A New Vascular Wilt Disease Caused in Crimson Clover by *Fusarium oxysporum*

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The author is grateful to R. B. Carroll, F. I. Frosheiser, D. J. Hagedorn, W. W. Hare, and J. M. Kraft for supplying seed and cultures of *Fusarium oxysporum* from soybean, alfalfa, bean, cowpea, and pea, respectively.

Accepted for publication 31 August 1981.

ABSTRACT

Pratt, R. G. 1982. A new vascular wilt disease caused in crimson clover by *Fusarium oxysporum*. Phytopathology 72: 622-627.

Crimson clover (*Trifolium incarnatum*) plants with stunted, chlorotic, and necrotic foliage and discolored vascular systems were observed in three fields in Mississippi. *Fusarium oxysporum* was isolated from symptomatic root tissue; few other fungi were obtained. Vascular wilt symptoms were reproduced in roots and crowns of plants grown in the greenhouse 4–6 wk after roots were inoculated with blended cultures, infested mixtures of cornmeal and sand, or suspensions of conidia. *F. oxysporum* was reisolated from roots and crowns of most symptomatic plants. It was also reisolated from flowering stems 12 wk or more after roots were inoculated. Mortality and severe foliar symptoms usually occurred only in plants inoculated in early spring and grown under increasing day lengths. Five isolates of *F. oxysporum* differed in virulence, but all caused wilt symptoms and death of some plants. Five cultivars of crimson clover differed slightly in susceptibility. No symptoms developed in alsike, arrowleaf, berseem, red, subterranean, or white clovers inoculated with *F. oxysporum* from crimson clover. Few or no wilt symptoms developed in crimson clover inoculated with *F. oxysporum* isolates from alfalfa, bean, cowpea, pea, or soybean, and isolates from clover caused few or no symptoms in those species. Results indicate that the wilt-inducing isolates of *F. oxysporum* from crimson clover are specialized in virulence to that host.

Additional key words: Glycine max, Medicago sativa, Phaseolus vulgaris, Pisum sativum, Trifolium alexandrinum, Trifolium hybridum, Trifolium incarnatum, Trifolium pratense, Trifolium repens, Trifolium subterraneum, Trifolium vesiculosum, Vigna unguiculata.

Fusarium wilt diseases, caused by forms of *Fusarium oxysporum* Schlecht. emend. Snyder & Hansen that are specialized in virulence to one or more hosts, have been described on seven leguminous crops in North America: alfalfa (4,18), bean (34), broadbean (14), chickpea (37), cowpea (2), pea (21), and soybean (10,16). No similar diseases have been observed on clover species on this continent. *F. oxysporum* was frequently isolated from plants of alsike (*Trifolium hybridum* L.), red (*T. pratense* L.), and white (*T. repens* L.) clovers with symptoms of root rot, but most isolates were only weakly virulent on plants beyond the seedling stage and did not induce vascular wilt symptoms (6,8,12,15,17,19, 23,24,26–28,31). Armstrong and Armstrong (3,5) obtained vascular wilt symptoms in alsike and crimson (*T. incarnatum* L.) clovers inoculated with *F. oxysporum* f. sp. *cassiae,* but such disease symptoms have not been reported in these species in the field.

A Fusarium disease of red clover was reported from Russia in 1917 (10) and later from Switzerland (30) and Czechoslovakia (1). The causal organism, described as *F. trifolii* Jacz. (10), was revised to *F. oxysporum* var. *trifoli* by Raillo (33) and later to *F. oxysporum* f. *trifolii* by Bilai (9). Snyder and Hansen (36) and Messiaen and Cassini (29) did not include *F. oxysporum* f. sp. *trifolii* in their lists of formae speciales, but it was included by Gordon (20) and Booth (10). Armstrong and Armstrong (6) were reluctant to consider *trifolii* as a forma specialis of *F. oxysporum* because symptoms of vascular wilt were not clearly described on diseased clover in the field.

From 1978 to 1980, symptoms of vascular discoloration of roots and stems, and wilting, chlorosis, and necrosis of foliage, were observed in crimson clover in the vicinity of Starkville, MS. *F. oxysporum* was isolated, and tracheomycotic symptoms (6) were reproduced in clover inoculated in the greenhouse. The purposes of this report are to describe the etiology of the Fusarium wilt disease of crimson clover in Mississippi, and to compare the pathogenicity and host specificity of *F. oxysporum* isolates from crimson clover and other legumes.

