P=0.01). This negative correlation implies the greater the number of shepherds

Table 16. Correlation coefficients between observations taken on experiment 812

	Fall stem count	Spring stem count	Number of shepherds crooks	Fifth cut yield[a]	Total yield[a]
Spring stem count	Ø.Ø85	----	Ø.Ø82	Ø.Ø35	Ø.146
Numbers of shepherds crooks	-Ø.1Ø2	Ø.ØØ2	----	Ø.ØØ3	Ø.2Ø5[b]
Fifth cut yield	Ø.Ø51	Ø.Ø35	Ø.ØØ3	----	----
Total yield	Ø.1Ø4	Ø.146	Ø.2Ø5[b]	----	----

[a]Metric tons per hectare.
[b]Significant at P=Ø.Ø5 (F-test).

Table 17. Yield (fifth cut and total) and numbers of shepherds crooks for each cultivar in experiment 901

Cultivar	Yield[x]		Numbers of shepherds crooks/plot[y]
	Fifth cut	Total	
555	2.60a[z]	21.22a	16.2a
PCc 77-122Br	2.52a	19.98ab	10.2abcdef
PCc 77-122	2.35abc	19.75ab	7.2cdef
PCc 77-122	2.54a	19.56b	10.7abcdef
WL312	2.25abcd	19.25bc	15.7ab
Trident	2.38ab	19.17bc	10.3abcdef
Hi-phy	2.30abc	19.10bc	6.0def
Buffalo	2.51a	19.10bc	12.3abcd
Advantage	2.34abc	18.96bcd	13.2abc
Apollo	2.34abc	18.84bcd	12.2abcde
Cimarron	2.35abc	18.63bcd	10.0abcdef
WL318	2.34abc	18.50bcde	10.7abcdef
Vangard	2.04bcdefg	17.80cdef	9.2bcdef
Kanza	2.09bcde	17.37defg	9.0bcdef
K7-29	1.83efgh	17.02efg	6.3def
G7730	2.08bcdef	18.83fgh	9.7abcdef
Dawson	1.87defgh	16.78fgh	15.3ab
Riley	1.99bcdef	16.77fgh	8.2cdef
Arc	1.74efgh	16.74fgh	6.3def
Classic	1.95cdefg	16.63fgh	6.3def
Baker	1.55h	15.85gh	7.3cdfg
Liberty	1.68fgh	15.26hi	5.5ef
Gladiator	1.81efgh	14.12i	4.7f
NK-78010	1.66gh	14.05i	5.2f

[x]Metric tons of dry matter/hectare.
[y]Plots were $5m^2$.
[z]Means followed by the same letter are not significantly different at P=0.05 (Duncan's multiple range test).

Table 18. Yield (fifth cut and total) and numbers of shepherds crooks for each cultivar in experiment 941

Cultivar	Yield[x]		Numbers of shepherds crooks/plot[y]
	Fifth cut	Total	
555	1.40abcd[z]	14.23a	2.3a
Buffalo	1.64a	13.8ab	1.0abc
PCc 77-122	1.55ab	13.82ab	1.2abc
Kanza	1.48abc	13.51ab	1.3abc
WL318	1.45abc	13.43abc	0.7abc
PCc 77-122Br	1.38abcd	13.36abcd	0.8abc
Trident	1.33abcd	13.32abcd	1.0abc
PCc 77-122	1.44abc	13.23abcd	1.7abc
Apollo	1.23cd	13.10abcd	0.5abc
WL312	1.30bcd	13.09abcd	1.3abc
Hi-phy	1.47abc	13.07abcd	0.0c
Riley	1.23bcd	12.93abcd	0.5abc
Gladiator	1.4,abcd	12.78abcd	2.2ab
Cimarron	1.28bcd	12.77abcde	0.0c
Vangard	1.27bcd	12.68abcde	0.0c
Baker	1.17cd	12.67abcde	0.3bc
Arc	1.30bcd	12.47abcde	0.3bc
Advantage	1.34abcd	12.47abcde	0.3bc
Dawson	1.20cd	12.34bcdef	1.3abc
K7-29	1.29bcd	11.65cdef	0.7abc
Classic	1.35abcd	11.59def	0.0c
NV-78010	1.35abcd	10.96efg	0.5abc
Liberty	1.32bcd	10.77fg	0.7abc
G7730	1.11d	9.42g	0.0c

