Effects of Dinitroaniline Herbicides, Carboxin-Pentachloronitrobenzene Seed Treatment, and Rhizoctonia Disease on Soybean

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ABSTRACT

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The effect of trifluralin, pendimethalin, and ethalfluralin and disease caused by *Rhizoctonia solani* on soybean (*Glycine max*) was determined with field and growth chamber studies. In field studies, oats infested with *R. solani* placed in furrow at planting significantly reduced plant growth variables. No significant interactions were observed between herbicides and inoculum treatments. Effects of inoculum were significantly reduced with carboxin-pentachloronitrobenzene seed treatment. In growth chamber studies, steamed and nonsteamed field soil were used. No significant interactions were found between herbicides and plant growth variables in steamed soil. Significant interactions between herbicides and inoculum treatments affecting root and hypocotyl weight occurred in nonsteamed soil. Root and hypocotyl weights were significantly lower in infested soil than in noninfested soil in the absence of herbicides. No similar significant differences were observed in treatments where herbicides were added to the soil.

Rhizoctonia solani Kühn is a soilborne pathogen with excellent saprophytic ability that causes root rot, stem decay, and damping-off disease on soybean (*Glycine max* (L.) Merr.). Typical symptoms include reddish brown lesions on the stem at the soil line, necrotic roots, wilting, and stunting. Rhizoctonia disease often is controlled by the use of pentachloronitrobenzene (PCNB) seed treatment on vegetable crops (17), but this control practice is not widely used on soybean seed.

In the Midwest, growers routinely apply dinitroaniline herbicides, such as trifluralin, ethalfluralin, and pendimethalin, to soybean production fields. These are preplant, soil-incorporated herbicides that are known to inhibit lateral root development (13). In growth chamber studies, trifluralin may delay emergence of soybeans, increasing the susceptibility of seedlings to damping-off diseases (8). From discussions with growers and our own observations, we suspected a detrimental interaction between Rhizoctonia disease and the dinitroaniline herbicides used on soybeans.

There is a considerable body of literature, including three reviews (1,2,11), on nontarget effects of herbicides and their possible effects on plant pathogens. Herbicides and/or herbicide/nematicide interactions have been shown to affect soybean cyst nematode and Phytophthora root rot on soybeans (3,7,16). Studies involving dinitroaniline herbicides, particularly trifluralin, and Rhizoctonia disease on several crops have been reported. Trifluralin increased disease caused by *R. solani* in cotton (6,10,12,14,19) but not in all cases (10,19). Ruppel et al (15) reported that Rhizoctonia root rot of sugar beet was unaffected by trifluralin. Trifluralin decreased disease caused by *R. solani* by 30–90% in eggplant, tomato, and pepper but had no effect on the disease in snap beans (10). Other workers reported an increase in the size and number of hypocotyl lesions caused by *R. solani* when trifluralin was used on snap beans (21).

Bowman and Sinclair (4) examined the effects of 13 herbicides, alone or in combination, on Rhizoctonia seedling disease of soybean in glasshouse experiments. They found pendimethalin did not affect any aspect of seedling development, whereas treatment with trifluralin gave different results in two experiments. Seedling emergence and dry shoot weight increased in trifluralin-treated soil infested with a single isolate of *R. solani*. In steamed soil infested with a mixture of five isolates of *R. solani*, stand counts were not affected by trifluralin. However, reduced emergence at 6 days resulted in lower shoot weight, shorter plant heights, and fewer seedlings with fully expanded, unifoliate leaves at 12 days. Because both the isolate (one or a mixture of five) and soil treatment (steamed or nonsteamed) varied between these experiments, the results cannot be attributed to either factor. Wiley and Ross (20) reported a 1-yr field study which indicated that in the presence of *R. solani*, trifluralin at 2.2 kg/ha caused a significant reduction in yield when compared with the treatment receiving no herbicide.

The first objective of our study was to determine the effects of three dinitroaniline herbicides and disease caused by *R. solani* on soybean. The study was done in the field as well as in a growth chamber, using both steamed soil and soil that was not steam-treated. The second objective was to determine the effect of carboxin-PCNB seed treatment on disease caused by *R. solani* in the field.

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MATERIALS AND METHODS

Field study. The effects of three dinitroaniline herbicides, carboxin-PCNB, and disease caused by *R. solani* on soybeans under field conditions were determined in 1987 and 1988 on a Drummer silt loam soil at the University of Illinois, Cruse Farm, in Champaign. The experiment also was done in 1988 at the Northern Illinois Research Farm in DeKalb on a Flanagan silt loam.