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MATERIALS AND METHODS

To isolate *F. oxysporum*, crimson clover plants from the field or greenhouse were removed from soil, crowns and taproots were bisected longitudinally, and cortical tissue was trimmed from large roots. Two to four pieces of discolored tissue (2–5 × 5–10 mm) from crowns and vascular cylinders of large roots, or whole half-sections of small roots, of each plant were surface disinfested in 1% sodium hypochlorite for 15–30 sec, rinsed twice in sterile distilled water, briefly blotted on sterile filter paper, and plated on Difco cornmeal agar. Colonies developing after 4–5 days were transferred to potato-sucrose agar and modified Bilai's medium for identification (10). Cultures contaminated with bacteria were grown either upon or through 2% water agar until aseptic. Cultures were incubated at 23–27 C for isolation, identification, and inoculum production, and were stored at 4 and 8 C in cornmeal agar slants overlaid with sterile mineral oil. Isolates of *Fusarium* were identified according to standards of Booth (10).

Inoculum of *F. oxysporum* consisted of blended cultures, infested cornmeal-sand mixture, and conidial suspensions. Blended-culture inoculum was prepared by cutting three 5-wk-old colonies on Bilai's medium into small pieces (~5 mm^2) with a scalpel and comminuting in 150 ml of distilled water for 10 sec in a Waring Blendor. Mixtures of cornmeal and sand in flasks (32) were infested and incubated for 5 wk. Conidia were obtained from colonies grown 2–5 wk on potato-sucrose agar by covering the agar surfaces with distilled water and scraping them with a microspatula. The spore suspensions were rinsed into a beaker, poured through a double-layer of cheesecloth to remove large hyphal fragments, and counted with a hemacytometer (five or six counts per suspension). Inoculum concentrations were adjusted to $3–30 \times 10^5$ conidia per milliliter in most experiments; these were similar to concentrations used with Fusarium wilt diseases of other

legumes (18,21,34).

Germinated seeds of clover and alfalfa (*Medicago sativa* L. 'Vernal') were planted in steamed (120 C for 12 hr) or unsteamed soil mixture (clay loam, sand, and peat, 1:1:1, v/v) in 10.5-cm-diameter pots. Species-specific Rhizobium inoculum (Nitragin Co., Milwaukee, WI 53209) was watered into the soil around the seedlings after 10 days. Plants were grown 6–7 wk before inoculation. Greenhouse temperatures ranged from 18–24 C from autumn to early spring and sometimes reached 30 C by midspring. Day lengths ranged from 11 hr in winter to 14.5 hr in spring.

For inoculations with blended cultures and cornmeal-sand, plants were grown around the rim of each pot (four per pot), soil was removed from a centerwell (3 cm in diamater, 6 cm deep), blended inoculum (50 ml) was poured into the well or cornmeal-sand was added to fill the well, and soil was added to cover the surface. Pots with control plants received uninfested blended agar and cornmeal-sand. For inoculations with conidia, plants and soil were removed from pots, roots were washed and dipped in stirred suspensions of conidia, and plants were transplanted back into the soil mixture (four per pot). Plants were grown for 6 wk after inoculation in most experiments.

Bean (*Phaseolus vulgaris* L. 'Bush Blue Lake 274'), cowpea (*Vigna unguiculata* (L.) Walp. 'Newton Silverskin'), pea (*Pisum sativum* L. 'M 410'), and soybean (*Glycine max* L. Merr. 'Essex') were planted in flats of steamed sand and watered with a nutrient solution (35) for the first 3 days in the greenhouse. After 8–12 days, the plants were removed from the flats, their roots were washed and trimmed to 4 cm from stems while immersed in suspensions of conidia, and the plants were transplanted back to the flats. Plants were watered once with 0.01 M ammonium nitrate when control plants appeared chlorotic. Ambient greenhouse temperatures were 24–30 C for cowpea and soybean and 18–30 C for bean and pea.