[x]Metric tons of dry matter/hectare.
[y]Plots were $5m^2$.
[z]Means followed by the same letter are not significantly different at P=0.05 (Duncan's multiple range test).

Table 19. Yield (fifth cut and total) and numbers of shepherds crooks for each cultivar in experiment Ø21

Strain or Cultivar	Yield[x]		Numbers of shepherds crooks/plot[y]
	Fifth cut	Total	
74-5T9	2.8Øa[z]	18.81a	6.7ab
555	2.6Øabc	17.8ab	8.5ab
WL314	2.48abcde	17.81ab	5.Øb
Riley	2.45abcdef	17.64ab	8.3ab
WL318	2.48abcde	17.19abc	4.5b
77T25	2.67ab	17.Ø7abcd	3.5b
OK9	2.59abcd	16.99abcd	1Ø.Øab
SYNXX	2.58abcd	16.72abcde	7.7ab
OK5	2.4Øabc	16.68abcde	5.Øb
WL313	2.7Øab	16.61bcde	5.7b
Baker	2.1Øefg	16.6Øbcde	5.2b
UC PX 1971	2.65ab	16.49bcdef	8.2ab
OK8	2.39bcdefg	16.45bcdef	4.2b
MSF_6CLS_4	2.31bcdefg	16.36bcdef	9.3ab
Teason	2.41bcdefg	16.36bcdef	4.2b
OK1Ø	2.39bcdefg	15.84bcdefg	5.3b
OK6	2.19defg	15.39cdefg	5.7b
UC176	2.14efg	15.25cdefg	9.7ab
Perry	2.Ø2g	15.19cdefg	5.5b
Corona	2.22cdefg	14.94defgh	6.7ab
Wash SNI	2.13efg	14.7Øefgh	8.8ab
Washoe	2.ØØg	14.39fgh	13.8a
OK4	2.Ø6fg	14.Ø3gh	9.Øab
Arc	2.Ø3g	14.Ø1gh	3.3b
OK11	2.Ø3g	13.11h	5.Øb

[x]Metric tons/hectare.
[y]Plots were $5m^2$.
[z]Means followed by the same letter are not significantly different at P=Ø.Ø5 (Duncan's multiple range test).

crooks in each plot, the lower the yield.

During the past three years, weather patterns were conducive for spread and infection of alfalfa by C. trifolii only during 1981. The older trials (812 and 901, showing positive yield-shepherds crook correlation) would have been differentially thinned by pests other than C. trifolii and environmental conditions. The pathogen would find more favorable conditions in the denser plots, and be more likely to become established. Plots in the youngest trial (021, showing a negative shepherds crook-yield correlation) would not have had time to be thinned by the same degree. The pathogen would have had a better chance to become established in each plot. In plots of susceptible cultivars, C. trifolii could then cause sufficient damage to result in yield reduction.

