Predisposition of Soybean Seedlings to Fusarium Root Rot with Trifluralin

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ABSTRACT

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Soil-incorporated trifluralin significantly increased the severity of Fusarium root rot of soybean seedlings (*Glycine max* 'Corsoy') in greenhouse and field trials. Hypocotyl swelling and cracking caused by trifluralin appeared to offer favorable sites for penetration by *Fusarium oxysporum*. Exposure of soybean seedlings to trifluralin before being transplanted to pathogen-infested soil also resulted in an increase in root rot severities. The predisposing effect of trifluralin decreased with increasing soil temperatures. No consistent in vitro effects of trifluralin on growth or reproduction of *F. oxysporum* were observed. These data support the hypothesis that the primary effect of trifluralin on the host-pathogen interaction is to predispose soybean seedlings to infection by *F. oxysporum*.

Fusarium root rot of soybean seedlings has been reported from the United States since 1961 (3-5,10,21). Affected plants may exhibit poor or slow emergence, and resultant seedlings often are stunted and weak (17). The fungus may penetrate directly through the epidermis or indirectly through hypocotyl stomata, lenticels, or through wounds arising during secondary root formation and cause necrotic lesions on lower stems and roots. Although the disease is favored by cool (14-23 C)

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temperatures and saturated soil conditions, affected plants may later wilt and die because of poor root development when soils dry. Severely affected seedlings may survive but often are permanently stunted and unthrifty. Yield losses to *Fusarium oxysporum* Schlechtend.: Fr. as great as 59% have been observed in controlled studies (11). Although several species of *Fusarium* have been isolated from affected plants, pathogenicity tests (21) usually implicate certain isolates of *F. oxysporum* as the primary causal agent. Although these isolates are morphologically and culturally identical to strains of *F. oxysporum* that cause a vascular wilt of soybeans, they are considered distinct strains. Resistance to Fusarium root rot of soybean has been reported, and effective screening techniques are available (11).

Trifluralin is a widely used, soil-incorporated herbicide for preplant application in soybean production. The effects of trifluralin on root disease development and plant pathogens have been reported for several pathosystems. Trifluralin reduces Aphanomyces root rot of pea (7,16,19) and Rhizoctonia damping-off of cowpea (12) but can predispose cotton to infection by *Rhizoctonia* sp. (13) and soybeans to *Phytophthora megasperma* Drechs. f. sp. *glycinea* T. Kuan & D. C. Erwin (2). Trifluralin may (1,15,22-24) or may not (6,8) reduce root rot severity of dry beans. Trifluralin also increases chlamydospore production and germination of *F. oxysporum* f. sp. *vasinfectum* (Atk.) W. C. Snyder & H. N. Hans. (18), inhibits mycelial growth by *Sclerotium rolfsii* Sacc. (14), reduces production of motile zoospores by *Aphanomyces euteiches* Drechs. (7,19), increases oospore production by *P. m.* f. sp. *glycinea* (2), and reduces linear growth of *Rhizoctonia solani* Kühn in culture (12).

Because Fusarium root rot has caused sporadic damage to soybean fields with a history of application of trifluralin in South Dakota, studies were initiated to determine the role of trifluralin in the etiology of Fusarium root rot of soybean. Objectives of our research were to: 1) determine the effect of trifluralin on the severity of Fusarium root rot of soybeans in the field and greenhouse; 2) examine effects of prior exposure to trifluralin on the susceptibility of soybean to Fusarium root rot; 3) determine the effect of soil temperature on the trifluralin-Fusarium root rot interaction; and 4) determine the effects of trifluralin on growth, spore

production, and spore germination of *F. oxysporum*. A preliminary report has been published (20).

MATERIALS AND METHODS

An isolate (of proven pathogenicity) of *F. oxysporum* from diseased soybeans in South Dakota was maintained on potato-dextrose agar (PDA) and used in all experiments. Inoculum consisted of 3- to 4-wk-old cultures of the fungus grown on sterile, autoclaved barley seed. Stock herbicide solutions were prepared by mixing a Treflan 4E herbicide formulation of trifluralin in sterile water. The soybean cultivar Corsoy was used in all experiments.