Soybean (Essex), pea (New Era and Dark Skin Perfection), and crimson clover (Chief) also were grown and inoculated in growth chambers (combined cool-white fluorescent and incandescent bulbs). Soybeans were grown at 27–29 C with an illuminance of $7.5–8.6 \times 10^3$ lx, a 16-hr photoperiod, and were inoculated 5 days after planting. Pea and crimson clover were grown at 23–25 C with an illuminance of $7–12 \times 10^3$ lx, and 16- and 12-hr photoperiods, respectively. Peas were inoculated 10 days after planting and clover was inoculated 6 wk after planting. Ammonium nitrate was added to soybean and pea plants 10 days after inoculation.

Disease was evaluated in plants of all species according to the extent of vascular discoloration in bisected stems or crowns and taproots. Scores were assigned to diseased plants as follows: 0 = no symptoms, 1 = vascular discoloration in taproot but not extending to crown, 2 = vascular discoloration extending throughout length of taproot and into crown or stem, and 3 = plant dead. In most experiments, disease was only evaluated in roots and crowns of crimson clover because stems were not present. These are only formed by mature plants grown with increasing day lengths in the spring (25). The association of *F. oxysporum* with vascular discoloration in roots, crowns, and stems of crimson clover was established by reisolations in several experiments.

RESULTS

Symptoms of disease and isolation and identification of *F. oxysporum*. Vascular wilt symptoms were observed in three stands of crimson clover near Starkville, MS. In April of 1978, many plants were killed in a seed production field of cultivar Chief; survivors were stunted, wilted, late-flowering, and had chlorotic, reddened, and necrotic foliage. Vascular tissues of lateral roots and taproots were discolored pink-orange to red-brown. In plants with mild foliar symptoms, light discoloration extended from lateral roots up and down one or both sides of the vascular cylinder in longitudinally sectioned taproots. Darker discoloration occurred throughout vascular cylinders and crowns of plants with severe foliar symptoms, and it frequently extended into stems of flowering plants (Fig. 1). Mortality and disease symptoms were most severe in areas where frost heaving of soil had occurred during the winter. Fungal isolates were obtained from 34 of 36 plants; 28 isolates were identified as *F. oxysporum* and six were *F. solani* (Mart.) Sacc., two unidentified *Fusarium* spp., *Epicoccum, Phoma,* and a nonsporulating fungus.

In April of 1980, similar root and foliar symptoms were observed throughout a seed production field of cultivar Tibbee and in a cover crop of Chief. The disease was not associated with known mechanical damage to roots in these stands. *F. oxysporum* was isolated from two of six symptomatic plants of Tibbee and one of five plants of Chief. Isolations were attempted after frequent rains late in the season, and root tissue of most plants was heavily contaminated with bacteria.

F. oxysporum was identified by numerous microconidia produced from short lateral phialides on surfaces of colonies and in false heads in agar; by the absence of conidial chains; and by occasional to frequent chlamydospores and macroconidia (10). Most isolates were very similar in morphology. Radial growth rates were 0.5–0.6 cm/day on potato-sucrose agar at 23–25 C and colonies were uniformly white with moderate aerial mycelium.

Evaluation of inoculation methods. In preliminary experiments, vascular wilt symptoms developed in crimson clover inoculated with blended cultures, infested cornmeal-sand, and suspensions of conidia of *F. oxysporum*. The organism was reisolated from most symptomatic plants. To compare efficiency of the inoculation methods, six pots of Chief grown in steamed and unsteamed soil