The experiment had a factorial arrangement of treatments in a split plot with a randomized complete block design and four replications. The whole plots consisted of one of three herbicide applications and an untreated control. Within each whole plot, there were six subplots, each of which received one of two seed treatments and one of three inoculum treatments. Subplots were 6.1 m long and four rows wide with rows 76 cm apart. Seeding rate was 30 seeds per meter at a depth of 2.5–5.0 cm.

Herbicides were applied at the following recommended rates: trifluralin at 1.12 kg a.i./ha, pendimethalin at 1.40 kg a.i./ha, and ethalfluralin at 1.12 kg a.i./ha. All materials were broadcast 40 cm above the soil with a sprayer equipped with flat-fan nozzles with a pressure of 1.2 kPa. Herbicides were incorporated to a depth of 10 cm with two passes of a disk harrow. In the control plots, weeds were removed by hand.

Before planting, half the Williams 82 seed was treated with carboxin-PCNB F (17% carboxin and 17% PCNB) at the recommended rate of 2.6 ml/kg of seed in a water slurry.

The isolate of *R. solani* (AG-2-2) used in all studies was obtained originally from diseased soybean material in Illinois. The isolate was grown on water agar for 3–4 days before use. Field inoculum was prepared by soaking oats in deionized water for 24 hr and draining the excess water. Oats were autoclaved for 60 min, allowed to cool for 24 hr, and autoclaved again for 60 min. Six 1-cm-diameter plugs from the actively growing edge of the fungal colony were placed with the sterilized oats, incubated for 15 days at room temperature, and the infested oats were air-dried for 7 days. Sterilized, air-dried oats and a no-oat treatment served as controls. Each plot received one of the three following inoculum treatments: oats infested with *R. solani*, oats without *R. solani*, or no amendment. Both infested and noninfested oats were placed in furrow with soybeans at planting at a rate of 60 cm^3 of oats per 6.1-m row.

Plant stands were determined at the V1–V2 growth stage (9) to determine the incidence of damping-off. At the V2–V3 stage, five plants were selected randomly from the two outer rows of each plot and disease severity was assessed using the scale developed by Cardoso and Echandi (5). Hypocotyl lesions were rated on a scale of 0–5 where 0 = no lesion, 1 = lesions <2.5 mm in diameter, 2 = lesions 2.5–5.0 mm in diameter, 3 = lesions >5.0 mm in diameter, 4 = lesions girdling plant and wilting visible on leaves, and 5 = seedlings damped-off or dead. The disease severity index (DSI) was calculated as a percentage of the maximum possible disease severity. The number of plants in each class was multiplied by that class number and divided by the total number of classes. The quotient was then multiplied by 100 to give a scale of 0 (no root rot) to 100 (completely rotted roots).

The heights of five random plants in 1987 and 10 random plants in 1988 were measured when the plants were in the R2–R3 stage. Pods were counted on 10 random plants in each plot, and late-season plant stands were counted at R8. The middle two rows of each plot were harvested mechanically for yield.

The data from all experiments were analyzed with the Statistical Analysis System (SAS Institute Inc., Cary, NC) general linear models procedure. Main effects and interactions were tested with analysis of variance and single degree-of-freedom comparisons. Six contrasts of main effects were made as follows: each of the three herbicides vs. no herbicide, fungicide seed treatment vs. no seed treatment, no amendment vs. oat amendment, and no amendment vs. oats infested with *R. solani*. Four contrasts were made involving interaction of main effects as follows: the interaction of each herbicide vs. no herbicide with *R. solani*-infested oats vs. no amendment, and the interaction of seed treatment vs. no seed treatment with *R. solani*-infested oats vs. no amendment. Preliminary analysis indicated that error terms and results were similar among the three field locations, so data were combined into a single analysis. Treatment and location were considered fixed effects.

Growth chamber study. The growth chamber was adjusted for 34-C day and 26-C night temperatures with a day length of 16 hr and light intensity of 120 $\mu E \cdot m^{-2} \cdot s^{-1}$. A factorial arrangement of treatments in a split plot with a completely randomized block design and four replications in time was used. Whole plots received a herbicide treatment, and subplots received an inoculum treatment.

A Drummer silt loam field soil was mixed with 10% perlite (9:1 by weight), steamed for 8 hr, and allowed to cool before use. In preliminary studies, the rates of trifluralin, pendimethalin, and ethalfluralin used in field studies were phytotoxic to soybeans in the growth chamber. Therefore, herbicides were applied at one-half of the recommended field rate. Application was followed by incorporation into the soil in a rotating drum mixer. Untreated soil served as a control.