Greenhouse studies of the trifluralin-Fusarium root rot interaction. The experiment consisted of a 4 × 3 factorial arrangement of four rates of trifluralin (0, 0.56, 1.12, and 2.24 kg a.i./ha; corresponding to 0, 50, 100, and 200% of the recommended application rate for soybeans) and three inoculum levels (0, 1, and 2% inoculum by weight in the soil mix) replicated three times in a randomized complete block design. Individual experimental units consisted of single 10-cm-diameter plastic pots filled with 465 g of 2:1:1 (v/v/v) mixture of silt-loam soil:sand:vermiculite planted with four soybean seeds. Trifluralin and finely ground inoculum were mixed thoroughly into the soil mixture in a rotating barrel mixer which was surface-disinfested with a 0.5% sodium hypochlorite solution between batches. Soil and air temperatures in the greenhouse averaged 13 and 18 C, respectively, during the course of the experiment. Whole seedlings were harvested 30 days after planting and washed under running tap water, and plant height, disease severity, and herbicide injury were recorded. Individual plants were rated for root rot severity on a 1–5 scale where 1 = no symptoms; 2 = <20% of root tissue discolored with scattered lesions, root system intact; 3 = 20–50% discoloration, coalescing lesions, some loss of root system; 4 = 50–75% of root system discolored, few lateral roots left; and 5 = tap root disintegrated and nonfunctional with little or no lateral root development. A disease severity index (DSI) which ranged from 0.2 to 1.0 was calculated for each experimental unit by the following formula: DSI = (number of plants in disease class × class value)/(total number of plants × 5).

Trifluralin injury to seedlings was assessed on a four-class scale where 1 = no visible damage; 2 = noticeable reduction in secondary roots; 3 = visible hypocotyl swelling with secondary root system reduced 50% or more; and 4 = extensive hypocotyl swelling and extreme stubbiness of root tips. Root and shoot dry weights and root:shoot ratios were determine by cutting sampled washed seedlings at the soil line, oven drying to complete dryness, and weighing them.

Field studies. Three identical experiments, representing three planting dates (7 May, 21 May, and 5 June), were planted on the South Dakota State University Agronomy Farm in 1981. Each experiment consisted of a 4 × 3 factorial arrangement of trifluralin treatments (0.00, 0.56, 1.12, and 2.24 kg a.i./ha) and inoculum levels (0, 100, and 200 g per row) in a randomized complete block design with three replications. An experimental unit consisted of four 3-m-long rows, with 76 cm between rows. Trifluralin was applied immediately before planting with a one-wheel bicycle sprayer and was incorporated to a depth of 5–7.5 cm with a rototiller. Inoculum was distributed evenly in the furrow at a 5-cm depth, and 50 Corsoy soybean seeds were hand-planted directly on top of the inoculum. Ten plants were randomly collected in each of the two outer rows of each plot 30 days after planting. Disease severity, herbicide injury, and root and shoot weights were determined for these plants as in the greenhouse experiment. Mean date of emergence and percentage of emergence were recorded for all four rows in each plot.

Effect of previous trifluralin exposure on Fusarium root rot development. An experiment consisting of a 3 × 4 × 3 factorial arrangement of trifluralin rates (0, 1.12, and 2.24 kg a.i./ha), length of trifluralin exposure (2, 4, 6, and 8 days), and soil inoculum levels (0, 1, and 2% by weight of soil) in a randomized complete design with three replications was conducted in the greenhouse. Trifluralin was incorporated into a 1:1 (v/v) sterilized sand:vermiculite mix. Fifteen Corsoy soybean seeds were planted in each 20-cm-diameter clay pot that contained one of the three trifluralin-treated mixes. After 2, 4, 6, and 8 days