Comparison of greenhouse and field resistance measurements. Percent resistant plants and the mean number of shepherds crooks per plot are plotted side by side for most cultivars and strains in trials 804, 812, 901, 941, and 021 in Figs. 13, 14, 15, 16, and 17 respectively. In each comparison, as the percent resistant plants contained in the cultivar decreases, the number of shepherds crooks counted tends to increase. This relationship is clearer at the ends of each graph (highly resistant cultivars and strains; and cultivars and strains with low to minimal resistance). Several cultivars, such as Baker and WL318 appear to contain adult plant resistance, as these cultivars produced

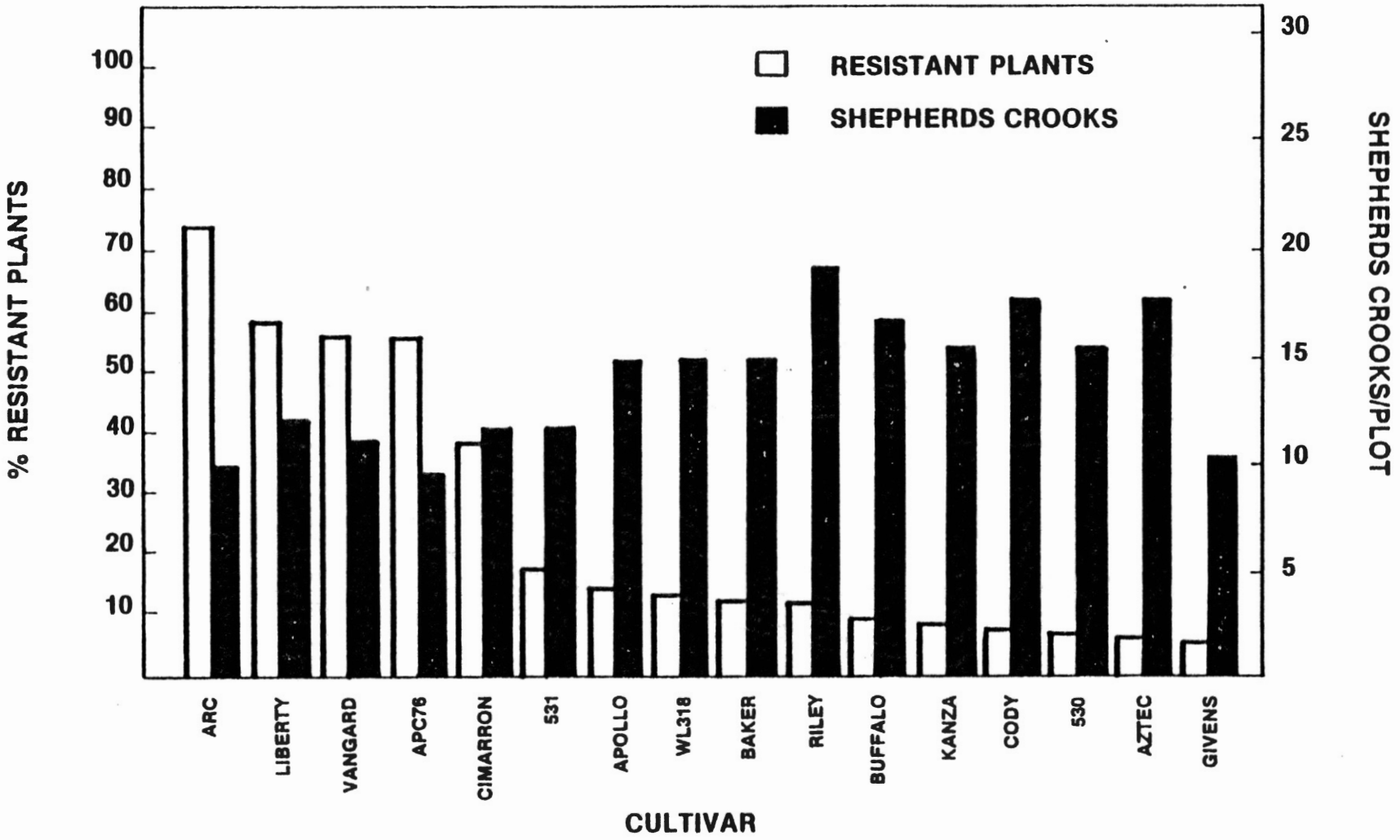

Fig. 13. Comparison of percent resistant plants (in greenhouse) and mean number of shepherds crooks per plot observed in experiment 804.