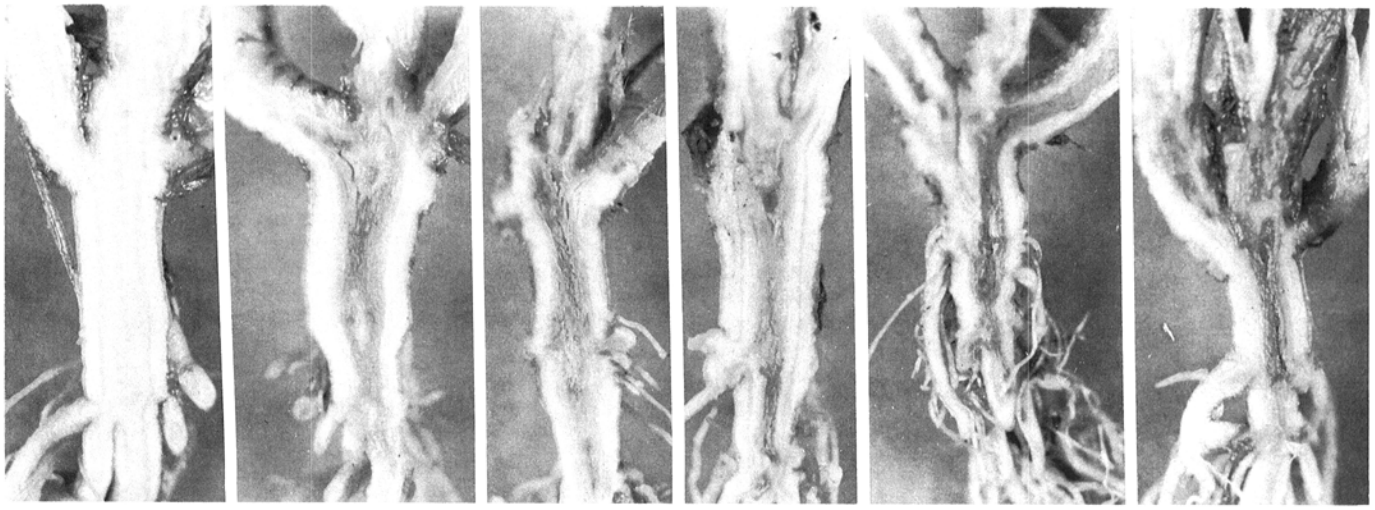

Fig. 1. Symptoms of vascular discoloration in longitudinally sectioned taproots and lower stems of crimson clover infected with *Fusarium oxysporum*. Plant on left is healthy; five plants on right are diseased and show pink-orange to red-brown vascular discoloration.

mix were inoculated by each method. Equal numbers of control plants received uninfested agar, cornmeal-sand, and root dip treatments. Inoculum of three randomly chosen isolates was composited in inoculations. Plants were inoculated in October.

Vascular wilt symptoms developed at similar frequencies in plants inoculated by the three methods and were identical to those observed in the field (Table 1) (Fig. 1). Some plants developed no symptoms. Foliage of most infected plants was stunted compared to that of the controls, especially where roots had been inoculated by dipping them in suspensions of conidia, but few other top symptoms were observed. Portions of taproots of some control plants were discolored, but this limited discoloration was purple to black and not typical of that observed in inoculated plants. One inoculated plant was dead 6 wk after inoculation.

Disease development over time. Plants of Chief were inoculated in January by root dips with composited inoculum of two randomly selected isolates (20×10^5 conidia per milliliter). At 2, 3, 4, and 6 wk after inoculation, plants of six inoculated and six control pots were evaluated for disease symptoms, and a single section from the taproot and crown of each plant was plated. Vascular discoloration was first observed in taproots after 3 wk and in crowns after 4 wk (Table 2). The incidence of discoloration in roots and crowns was highest at 6 wk. *F. oxysporum* was frequently reisolated from roots and crowns 2–4 wk in advance of symptom development. One inoculated plant was dead after 4 wk.

Virulence of isolates. Virulence of five randomly selected isolates was compared on Chief plants grown and inoculated in the growth chamber. Three isolates from field-infected plants were stored at 4 C for 3 yr and two were freshly reisolated from diseased plants in the greenhouse. Twenty-four plants were inoculated with a spore suspension of each isolate containing 15×10^5 conidia per milliliter. Roots of 24 control plants were dipped in water. The five isolates differed significantly in virulence ($P = 0.01$). The most virulent isolate caused the death of eight plants and no symptoms in one plant; the least virulent isolate caused the death of three plants and no symptoms in 10 plants. The three isolates from field-infected plants were the most virulent. No symptoms developed in controls.

Disease severity at different inoculum levels. Plants of cultivar Chief were inoculated in January with the most virulent isolate of *F. oxysporum* at inoculum levels of 13.50, 3.38, 0.84, 0.21, and 0.05 $\times 10^5$ conidia per milliliter. Twenty-four plants were inoculated at each dilution. Disease symptoms were similar and most severe at the two highest levels, similar and less severe at the intermediate levels, and slight at the lowest level (Table 3). No plants were killed in this experiment.