Table 1. Analyses of variance of the effects of four rates of trifluralin herbicide[a] and three inoculum levels of *Fusarium oxysporum*[b] on root rot severity (DSI), herbicide injury ratings, root and shoot weights, and root:shoot ratios of soybean (cv. Corsoy) in the greenhouse

		F ratio				
Source of variation	df	DSI[c]	Herbicide injury[d]	Root weight	Shoot weight	Root:shoot ratio
Trifluralin level (T)	3	21.2**[e]	93.8**	0.7	1.2	0.5
Inoculum level (I)	2	242.9**	1.5	5.9**	6.3**	0.3
T × I	6	6.8**	0.3	1.0	1.4	0.6

[a] 0, 0.56, 1.12, and 2.24 kg a.i./ha.
[b] 0, 1, and 2% of soil mix by weight.
[c] Disease severity index (DSI) is based on disease ratings of individual plants on a 1–5 scale where 1 = no symptoms and 5 = complete disintegration of the primary root with no lateral root development and was calculated for each experimental unit (pot) by the formula: DSI = (number of plants in disease class × class value)/(total number of plants × 5).
[d] Herbicide injury ratings were made on a 0–4 scale where 0 = no visible injury and 4 = extensive hypocotyl swelling and cracking with little secondary root formation.
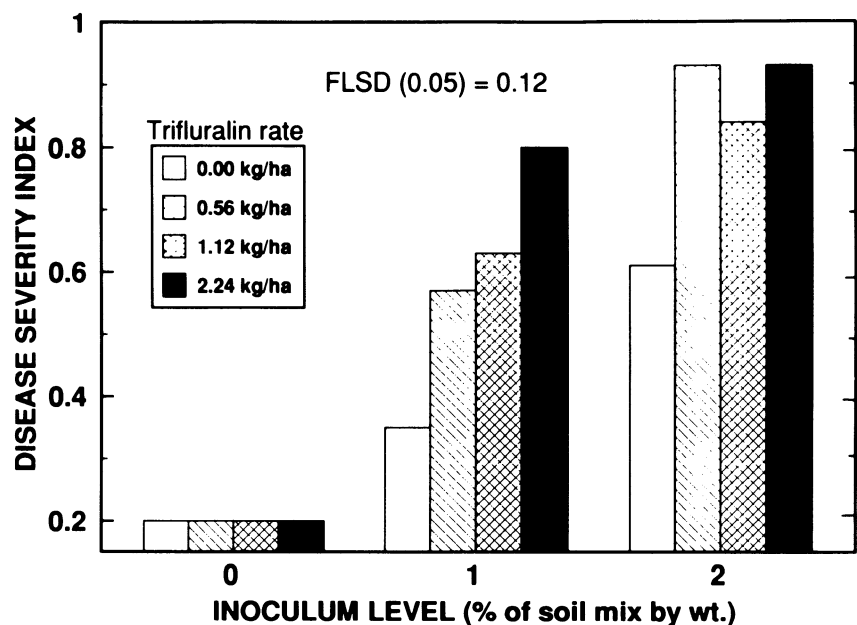[e] *F* ratio is significant at the $P \leq 0.01$ level.

Fig. 1. Effect of varied rates of trifluralin concentration and inoculum levels in soil on development of Fusarium root rot of soybean (cv. Corsoy) in the greenhouse. Disease severity index values can range from 0.2 (no symptoms) to 1.0.