Potato-dextrose broth (50 ml) was infested with the same *R. solani* isolate used in the field experiment and incubated at room temperature. After 8 days, the mycelial mats were removed, rinsed with deionized water, blotted with paper towels, and weighed. The mycelial mats were placed in 200 ml of deionized water, blended for 10 s, and incorporated by hand into the soil at a rate of 1 mg of wet mycelium per gram of soil. One-half of the soil receiving each herbicide treatment was infested in this manner.

Table 1. Main effects of three dinitroaniline herbicides, *Rhizoctonia solani* inoculum, and carboxin-pentachloronitrobenzene (PCNB) seed treatment on the soybean cultivar Williams 82 in the field

	Means[a]					
Treatment	**Early stand[b] (plants per m)**	**DSI[c]**	**Plant height[d] (cm)**	**Pods[e] (pods per plant)**	**Late stand[e] (plants per m)**	**Yield (kg/ha)**
Herbicide treatments						
No herbicide	14.8	29.2	52.2	79.9	13.8	2,647.2
Trifluralin	14.4	28.8	52.1	88.9	12.5*[f]	2,545.0
Pendimethalin	15.1	30.2	53.2	78.7	13.4	2,766.3
Ethalfluralin	14.9	28.7	51.4	85.2	12.7*[f]	2,556.2
Seed treatment						
No fungicide	13.9	35.3	51.8	97.9	12.3	2,397.1
Carboxin-PCNB	15.5**[g]	23.7**	52.7**	69.8**	13.9**	2,845.6**
Inoculum treatments						
No amendment	20.9	5.4	56.8	45.8	18.6	3,174.2
Oat amendment	19.6**[h]	5.5	56.4	48.4	17.7	3,234.4
R. solani-infested oats	3.4**[h]	77.5**	43.0**	166.8**	3.0**	1,400.2**

[a]Means are based on 96 plots in each of three experiments and are averaged over the other experimental factors.
[b]At V1–V2 stage.
[c]Disease severity index at V2–V3 stage.
[d]At R2–R3 stage.
[e]At R8 stage.
[f]Significantly different from no herbicide, * = $P < 0.05$.
[g]Significantly different from no herbicide, ** = $P < 0.01$.
[h]Significantly different from no amendment, ** = $P < 0.01$.

Table 2. Main effects of three dinitroaniline herbicides and *Rhizoctonia solani* on soybean cultivar Williams 82 seedlings in field soil under controlled conditions

	Means[a]		
Treatment	**Germination per cup[b]**	**Plant height[c] (cm)**	**Root and hypocotyl weight[d] (mg)**
Herbicide treatments			
No herbicide	2.5	15.9	146
Trifluralin	2.2	15.3	154
Pendimethalin	2.5	14.9	151
Ethalfluralin	2.2	16.5	178**[e]
Inoculum treatments			
Noninfested	2.3	15.7	161
R. solani	2.4	15.6	153

[a]Means are based on five seeds per cup with 128 cups in the experiment and are averaged over the other factor in the experiment.
[b]Number of seeds that germinated per cup.
[c]Height measured from the first lateral root to meristem.
[d]Oven dry weight of root and hypocotyl.
[e]Significantly different from no herbicide, ** = $P < 0.01$.

Soil from each of the eight treatments was placed into four 355-ml waxed paper cups with holes in the bottom for drainage. There were four cups per treatment and four replications for a total of 128 cups in the experiment. Five soybean seeds, cv. Williams 82, were planted 2.5 cm deep in each cup. After 18 days, the germination per cup and stem length from the first lateral roots to the meristem was recorded. To determine the dry weight of the hypocotyl and root, seedlings were cut off at the cotyledons and dried at 80 C for 48 hr and weighed.

This experiment was repeated using the same methods on a Drummer silt loam field soil that had not been steam-treated. The data from both experiments were analyzed with the SAS general linear models procedure. Main effects and interaction were tested with analysis of variance and single degree-of-freedom contrasts. Treatments were considered fixed effects. Four contrasts were made of main effects as follows: each of the three herbicides vs. no herbicide and noninfested vs. infested with *R. solani.* The interaction of these main effects also was tested.

RESULTS AND DISCUSSION

Field study. Infesting the soil with *R. solani* significantly affected all variables measured at all locations when contrasted with the no amendment treatment (Table 1). Plant stands, plant height, and yield were reduced. The DSI increased. The number of pods per plant increased as a result of reduced plant stands and compensation.

Both trifluralin and ethalfluralin slightly reduced late plant stands (Table 1). No significant interactions occurred between herbicide treatments and inoculum treatments (*data not shown*). All variables had similar response to the inoculum treatment in both the presence and the absence of the herbicides.