Symptoms of disease and presence of *F. oxysporum* in stems. Plants of cultivars Chief and Tibbee were inoculated by root dips in February and grown (four per pot) until two or more flowering stems developed. Controls were not inoculated. Surviving plants were removed from pots 12–18 wk after inoculation and examined

TABLE 1. Severity of vascular discoloration in crimson clover plants grown in steamed and nonsteamed soil mixtures and inoculated with *Fusarium oxysporum* by three methods

Soil mixture[a]	Inoculation[b] method and inoculum	Severity of disease symptoms[c]		
			Vascular discoloration	
		No symptoms	In portion of taproot	Throughout taproot and in crown
Steamed	Root dip (−)	18	6	0
	(+)	7	3	14
	Blended cultures (−)	18	6	0
	(+)	7	2	15
	Cornmeal-sand (−)	19	5	0
	(+)	4	6	14
Not steamed	Root dip (−)	15	9	0
	(+)	5	4	15
	Blended cultures (−)	20	4	0
	(+)	9	2	13
	Cornmeal-sand (−)	16	6	2
	(+)	10	4	10

[a] Soil mixture consisted of clay loam, sand, and peat (1:1:1, v/v) and was steamed at 120 C for 12 hr or not steamed.
[b] Root dip = plants removed from soil mixture, dipped in a suspension of conidia (4.4×10^5/ml) (+) or water (−), and transplanted back into soil mixture; blended cultures = colonies on agar (+) or uninfested agar (−) comminuted in water and poured into centerwells of pots; cornmeal-sand = infested (+) or uninfested (−) mixtures of cornmeal and sand added to fill centerwells of pots. All plants were grown four per pot for 6 wk before and after inoculation.
[c] Data indicate numbers of plants. Vascular discoloration was observed in longitudinally sectioned taproots and crowns. The occasional discoloration in taproots of control plants was not similar to that observed in inoculated plants.

TABLE 2. Severity of vascular discoloration in crimson clover plants and reisolation of *Fusarium oxysporum* 2–6 wk after inoculation[a]

Weeks after inoculation	Inoculum	Severity of disease symptoms[b]			*F. oxysporum* reisolated[c]	
			Vascular discoloration			
		No symptoms	In portion of taproot	Throughout taproot and in crown	Taproot	Crown
2	(−)	24	0	0	0	0
	(+)	24	0	0	6	1
3	(−)	24	0	0	0	0
	(+)	22	2	0	1	3
4	(−)	23	1	0	0	0
	(+)	19	1	4	4	7
6	(−)	21	2	1	1	0
	(+)	12	0	12	5	9

[a] Plants grown four per pot for 6 wk in a soil mixture were removed from pots, roots were dipped in a suspension of conidia (20×10^5/ml) (+) or water (−), and plants were transplanted back into the soil mixture.
[b] Data indicate numbers of plants. Vascular discoloration was observed in longitudinally sectioned taproots and crowns.
[c] Data indicate numbers of plants. One section of taproot and one piece of crown tissue were plated from each plant.

for vascular discoloration in roots, crowns, and stems. Isolations were attempted from 10 plants of each cultivar in which vascular discoloration extended at least to crowns, and from five control plants of each cultivar which had no discoloration. For each plant, one section of tissue from the mid-taproot and crown, and two sections from each of the lower, middle, and upper thirds of two flowering stems, were surface-disinfested and plated on cornmeal agar.

Vascular discoloration extended into stems of six plants of Chief and five plants of Tibbee. Discoloration was usually difficult to trace beyond lower thirds of stems, but in four plants it was visible in upper halves, and in one plant it was visible up to flower heads 17 and 27 cm above crowns. Vascular discoloration also occurred throughout other stems that were killed before flowers developed. *F. oxysporum* was reisolated from roots and crowns of all inoculated plants and from stems of eight plants of each cultivar. In nearly all instances, whenever vascular discoloration was present in a portion of stem, *F. oxysporum* was reisolated. It was also frequently reisolated from basal stem sections of plants in which discoloration did not extend beyond crowns, and from asymptomatic tissue above discolored stem sections. *F. oxysporum* was reisolated from the upper one-thirds of 10 stems of inoculated plants of both cultivars, ⩾ 8–18 cm above crowns. It was not isolated from roots, crowns, or stems of the control plants.

Susceptibility of cultivars of crimson clover. Forty plants of each of five cultivars of crimson clover were inoculated in March with composited inoculum of the two most virulent isolates of *F. oxysporum* (3×10^5 conidia per milliliter). Disease symptoms developed in 70–95% of plants of all cultivars (Table 4), and many plants were killed. Disease scores of Talladega plants were lower than those of the other four cultivars ($P = 0.05$).