Table 2. Analyses of variance of effects of four trifluralin and three inoculum levels of *Fusarium oxysporum* on the root rot severity (DSI), root dry weights, root:shoot ratio, emergence percentage, and herbicide injury[a] of soybean cv. Corsoy planted in the field on three planting dates in 1981

		F ratio														
		Planting date														
		7 May					**21 May**					**5 June**				
Source of variation	df	DSI	Root weight (g)	Root: shoot	Emergence (%)	Herbicide injury	DSI	Root weight (g)	Root: shoot	Emergence (%)	Herbicide injury	DSI	Root weight	Root: shoot	Emergence (%)	Herbicide injury
Trifluralin levels (T)	3	9.3**[b]	0.5	0.9	4.1*	49.6**	6.4**	7.1**	0.5	5.9**	82.6**	11.4**	9.8**	0.7	3.2*	49.1**
Inoculum levels (I)	2	13.9**	0.2	0.1	0.1	4.6*	11.9**	0.7	0.6	0.5	0.6	14.1**	1.0	0.6	0.2	0.3
T × I	6	0.8	0.8	0.2	2.0	2.0	0.6	0.4	0.6	1.0	0.3	0.3	0.6	0.4	1.0	0.9

[a] Herbicide injury ratings were made on a 0–4 scale where 0 = no visible injury and 4 = extensive hypocotyl swelling and cracking with secondary root formation.
[b] * = F ratio is statistically significant at P = 0.05; ** = F ratio is statistically significant at P = 0.01.
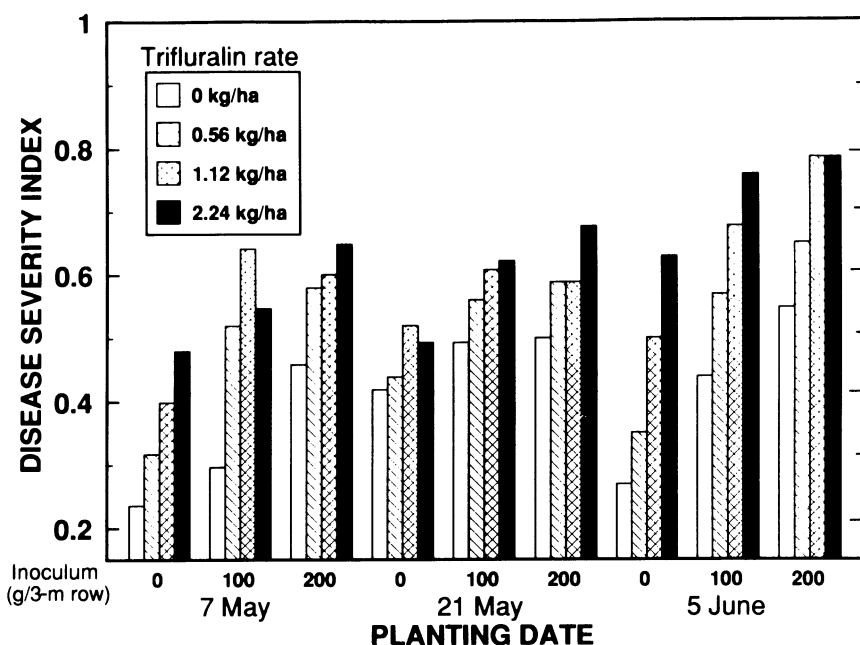

Fig. 2. Disease severity indices of soybean (cv. Corsoy) planted in soil treated with four rates of trifluralin and three levels of inoculum of *Fusarium oxysporum* on each of three dates in 1981. Disease severity index values can range from 0.2 (no symptoms) to 1.0.

Table 3. Analyses of variance of disease severity indices (DSI) and herbicide injury ratings of soybean cv. Corsoy exposed to three levels of trifluralin[a] for four exposure times[b] and three levels of inoculm of *Fusarium oxysporum*[c] in the greenhouse

		F ratios	
Source of variation	df	DSI	Herbicide injury[d]
Exposure time (E)	3	19.0**[c]	35.1**
Trifluralin rate (T)	2	105.4**	383.8**
Inoculum level (I)	2	601.0**	2.1
E × T	6	1.0	8.9**
E × I	6	5.8**	0.5
T × I	2	26.9**	0.8
E × T × I	12	0.9	0.6

[a] 0, 1.12, and 2.24 kg a.i./ha.
[b] 2, 4, 6, and 8 days.
[c] 0, 1, and 2% of soil mix by weight.
[d] Herbicide injury ratings were made on a 0–4 scale where 0 = no visible injury and 4 = extensive hypocotyl swelling and cracking with little secondary root formation.
[c] ** = F ratio is statistically significant at P = 0.01 level of probability.

of exposure to trifluralin, seedlings were transplanted to 10-cm-diameter clay pots (four per pot) that contained a 1:2 (v/v) sterilized sand:vermiculite mix and one of three inoculum levels. Transplanted seedlings were grown until the two- to three-trifoliolate leaf stage, harvested, and assessed as described pre-

viously for disease severity, herbicide injury, and root and shoot weight.