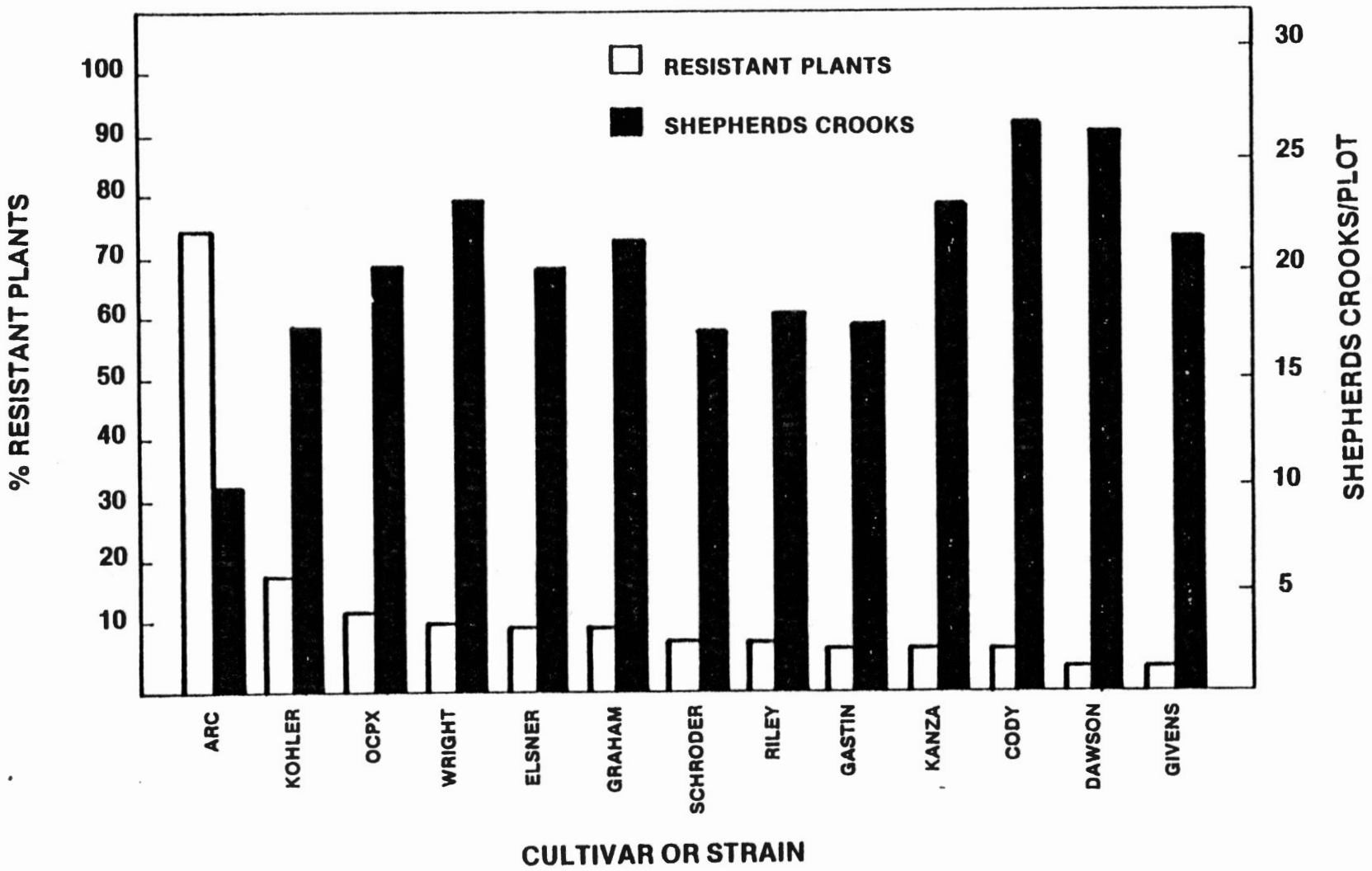

Fig. 14. Comparison of percent resistant plants (in greenhouse) and mean number of shepherds crooks per plot for experiment 812.