Wiley and Ross (20) suggested that trifluralin in the presence of high levels of *Rhizoctonia* inoculum could cause a significant increase in Rhizoctonia root rot of soybeans with a consequent reduction in yield. This study does not support this conclusion. The herbicides had no significant effect on yield, either in the presence or absence of inoculum. The 2 yr in which this study was done did not have similar weather patterns: 1987 was a typical year and 1988 was characterized by severe drought. Under the field conditions in this study, the herbicide-disease interaction may not be economically important.

The carboxin-PCNB seed treatment significantly increased early and late plant stands, height, and yield and reduced the DSI and the number of pods per plant when compared with no fungicide treatment (Table 1). Interactions between fungicide seed treatments and the *R. solani* inoculum treatments were significant for early and late plant stands, DSI, height, pods per plant, and yield (*data not shown*). As expected, carboxin-PCNB had little or no effect in noninfested plots, and it was effective in plots infested with *R. solani.* In the field test, disease severity was high. The carboxin-PCNB seed treatment did provide effective protection from Rhizoctonia damping-off disease.

Growth chamber study. In steam-treated soil, the herbicides did not affect germination per cup, plant height, or root and hypocotyl dry weight (*data not shown*). However, germination was significantly reduced ($P < 0.01$) from 4.5 to 2.0 seeds per cup when soil was infested with *R. solani.* Plant height was reduced ($P < 0.01$) from 16.6 to 12.1 cm, and root and hypocotyl dry weight was reduced ($P < 0.01$) from 130 to 75 mg in infested soil. Characteristic lesions were apparent on plants in infested soil. There were no significant interactions between herbicides and inoculum for the variables measured, indicating that the herbicides had no effect on the response of the seedling to *R. solani.*

In the nonsteamed soil, neither herbicides nor inoculum significantly affected germination, plant height, or root and hypocotyl dry weight, even though lesions were present on plants grown in infested soil (Table 2). In nonsteamed soil, significant ($P < 0.01$) interactions between herbicide treatments and inoculum treatments affected root and hypocotyl dry weight. In the no-herbicide treatment with noninfested soil, root and hypocotyl weights averaged 182 mg, and in the no-herbicide treatment with infested soil, they averaged 109 mg (Table 3). However, in the presence of the herbicides, the root and hypocotyl weights did not differ significantly ($P <$ 0.05) between noninoculated and inoculated treatments.

The growth chamber experiments produced inconsistent results in this study, although replications within each experiment were consistent. In the steam-treated soil, no interactions between the four herbicide treatments and Rhizoctonia disease were observed. In nonsteamed field soil, root and hypocotyl dry weight was reduced by the *R. solani* inoculum in the absence of the herbicides. In the presence of the herbicides, root and hypocotyl dry weight increased slightly, although this increase was not statistically significant. Many biotic and physical changes occur when soil is steamed (18), and these changes appear to have affected the herbicide-disease interaction.

In nonsteamed soil, the variables measured were not affected by the inoculum treatment even though lesions were visible on the roots and stems. Steam treatment destroys mycorrhizae and rhizobia associated with soybeans. Zambolim and Schenck (22) studied the interaction between the mycorrhizal fungus, *Glomus mosseae* (Nicol. & Gerd.) Gerdemann & Trappe, and *R. solani.* They reported that soybean plants infected by *G. mosseae* and *R. solani* had growth responses similar to noninoculated controls and were able to withstand infection better than plants without *G. mosseae.* This may explain why germination per cup, plant height, and root and hypocotyl weight were not affected by the inoculum; however, it does not explain the response of root and hypocotyl weight to the herbicide treatments. Root and hypocotyl weight was not measured in the field, and the response of this variable to the herbicide and inoculum treatments under field conditions remains to be determined. Additional research on the soil conditions in which dinitroaniline-Rhizoctonia disease interactions are significant will determine if these interactions are of economic interest.

Table 3. Interaction of three dinitroaniline herbicides and *Rhizoctonia solani* on root and hypocotyl dry weight of soybean cultivar Williams 82 seedlings in field soil

Treatment	Noninfested	*R. solani*	Difference[a]
No herbicide	182[b]	109*[c]	73
Trifluralin	139	167	−28
Pendimethalin	150	152	−2
Ethalfluralin	173	183	−10

[a] Difference between root and hypocotyl weight in noninfested soil and *R. solani*-infested soil.
[b] Mean oven dry weight of root and hypocotyl in milligrams.
[c] Significantly different from the noninfested treatment, * = $P < 0.05$.

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