TABLE 3. Severity of vascular discoloration in crimson clover plants inoculated with *Fusarium oxysporum* at different inoculum levels[a]

Inoculum level (conidia/ml × 10^5)	Severity of disease symptoms[b]		
		Vascular discoloration	
	No symptoms	In portion of taproot	Throughout taproot and in crown
13.50	2	4	18
3.38	7	1	16
0.84	13	1	10
0.21	12	2	10
0.05	19	4	1
0	22	2	0

[a] Plants grown four per pot for 6 wk in a soil mixture were removed from pots, roots were dipped in a suspension of conidia or water, and plants were transplanted back into the soil mixture.
[b] Data indicate numbers of plants. Vascular discoloration was observed in longitudinally sectioned taproots and crowns.

Host range of *F. oxysporum*. Plants of the following clover species were inoculated in March: alsike (unknown cultivar), arrowleaf (*T. vesiculosum* Savi 'Meechee' and 'Yuchi'), berseem (*T. alexandrinum* L., a winter-hardy selection), crimson (cultivars Chief and Tibbee), red (cultivars Kenland and Kenstar), white (cultivars Lucky and Regal), and subterranean (*T. subterraneum* L. 'Mt. Barker' and 'Woogenellup'). Six pots of each cultivar or selection were inoculated with composited inoculum of the two most virulent *F. oxysporum* isolates (16.5×10^5 conidia per milliliter). Symptoms of disease were severe in crimson clover; all plants of Chief had root or crown symptoms, and 13 of 24 plants were killed; 21 of 24 plants of Tibbee had symptoms and three plants were killed. No symptoms of disease were observed in any other clover species.

Host specificity of *F. oxysporum* from crimson clover and other legumes. Plants of crimson clover and other legumes were grown in the greenhouse and inoculated with *F. oxysporum* isolates from crimson clover and the corresponding host species. Forty plants of clover and alfalfa, and 20 plants of the other four species, were inoculated in each treatment. Control plants received root dips in water. One or two *F. oxysporum* isolates from each host were utilized in inoculum, and conidial suspensions were adjusted to $25–30 \times 10^5$/ml. Disease was evaluated after 6 wk in alfalfa and clover; approximately 4 wk in cowpea, pea, and soybean; and 16 days in bean.

Severe vascular wilt symptoms occurred in clover, alfalfa, bean, and cowpea plants inoculated with *F. oxysporum* from the same hosts (Table 5). All bean plants were killed and all cowpeas were killed or largely necrotic. Most alfalfa and clover plants were killed or severely diseased, but some plants of both species showed few or no symptoms. Pea and soybean plants did not develop external symptoms of vascular wilt. All pea plants inoculated with an isolate received as Race 6 of *F. oxysporum* f. sp. *pisi* (21) had symptoms of root rot and dark necrosis around collars of stems, but discoloration did not extend into vascular tissues of roots or stems. Soybean plants had slight vascular discoloration only in lowermost stems.

Few or no symptoms of disease developed in most plants inoculated with *F. oxysporum* isolates from other species. Isolates from soybean and alfalfa frequently caused vascular discoloration in lower taproots of crimson clover, but this seldom extended to crowns; no external symptoms of disease were evident in these plants. Isolates from bean, cowpea, and pea caused only occasional discoloration in lower taproots of crimson clover plants. The clover isolates caused no symptoms in alfalfa and cowpea, and only slight discoloration in roots of bean, pea, and soybean.

Crimson clover, pea, and soybean plants also were grown in growth chambers and inoculated with *F. oxysporum* isolates ($25–30 \times 10^5$ conidia per milliliter). Ten to 20 plants were used in each treatment. Isolates from crimson clover caused severe

TABLE 4. Severity of disease symptoms in five cultivars of crimson clover inoculated with *Fusarium oxysporum*[a]

Cultivar	Inoculum	Severity of disease symptoms[b]			
			Vascular discoloration		
		No symptoms	In portion of taproot	Throughout taproot and in crown	Plants dead
Autauga	(−)	15	1	0	0
	(+)	4	4	15	17
Chief	(−)	16	0	0	0
	(+)	2	8	9	21
Dixie	(−)	14	2	0	0
	(+)	4	3	14	19
Talladega	(−)	16	0	0	0
	(+)	12	5	17	6
Tibbee	(−)	15	1	0	0
	(+)	3	6	19	12

