Trifluralin Enhancement of Phytophthora Root Rot of Soybean

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ABSTRACT

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Trifluralin used on soils infested with *Phytophthora megasperma* var. *sojae* increased Phytophthora root rot of soybean (*Glycine max*) plants. The increase ranged from slight to more than 80% death, depending on the inoculum density and the soil condition.

Additional key words: herbicides, soilborne disease, synergism

When incorporated into soil, trifluralin herbicides interact with microorganisms as well as plants. In various studies, trifluralin enhanced chlamydospore production and spore germination of *Fusarium oxysporum* f. sp. *vasinfectum* in soil (11), inhibited production of mycelium by *Sclerotium rolfsii* (9), reduced the number of motile zoospores produced by *Aphanomyces euteiches* in culture (1,5,12), and served as a nitrogen source for several bacteria and fungi (8).

Besides affecting the growth habits of soil organisms, trifluralin can influence disease development. Trifluralin compounds reduced Aphanomyces root rot of pea (1,2,4,12) but predisposed cotton to infection by *Rhizoctonia* sp. and *Fusarium* sp. (6,7).

In view of these studies and the widespread use of trifluralin in soybean fields, we attempted to determine the effect of this compound on Phytophthora root rot, a severe disease of soybean (*Glycine max* (L.) Merr.) caused by *Phytophthora megasperma* (Drechs.) var. *sojae* A. A. Hildebrand (3).

MATERIALS AND METHODS

Isolates of *P. megasperma* var. *sojae* race 1 (Pms_1) from Indiana were grown for 35 days in a liquid culture of 200 ml of V-8 juice, 3 g of calcium carbonate, and 800 ml of distilled water. The mycelium produced was blended for 15 sec at high speed in a blender, and half of the mycelium was autoclaved.

Autoclaved mycelium (2.1 g fresh weight) was thoroughly incorporated by hand into 1,750 g of a 1:1 mixture of sand and field soil that had been autoclaved as part of a larger mixture used throughout the experiment. Live mycelium was incorporated in the same manner and at the same rate into another portion of the autoclaved sand-soil mix.

Duplicate live mycelium and autoclaved mycelium sand-soil mixtures were prepared, but trifluralin, as the 44.5% emulsifiable concentrate Treflan (Elanco Products Co.), was also thoroughly incorporated into these samples. Trifluralin was applied as an aqueous solution at 0.5 ppm active ingredient, a rate comparable to field applications recommended on the product label.

The soil mix samples were divided into five 350-g aliquots, each placed into a 12-oz waxed paper cup with a drainage hole 4 cm in diameter in its side near the base. Five Harosoy (Pms_1-susceptible) and five Harosoy 63 (Pms_1-resistant) soybean seeds were planted in each cup.

Thus, the four soil mix treatments were 1) autoclaved Pms_1 mycelium, 2) autoclaved Pms_1 mycelium plus trifluralin, 3) viable Pms_1 mycelium, and 4) viable Pms_1 mycelium plus trifluralin. Each treatment was replicated five times in a factorial design, for a total of 25 plants of each cultivar per treatment.

After seeds germinated and cotyledons opened (about 6 days), each cup was set in a second waxed cup without a drainage hole and flooded for 2 days with distilled water to simulate flooded field conditions. The lower cup was then removed, the soil was drained, and the plants were incubated for 16 days with periodic watering. All experiments were conducted in a growth chamber with 14-hr days (25 C and light intensity of 26,000 lux at bench height) and 10-hr nights (20 C).

At the end of the experiment, the cups were peeled away, and the roots were soaked and washed in water to remove soil. Severity of disease was rated by the number of surviving plants and dry weight of the whole plant.

Another experiment with similar treatments used unautoclaved sand-soil mix to simulate field conditions and the interaction of soilborne microorganisms. Because we have observed that Pms_1 does not attack plants as severely in unautoclaved soils as in autoclaved soils, we used 3.5 g of Pms_1 mycelium per 1,750 g of sand-soil mix to increase disease development.

RESULTS AND DISCUSSION

In the experiments using autoclaved sand-soil mix, an average 4–16% of the Harosoy 63 plants died (Table 1). Harosoy 63 (a line near-isogenic to Harosoy) is resistant to Pms_1, so these losses were caused by factors other than the fungus. Because similar losses

Table 1. Survival and total dry weight of soybean plants grown in autoclaved soil treated with trifluralin and/or *Phytophthora megasperma* var. *sojae* race 1 (Pms_1)[a]

Treatment	Survival (%)		Mean treatment dry weight (g)	
	Harosoy	Harosoy 63[b]	Harosoy	Harosoy 63[b]
Dead Pms_1	84	96	0.23	0.26
Trifluralin plus dead Pms_1	92	88	0.22	0.23
Live Pms_1	60[c,d]	84	0.19[e]	0.22
Trifluralin plus live Pms_1	12[c,d]	92	0.12[e]	0.25

[a] 50 plants per treatment (25 Harosoy, 25 Harosoy 63).
[b] Harosoy 63 is a Pms_1-resistant, near-isogenic line to Harosoy.
[c] Values are significant according to an analysis of variance of a factorial-designed experiment ($P<0.01$).
[d] Values are significantly different from each other according to a Tukey student test ($P<0.001$).
[e] Values are significant according to an analysis of variance on a factorial-designed experiment ($P<0.05$).