Effect of soil temperature on the trifluralin-Fusarium root rot interaction. Six Wisconsin-type soil temperature tanks (9) with regimes at 5 C intervals from 10 to 35 C (± 1 C) were used to test the effect of soil temperature on the

trifluralin-Fusarium root rot interaction. Within each temperature tank, there was a 2 × 2 factorial arrangement of trifluralin treatments (0 and 1.12 kg a.i./ha) and soil inoculum levels (0 and 1% by weight of soil) in a completely randomized design with two replications. An experimental unit consisted of a single cylindrical plastic watertight container (10 cm diameter × 21.5 cm deep) planted with four soybean seeds in a 2:1 (v/v) sterilized soil:sand mix. Plants were harvested 30 days after planting and were rated for disease severity and trifluralin injury as described previously. Analyses of variance were performed on data from each temperature tank. Estimates of experimental error within each temperature tank were pooled and an FLSD statistic to compare treatment means within temperature tanks was calculated. Regression analysis was used to analyze the response of the trifluralin × inoculum interaction effect on temperature.

In vitro effects of trifluralin on *F. oxysporum*. Single 5-mm-diameter plugs of a 2-wk-old PDA culture of *F. oxysporum* were added to 300-ml flasks containing 100 ml of potato-dextrose broth amended with either 0.00, 0.10, 0.25, 0.50, 1.00, or 2.00 μg a.i./ml of trifluralin and grown for 7 days on a mechanical shaker at 150 rpm and 25 ± 3 C. Each concentration of trifluralin was replicated four times. At the end of 7 days, contents of the flasks were filtered through a single layer of Dacron gauze (Du Pont Company, Wilmington, DE). The mycelium was scraped and washed from the gauze onto a piece of Whatman No. 4 filter paper in a petri dish, oven-dried at 65 C to a constant dry weight, and the final weight was recorded. The experiment was repeated once. The second experiment was identical to the first, except that in addition to measuring mycelial growth, the filtrate from each flask was collected in sterile 300-ml flasks and the concentration of total microconidia and germinated microconidia of *F. oxysporum* was determined with a hemacytometer.

The effect of trifluralin on chlamydospore production by *F. oxysporum* in sterile water was assessed by adding two 5-mm-diameter plugs of a 13-day-old PDA culture of *F. oxysporum* to 250-

ml flasks containing 100 ml of sterile water amended with 0.00, 0.20, 0.25, 0.50, 1.50, or 2.00 µg a.i./ml of trifluralin. Each trifluralin concentration was replicated twice. After 6 wk of growth at room temperature (25 ± 3 C) under fluorescent light (12 hr light and 12 hr dark), the concentration of chlamydospores in the flasks was determined with a hemacytometer.

Chlamydospore production by *F. oxysporum* in soil was determined by infesting 50 g of sterile, slightly moistened loam soil contained in autoclaved 125-ml flasks with 1 ml of a suspension of 2.4×10^6 microconidia per milliliter of *F. oxysporum*. Flasks were incubated at room temperature (25 ± 3 C) for 2 days, and trifluralin stock solutions were added to the flask to attain concentrations of 0.00, 0.10, 0.25, 0.50, 1.00, 2.00, or 5.00 µg/g of soil. Each trifluralin treatment was replicated three times. After 6 wk of incubation at room temperature, 40 ml of water was added to each flask, which was then shaken on a mechanical shaker for 30 min. Single 10-ml samples of the soil suspension from each flask were mixed with 40 ml of water and heavier particles were allowed to settle for 1 min. Two 10-ml samples of the resulting suspension were diluted 1:1 (v/v) with water. Chlamydospore concentrations in the final suspension were determined with a hemacytometer. Chlamydospores were easily distinguished from soil particles without staining.