[a] Plants grown four per pot for 6 wk in a soil mixture were removed from pots, roots were dipped in a suspension of conidia (3×10^5/ml) (+) or water (−), and plants were transplanted back into the soil mixture.
[b] Data indicate numbers of plants. Vascular discoloration was observed in longitudinally sectioned taproots and crowns.

vascular wilt symptoms in most Chief crimson clover plants, but no symptoms or only slight vascular discoloration in roots of Essex soybeans and New Era and Dark Skin Perfection peas. Race 2 and Race 5 isolates of *F. oxysporum* f. sp. *pisi* caused severe vascular wilt symptoms in Dark Skin Perfection and New Era peas, respectively, and most plants were killed. The pea isolates caused no symptoms in crimson clover. The soybean isolates only caused slight vascular discoloration in roots of soybean plants.

DISCUSSION

Results of this study demonstrate the occurrence of a vascular wilt disease of crimson clover caused by a host-specific form of *F. oxysporum*. This is believed to be the first report of a clearly defined and naturally occurring Fusarium wilt disease on any species of *Trifolium*. The symptoms of vascular discoloration in roots and stems, accompanied by stunting, wilting, reddening, chlorosis and necrosis of foliage, and death of plants, represent a classical tracheomycosis (6). *F. oxysporum* was the principal organism isolated from plants with these symptoms in the field. Symptoms similar to those observed in the field developed in plants inoculated with virulent isolates under favorable conditions in the greenhouse, and *F. oxysporum* was reisolated.

Isolates of *F. oxysporum* from crimson clover differ in pathogenicity from those reported from red, white, and alsike clovers in North America (8,12,15,17,19,26–28) and from subterranean clover in Australia (11,22). Most of these isolates caused slight to moderate root rot on original hosts, but they did not cause distinct vascular wilt symptoms. The isolates from crimson clover, in contrast, did not cause root rot in seven other clover species but did cause a vascular wilt in crimson clover.

Chi and Hanson (13) reported wilting and vascular plugging of red clover infected with *Fusarium* spp. in the field. However, similar symptoms were reproduced only in excised stems dipped in culture filtrates. Wilt-type symptoms also were caused by *F. solani* and *F. roseum* under these conditions (13). Other reports also have described vascular discoloration in roots of naturally and artificially infected red and alsike clovers (15,17,19,24,26,36). However, the discoloration in these instances was only one part of a broad syndrome of symptoms associated with Fusarium or common root rot, including feeder root necrosis, cortical rot, crown rot, and vascular decay. These other symptoms frequently graded into vascular discoloration and often originated from insect damage on taproots and crowns. Vascular discoloration also may occur with internal breakdown, an apparent physiogenic condition in red clover (15,17,36). In the Fusarium wilt disease of crimson clover, in contrast, vascular discoloration was not associated with feeder root necrosis or cortical rots, did not originate from crowns, and was not related to mechanical damage to large roots. Symptoms observed in the field and greenhouse were identical, consistent, and similar to those caused by *F. oxysporum* f. sp. *medicaginis* in alfalfa (Table 5) (4,18).

The isolates of *F. oxysporum* from crimson clover caused few or no symptoms in six other *Trifolium* spp., and in five other legumes. Similarly, isolates from the other legumes caused only slight discoloration in roots of crimson clover, and no external symptoms except for slight stunting. These results suggest that the crimson clover isolates are host-specific in virulence and are distinct from the formae speciales *medicaginis, phaseoli, pisi,* and *tracheiphilum*. Comparisons with soybean isolates are less certain because adequate wilt symptoms did not develop with these isolates on soybean. Nevertheless, the clover isolates caused only occasional vascular discoloration in plants of a soybean cultivar reported susceptible to *F. oxysporum* (16). This suggests that the clover isolates are also distinct from *F. oxysporum* f. sp. *glycines*.

Armstrong and Armstrong (3,5) reported that *F. oxysporum* f. sp. *cassiae* caused severe vascular wilt in alsike and crimson clovers in the greenhouse. However, f. sp. *cassiae* has a wide host range and also causes vascular wilt in alfalfa (4,5). Since the crimson clover isolates did not cause disease in alfalfa or alsike clover, they are not representative of f. sp. *cassiae*. Forma specialis *vasinfectum* also causes severe wilt in alfalfa (4), and therefore it also is distinct from the crimson clover isolates. Most other formae speciales of *F. oxysporum* are known to cause disease only in original hosts (7,10).