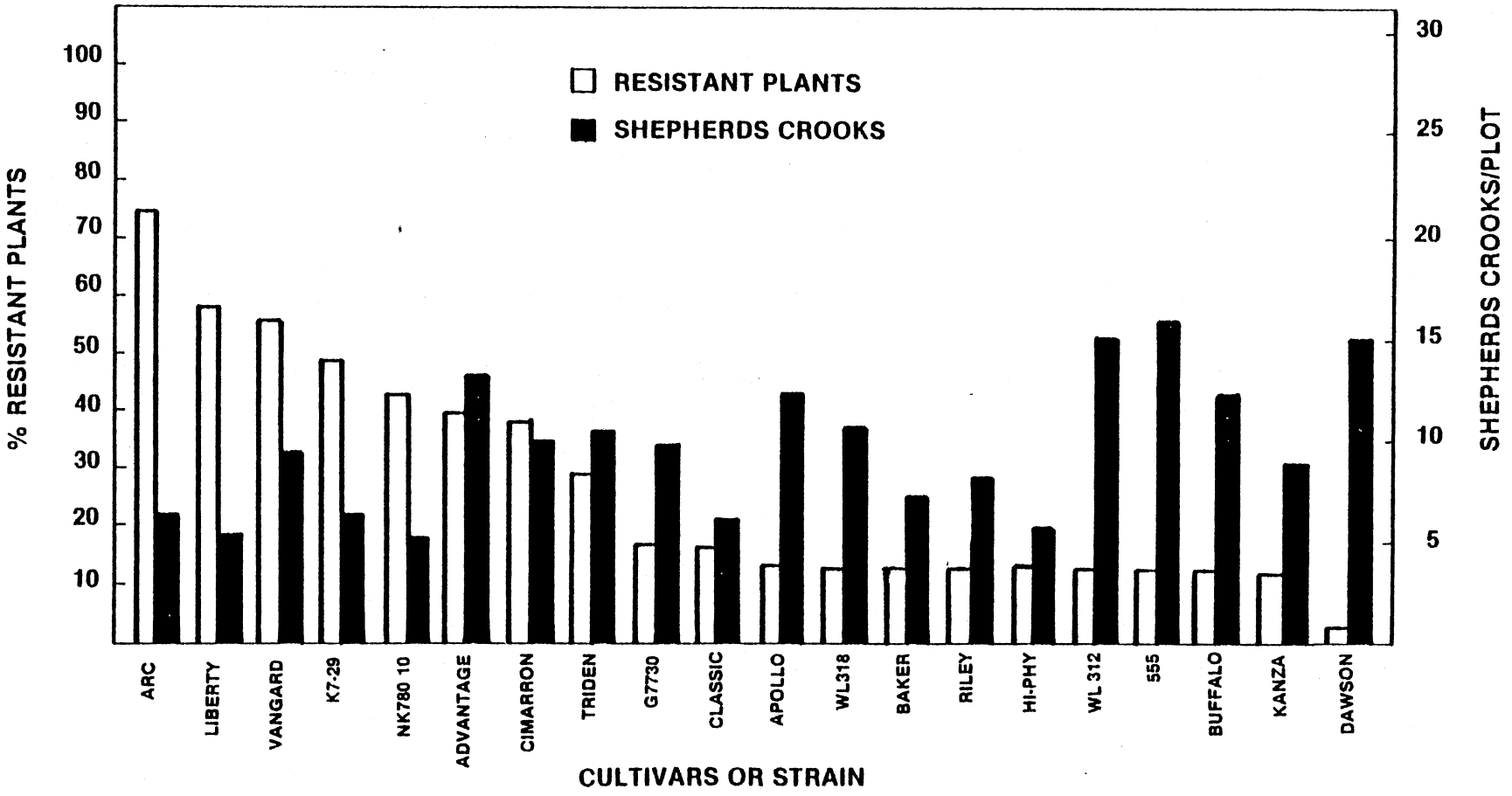

Fig. 15. Comparison of percent resistant plants (in greenhouse) and mean number of shepherds crooks per plot for experiment 901.