RESULTS

In the initial greenhouse experiment, DSI increased with increasing levels of both trifluralin and inoculum of *F. oxysporum* (Table 1, Fig. 1). The trifluralin × inoculum interaction was also significant, indicating that the effects of these factors were not independent of each other (Table 1). Herbicide injury ratings increased only in response to trifluralin concentration (Table 1). Both plant root and shoot dry weights were significantly reduced by inoculum levels and not trifluralin, but root:shoot ratios were not affected by trifluralin or inoculum level (Table 1).

In the field experiment, trifluralin significantly increased the DSI across all inoculum levels at each planting date (Table 2, Fig. 2). With the exception of the 7 May planting date, herbicide injury was influenced only by trifluralin concentration (Table 2). Similarly, trifluralin reduced the percentage of seedling emergence but the level of inoculum of *F. oxysporum* did not. Root dry weights were reduced only by trifluralin in the 21 May and 5 June plantings but not the 7 May planting dates (Table 2). Root:shoot ratios were not significantly affected by any treatment on any date of planting (Table 2).
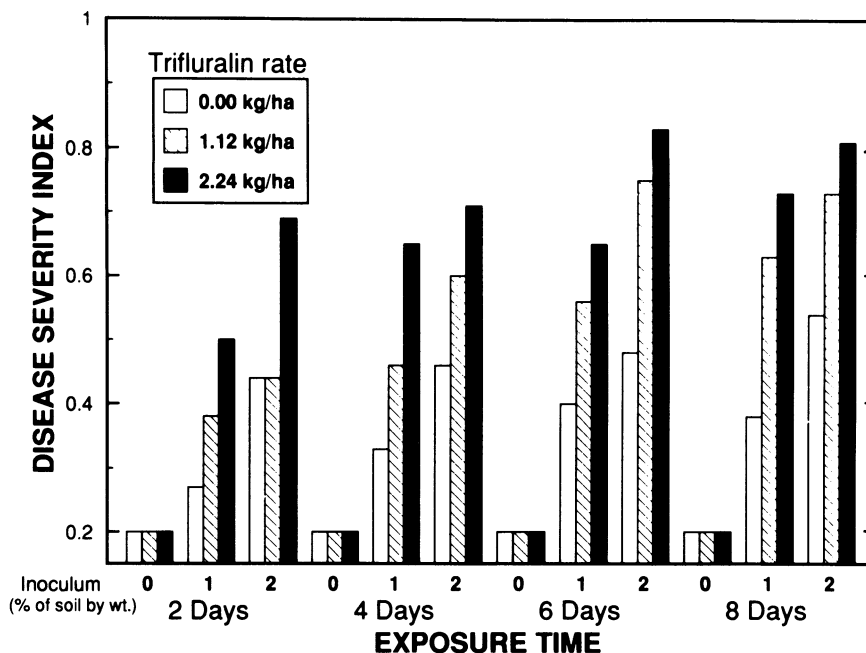
DSI values were increased signifi-