The isolates of *F. oxysporum* from crimson clover should not be considered similar to f. sp. *trifolii* (9) because that form is not

TABLE 5. Severity of disease symptoms in crimson clover and other legumes inoculated with *Fusarium oxysporum* isolates from crimson clover and corresponding hosts

Plant species[a]	Source hosts of isolates[b]	Severity of disease symptoms[c]			
			Vascular discoloration		
		No symptoms	In portion of taproot	Throughout taproot and in crown or stem	Plants dead
Crimson clover	0 (control)	39	0	1	0
	Crimson clover	3	6	19	12
	Alfalfa	19	19	2	0
	Bean	36	4	0	0
	Cowpea	37	3	0	0
	Pea	34	6	0	0
	Soybean	23	16	1	0
Alfalfa	0 (control)	40	0	0	0
	Alfalfa	1	7	13	19
	Crimson clover	40	0	0	0
Bean	0 (control)	20	0	0	0
	Bean	0	0	0	20
	Crimson clover	18	2	0	0
Cowpea	0 (control)	20	0	0	0
	Cowpea	0	0	5	15
	Crimson clover	20	0	0	0
Soybean	0 (control)	18	2	0	0
	Soybean	5	7	8	0
	Crimson clover	14	6	0	0

[a] Cultivars of species in order of listing were Chief, Vernal, Bush Blue Lake 274, Newton Silverskin, and Essex. Forty plants of clover and alfalfa were inoculated in each treatment; 20 plants of bean, cowpea, and soybean were inoculated in each treatment. Clover and alfalfa were inoculated at 6 wk, bean and soybean at 8 days, and cowpea at 12 days. All plants were inoculated by root dips in suspensions of conidia or water (controls).

[b] One or two isolates of *F. oxysporum* from each host were utilized in inoculum. Conidial suspensions were adjusted to $25–30 \times 10^5$/ml in all treatments. Races of isolates from bean, cowpea, and pea were U.S., 3, and 6, respectively.

[c] Data indicate numbers of plants. Vascular discoloration was observed in longitudinally sectioned taproots, crowns, and stems. Alfalfa and clover were evaluated 6 wk after inoculation, cowpea and soybean were evaluated at 4 wk, and beans were evaluated at 16 days.

justified by present standards (6). The original description of *F. trifolii* was not located. Subsequent reports did not clearly establish that the organism caused a vascular wilt disease rather than a root rot of red clover (1,9,30,33). Other reports of *F. oxysporum* on red clover in Europe have not suggested the occurrence of a vascular wilt disease (38).

F. oxysporum caused discoloration throughout vascular tissues of roots and crowns of many crimson clover plants in all experiments, but severe top symptoms and mortality usually occurred only in plants inoculated in early spring. Symptoms also were observed in the field only in mature stands in spring, near the end of the growing season. These observations indicate that symptom expression of Fusarium wilt in crimson clover is related to the physiology and growth stage of the host. During fall and winter, with cool temperatures and short day lengths, crimson clover produces foliage from buds at the crowns and retains a bushy growth habit without development of stems. As temperatures and day lengths increase in the spring, stems develop and elongate, and flowering commences (25). Plants severely infected in the winter, in both the greenhouse and field, often show no external symptoms apart from stunting. When plants are infected in the spring, however, vascular discoloration progresses into developing stems; foliage then becomes chlorotic, reddened and necrotic, and many plants wilt and die (Tables 4 and 5).

The fact that the Fusarium wilt of crimson clover occurred in three separate fields near Starkville, MS, suggests that it may be widespread. However, information on the occurrence and distribution of diseases of crimson and other annual clovers is difficult to obtain because these crops are utilized only in mixed stands with grasses and are continually grazed from late autumn to early spring. It is likely that in grazed pastures, the principal symptoms of Fusarium wilt would be a gradual decline and disappearance of clover in the stand with little or no reestablishment the following autumn. These symptoms would be similar to those of Phytophthora root rot (32). Foliar symptoms are conspicuous only in seed production fields, cover crops, or experimental plots where the clover is grown in pure stands without grazing.

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