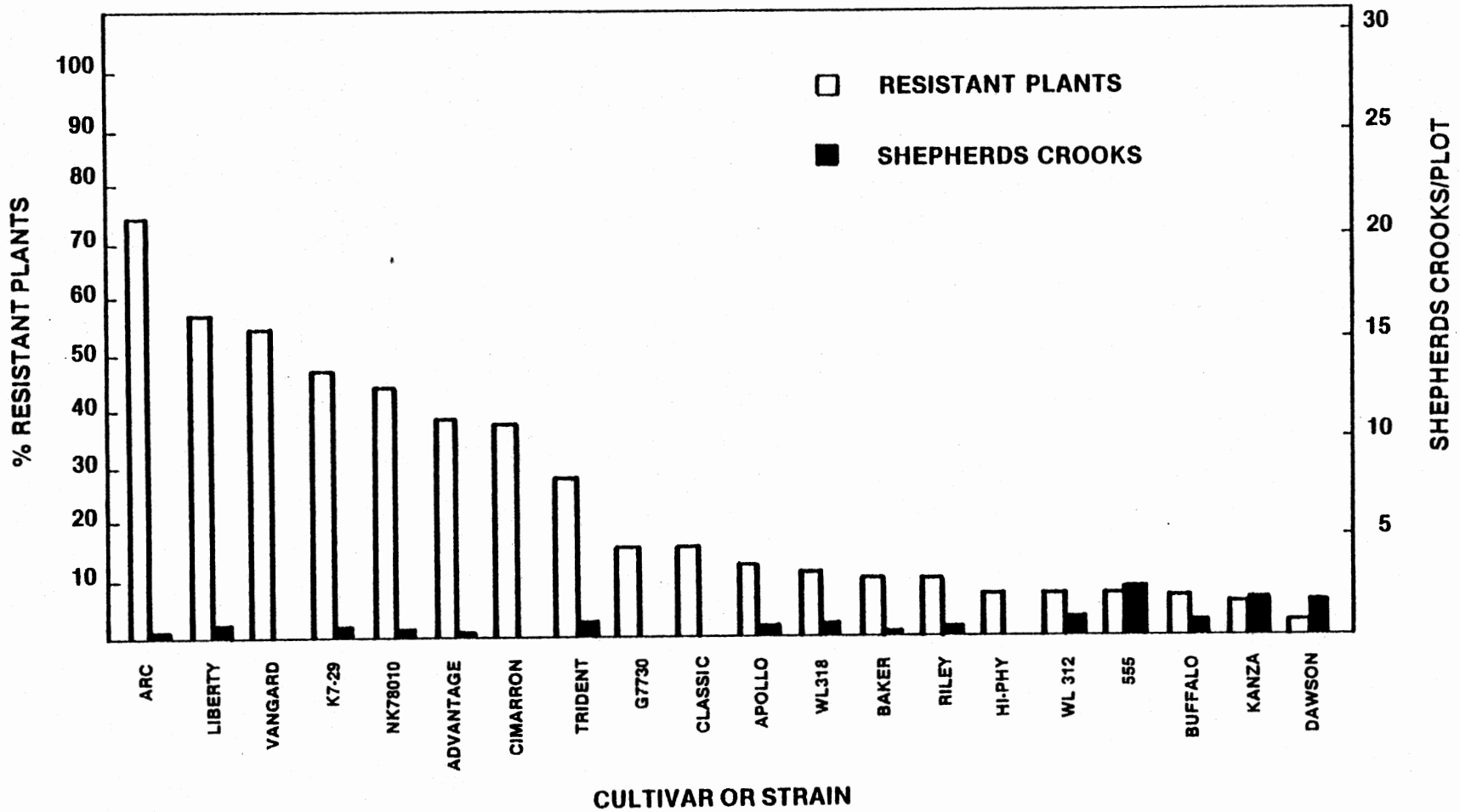

Fig. 16. Comparison of percent resistant plants (in greenhouse) and mean number of shepherds crooks per plot for experiment 941.