Fig. 3. Effect of four exposure durations of soybean cv. Corsoy to three levels of trifluralin before exposure to three inoculum levels of *Fusarium oxysporum* on disease severity indices. Disease severity index values can range from 0.2 (no symptoms) to 1.0.
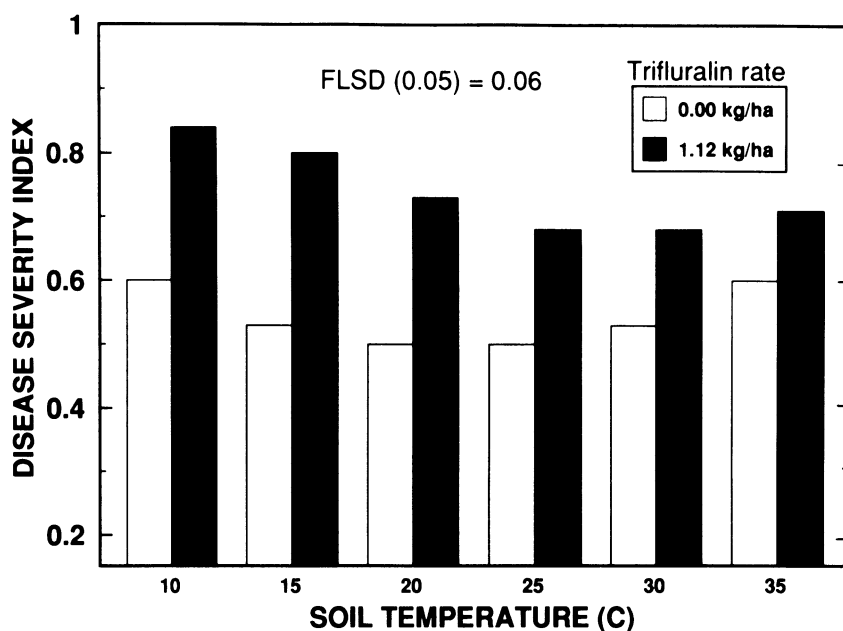

Fig. 4. Effect of soil temperature and trifluralin on Fusarium root rot severity indices of soybean cv. Corsoy. The Fisher's protected LSD (FLSD) is valid only for comparison between treatments within a given temperature.

Table 4. Effect of trifluralin concentration on mycelial growth of *Fusarium oxysporum* and microconidia concentration and germination after 7 days of growth in potato-dextrose broth at 25 C

Trifluralin (µg/ml)	Mycelial dry weight (g)	Microconidia/ml ($\times 10^6$)	Germination of microconidia (%)
0.00	0.98[y]	2.40[y]	7.1 c[z]
0.10	1.04	2.97	10.4 b
0.25	1.02	2.84	7.3 c
0.50	1.06	2.54	11.3 b
1.00	1.03	2.85	17.5 a
2.00	1.02	3.03	17.0 a

[y] Means in these columns are not significantly different from each other at the $P = 0.05$ level.
[z] Means followed by the same letter are not different from each other at $P = 0.05$ according to Duncan's multiple range test.

cantly by the length of prior exposure of seedlings to trifluralin before transplanting to soil containing inoculum of *F. oxysporum* (Table 3, Fig. 3). DSI was increased with increasing rates of trifluralin and inoculum. The exposure time × inoculum interaction and the trifluralin × inoculum level interaction were also significant because there was no disease in the absence of inoculum, regardless of trifluralin concentration on exposure time (Table 3). Herbicide injury ratings generally increased with increased levels of trifluralin and lengths of exposure of seedlings, although the effects of these two factors were not independent (Table 3).

The effect of trifluralin on DSI increased with decreasing soil temperatures (Fig. 4). At all temperatures tested, trifluralin significantly increased DSI (Fig. 4). The correlation coefficient between soil temperature and the difference in DSI between trifluralin-treated and untreated seedlings grown in infested soil was $r = -0.99$ ($P \leq 0.01$).

Trifluralin concentration in liquid medium had no effect on dry weight of mycelium or production of microconidia of *F. oxysporum* (Table 4). Although media containing trifluralin generally supported greater germination of microconidia, there appeared to be no consistent increase in the percentage of germination with increasing trifluralin concentration (Table 4). Similarly, the effects of trifluralin on chlamydospore production in sterile water and soil also appeared to be somewhat inconsistent (Table 5). In sterile water, only the two highest trifluralin concentrations (1 and 2 μg/ml) significantly reduced chlamydospore production compared with the control (Table 5). In sterile soil, chlamydospore production after 6 wk was reduced in all soils containing trifluralin, but only the 0.25 and 5.00 μg/g treatments were significantly different from the control treatment (Table 5).