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Table 2. Survival and average dry weight of Harosoy soybean plants grown in unautoclaved soil treated with trifluralin and/or *Phytophthora megasperma* var. *sojae* race 1[a]

Treatment	Survival (%)	Average dry weight of plant (g)[b]
Dead Pms_1	100	0.25
Trifluralin plus dead Pms_1	93	0.26
Live Pms_1	93	0.22
Trifluralin plus live Pms_1	73[c]	0.21

[a] 25 plants per treatment.
[b] Values are not significantly different according to an analysis of variance of a factorial-designed experiment.
[c] An analysis of variance on arcsin-transformed data indicated significance at the $P < 0.10$ level.

occurred in all treatments, factors other than Pms_1 and trifluralin did not obscure the interaction among the plant, the pathogen, and the herbicide.

The controls, all of which contained autoclaved Pms_1 mycelium and half of which contained trifluralin, also had a 4–16% mortality of Harosoy plants (Table 1). That is, trifluralin alone had no detectable effect on the soybean plants, and something besides Pms_1 or trifluralin was killing some plants of both cultivars. These data gave us a reference death rate with which to compare living and dead mycelium.

Treatments with the live fungus and without trifluralin reduced the Harosoy plant population 40% (Table 1). This loss was 2.5–5 times as great as the losses in the Harosoy controls and in the Harosoy 63 plants and verifies the pathogenic effect of Pms_1 on Harosoy in this experimental system.

In treatments with both trifluralin and viable Pms_1 (Table 1), 88% of the Harosoy plants died, a loss 5.5–11 times as great as in the controls and twice that in treatments with the fungus only. Hence, the results of the experiment in autoclaved sand-soil mix (Table 1) suggest a synergistic effect between trifluralin and Pms_1 in killing Harosoy soybean plants.

Statistical analysis of dry weight measurements of the surviving plants (Table 1) did not reflect this synergism, even though the trend is the same as with plant survival. An analysis of variance of the dry weights showed significant differences at $P = 0.05$ between any treatment without Pms_1 and any treatment with the fungus, which indicated that the pathogen was active. Dry weights were not significantly different between treatments containing Pms_1. This lack of statistical significance between treatments probably resulted from increased growth of the surviving soybean plants due to the reduction of competition in each cup (3). These results do not discount the synergism between trifluralin and Pms_1, but they suggest that the final yield loss in a soybean field might not be as great as the number of plants killed would indicate, if the plants were initially crowded and if some plants survived.

When unautoclaved soil was used in the experiment and the inoculum density was increased to partially overcome any antagonistic factors in the soil (Table 2), the treatment with live Pms_1 alone caused a plant loss similar to that of the controls. The combination of trifluralin and Pms_1 resulted in a loss 4.5 times greater than either the controls or the fungus alone. The same trend was observed in the average dry weight (Table 2), although here again, competition probably limited the statistical significance of the values.

These data suggest that Pms_1 and trifluralin are synergistic in field soil as well as autoclaved soil. The low death rate of plants treated with Pms_1 shows that more than twice the inoculum used in autoclaved soil is required to obtain adequate plant death in field soil. Doubling the inoculum did not influence the synergism between trifluralin and Pms_1 (Table 2).

These experiments demonstrate that trifluralin does not affect the soybean plant directly; consequently, we suggest that a direct trifluralin–Pms_1 interaction increases disease loss. The nature of the interaction between Pms_1 and trifluralin is unclear. Several bacteria and fungi, perhaps including Pms, can use trifluralin as a nitrogen source.

Alternatively, we have noted a reduction in sporangium formation but an increase in oospore production by Pms_1 in media containing trifluralin (*unpublished*). Blending the mycelium produces hyphal fragments that might produce oospores in the presence of trifluralin. Because oospores are more durable in soil than hyphal fragments (10), the synergism might be the result of an increased inoculum potential.

Whatever the mechanism, the interaction between trifluralin and Pms_1 is especially significant in Illinois, where most of the soybeans planted are the Pms_1-tolerant cultivar Williams, more than 60% of the soybean acreage is treated with trifluralin (Fred Slife, *personal communication*), and Pms_1 is widely distributed (*unpublished*). Under these circumstances, large economic losses may occur with certain environmental conditions, particularly in the spring when cool, wet conditions needed by the fungus for infection are most prevalent. In areas with a history of Phytophthora root rot of soybeans, it is advisable to use an appropriate resistant cultivar and to monitor weather conditions so as to apply herbicides other than trifluralin when conditions are favorable for the disease.

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