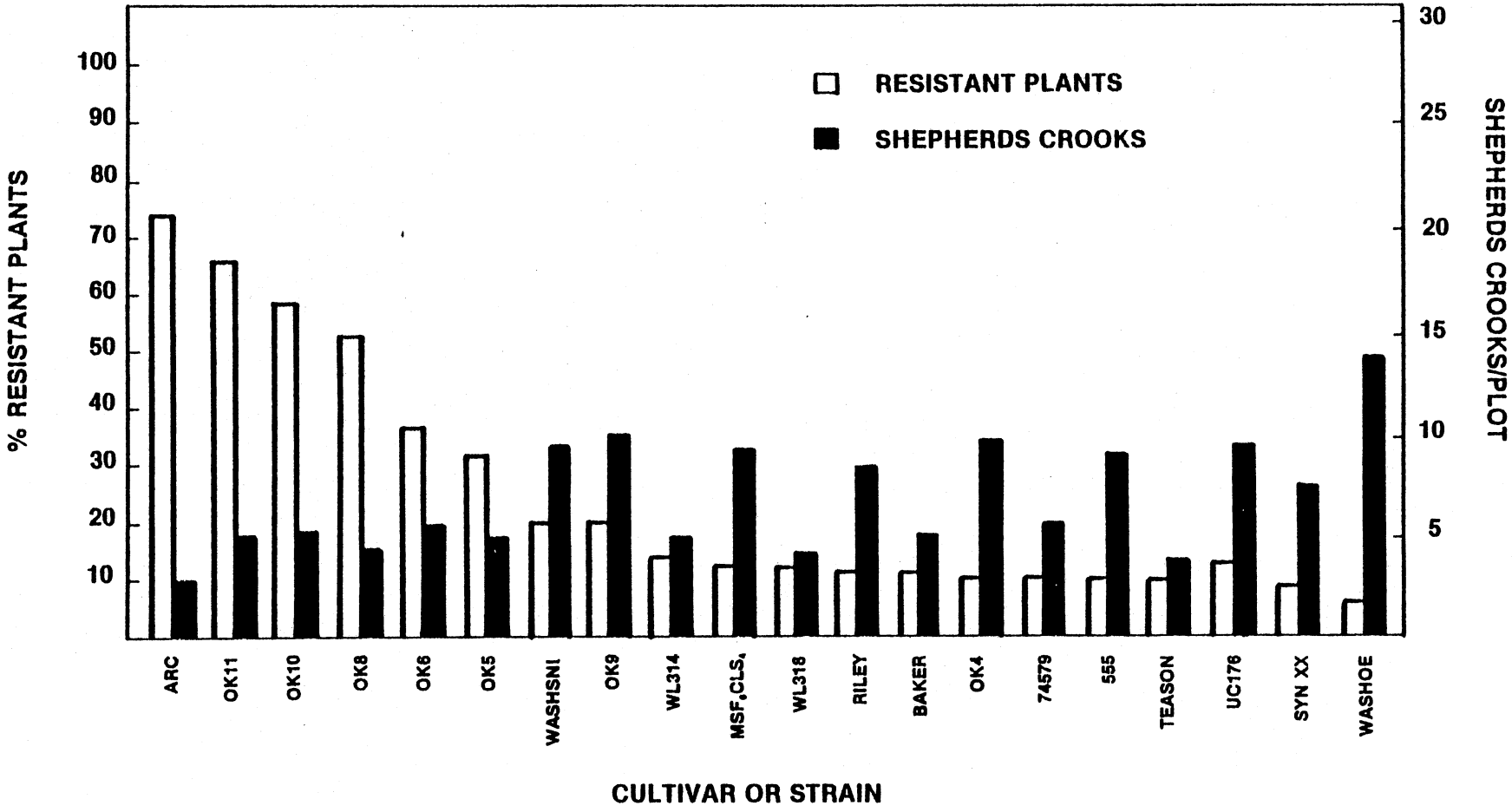

Fig. 17. Comparison of percent resistant plants (in greenhouse) and mean number of shepherds crooks per plant for experiment Ø21.

relatively fewer shepherds crooks than expected in experiments 901 and 012. However, since significant differences existed among cultivars only in experiment 901, no conclusions can be drawn without additional data.

Workers who have successfully found resistance to C. trifolii under field conditions such as Smith (108) have had an epidemic of southern anthracnose to study. Although weather conditions in Oklahoma during 1981 were favorable for the development of southern anthracnose, infection was mostly sporadic. Weather conditions in Oklahoma will only rarely, if ever, cause an epidemic of southern anthracnose. Probably such conditions are necessary to study field resistance.

CHAPTER VIII

SUMMARY AND CONCLUSIONS

The research reported in this thesis will help in understanding the importance of southern anthracnose as an alfalfa disease in Oklahoma. The information collected during each study should also lay the framework for integrating resistance to Colletotrichum trifolii into the alfalfa breeding program at the Oklahoma Agricultural Experiment Station.

Although race 1 has been isolated in Oklahoma, the new race described here, race 3, appears to be the predominant race in the State. Cultivars which are resistant to race 1 of C. trifolii are also resistant to race 3. Therefore, sources of resistance to race 1 are effective against race 3. The pathogenic characteristics of race 3 suggest that resistance to C. trifolii in alfalfa is conditioned by a major gene along with one or more modifying genes.

Two other Colletotrichum spp., C. destructivum and C. dematium f. sp. truncata are commonly isolated from alfalfa tissue. Isolates of both fungi were tested for pathogenicity and no cultivar tested was susceptible to either fungus. Therefore, neither fungus appears to be a pathogen of alfalfa in Oklahoma.