Table 5. Effects of trifluralin concentration on chlamydospore production of *Fusarium oxysporum* in sterile water or sterile soil after 6 wk incubation at 25 C

Trifluralin μg/ml (water) or μg (soil)	Chlamydospores/ml ($\times$ 10^4)	
	Water	Soil
0.00	5.60 a[y]	5.22 a
0.10	5.40 ab	4.83 a
0.25	5.10 ab	4.05 b
0.50	5.85 a	4.83 a
1.00	4.65 bc	5.02 a
2.00	3.95 c	4.83 a
5.00	...[z]	3.93 b

[y] Means followed by the same letter within a column are not significantly different at $P = 0.05$ level of probability according to Duncan's multiple range test.

[z] The effect of this concentration of trifluralin in sterile water was not tested.

DISCUSSION

Based on both field and greenhouse experiments, the severity of Fusarium root rot of soybeans was increased by the presence of trifluralin. The inoculation technique and the use of sterile soil in the greenhouse experiments represent an artificial situation; however, results of the field experiment support our findings from greenhouse trials. In field plots receiving no inoculum, DSI values were consistently higher in plots treated with trifluralin. *F. oxysporum* was consistently isolated from affected plants in these plots, indicating that infection probably arose from native populations of *F. oxysporum*.

Our results also support the hypothesis that the primary effect of trifluralin is a predisposition of soybeans to infection by *F. oxysporum* and not a direct stimulation of the pathogen. Prior exposure to trifluralin from 2 to 8 days before being transplanted into pathogen-infested soil resulted in DSIs related directly to exposure time and concentration of trifluralin, despite the fact that there was no direct contact between the pathogen and herbicide. In all of our greenhouse studies, seedling injury from exposure to trifluralin was evident in the absence of the pathogen. Symptoms of injury included extensive swelling and visible cracking of the hypocotyl as well as poor secondary root formation. The hypocotyl cracks particularly appeared to be favorable sites for ingress by the pathogen because lesions typically occurred at such cracks. Our hypothesis is further supported by the results of in vitro tests on the effects of varied levels of trifluralin on *F. oxysporum*, which suggest that the herbicide has little direct effect upon the pathogen.

Soil temperature appears to be an important environmental factor affecting the predisposition of soybean to Fusarium root rot by exposure to trifluralin. At lower soil temperatures (10–15 C), which are unfavorable for rapid germination and establishment of soybeans, the adverse effect of trifluralin on the severity of Fusarium root rot was most pronounced. In the absence of trifluralin, the lowest DSI was observed at temperatures considered optimal for germination and emergence of soybeans (20–25 C). At higher temperatures (30–35 C), the DSI of seedlings not exposed to trifluralin increased but the predisposing effect of trifluralin decreased, which indicates that less herbicide injury occurred at these temperatures. One objective of the three planting dates of the field experiment was to determine the effects of various soil temperatures at planting on the trifluralin-Fusarium root rot interaction. Although soil temperatures did increase with successive planting dates, none of the average afternoon soil temperatures observed would be stressful to soybean. Mean afternoon soil temper-

atures at the 5-cm depth were 15.1, 18.6, and 22.7 C for the 10 days after the 7 May, 21 May, and 5 June plantings, respectively. These results support our observations and those of others that Fusarium root rot is increased by cool soil temperatures at planting (3–5,10,17).

The application of trifluralin herbicide is an important part of many growers' soybean cultural practices in most of the major soybean production areas of the United States. Despite our demonstration of the role trifluralin stress may play in the development of Fusarium root rot, trifluralin continues to be used without apparent problems. Growers using trifluralin herbicide should closely follow label recommendations for application rates and depth of soil incorporation to minimize stress to soybean seedlings. Growers should also avoid planting soybeans in trifluralin-treated soils if soil temperatures are suboptimal for rapid germination and emergence.

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