Recurrent phenotypic selection, a form of mass selection, can be utilized in developing cultivars with resistance to C. trifolii. Resistant selections from cultivars Buffalo, Riley, Baker and Oklahoma common (Kohler) were intercrossed. Progeny from this polycross (OK12) contained significantly more resistance to C. trifolii than did any of the parents.

A good source of resistance for production of alfalfa cultivars resistant to C. trifolii are Oklahoma common strains. These commons are naturalized to the environmental conditions present in Oklahoma, and contain low resistance to C. trifolii. By utilizing recurrent mass selection, the frequency of the resistant gene(s) can be increased without altering beneficial agronomic characteristics if care is taken to maintain large populations. Several public cultivars, some of which contain high resistance to one or more pests, also contain resistance to C. trifolii. These alfalfas can be used in breeding multiple-pest resistant cultivars.

An attempt was made to determine if resistance in alfalfa to C. trifolii could be measured in the field. Weather patterns in Oklahoma are not conducive to epidemics of southern anthracnose. Apparently such an outbreak is necessary to measure resistance in the field.

Southern anthracnose is not a devastating alfalfa disease in Oklahoma, but probably contributes to stand decline. The best means to control the disease is through

the use of host plant resistance. Resistance to C. trifolii is currently being incorporated into alfalfa germplasm adapted to Oklahoma. Several other disease problems, i.e. Phytophthora root rot and Fusarium root rot and wilt appear to be serious problems, and deserve study.

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