The Influence of Alachlor, Trifluralin, and Diazinon on the Development of Endogenous Mycorrhizae in Soybeans

L. L. Burpee and H. Cole, Jr.
Pennsylvania State University
University Park, Penn. 16802

Several species of soil-borne fungi invade plant roots forming vascular-arbuscular (VA) mycorrhizae which aid in growth and nutrient element absorption (GERDEMANN, 1968, MOSSE, 1973a). Certain fungicides in soil such as thiram, captan, PCNB, and lanstan, have been shown to inhibit VA mycorrhizae (NESHEIM and LINN, 1969). In addition, soil fumigants including methyl bromide, Vapam, and mylone have had detrimental effects on mycorrhizal development (KABIN and KAHN, 1972, NESHEIM and LINN, 1969).

Increases in soybean yields and phosphorus accumulation have been attributed to VA mycorrhizae (ROSS and HARPER, 1970). Reports dealing with the effect of commonly used soybean pesticides are limited to information regarding the influence of these pesticides on growth of soybeans (OLIVER and FRANS, 1968) and soil fungi (BREAZEALE and CAMPER, 1970, RODRIGUEZ-KABANA et al., 1969, TU, 1970, KABIN and KHAN, 1972) exclusive of the fungi that form VA mycorrhizae. In view of this lack of knowledge the following study was undertaken to investigate the influence of the herbicides alachlor (2-chlor-2', 6'-diethyl-N-(methoxymethyl) acetanilide) and trifluralin (a,a,a,-tri-fluro-2,6-dinitro-N_1N_1 dipropyl-p-toluidine) and the insecticide diazinon (0-0-Diethyl-0 (2 isopropyl-6-methyl-5-pyrimidinyl) phosphorothioate) on soybean mycorrhizae.

MATERIALS AND METHODS

The research was conducted at The Pennsylvania State University Research Farm at the Rock Springs Plant Science Research Center. The experiment was a factorial design with factor A; pesticides and rates of application; B, soil phosphorus level; C, replication. A 16 x 18 m seed bed divided into 72 - 2 x 2 m plots was prepared in an Andover clay loam soil having a pH of 6.8, a C.E.C. of 11.7 and a mean available phosphorus content of 18 ppm (soluble in 0.025 N HCl). Treatments were replicated four times

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and arranged in a randomized complete block design. Phosphorus was added to the appropriate plots as a broadcast application of superphosphate at the rate of 90 kg/ha. Pesticide rates for alachlor, trifluralin, and diazinon were 2 or 4 kg active ingredient per hectare equivalent. Pesticides were applied as a soil surface spray. All soil treatments including phosphate were incorporated to a 4-6 inch depth by discing immediately after application. The following day soybeans (Glycine max (L.) Merr. 'Chippewa 64') were planted at a rate of 112 kg/ha, with a row spacing of 35.5 cm. Weeds were controlled by hand cultivation at regular intervals throughout the test period.

Three plants were randomly selected from center of each plot 25 and 60 days after planting. The roots were washed, cut into 2 cm sections, and fixed in FAA (13 ml formalin, 5 ml glacial acetic acid, 200 ml 50% ethanol). The shoots were washed in 0.1 N HCl, rinsed in distilled water, and dried for 24 hr at 105 C. Shoot weights were recorded. Leaves were ground and samples were analyzed for phosphorus by the method of BAKER et al., 1964.

Root segments were cleared and stained for microscopic examination according to the methods of PHILLIPS and HAYMAN, 1970. Mycorrhizal development was assessed using procedures similar to those of NICOLSON, 1960. Ten microscope fields (50X) were examined per root segment. The presence of vesicles or arbuscules was recorded as a percentage of the fields observed.

At the time of the two harvests, soil samples were randomly taken and analyzed for phosphorus using the Bray No. 1 method (BRAY and KURTZ, 1945). Spores of mycorrhizal fungi were separated from the soil samples using the floatation-adhesion technique of SUTTON and BARRON, 1972. The spores were observed microscopically to ascertain which species of mycorrhizal fungi were most prevalent on the experimental site.

Data were treated statistically employing the analysis of variance and means were separated by use of Duncan's modified (Bayesian) least significant difference test (DUNCAN, 1965).

RESULTS

None of the test pesticides when applied at the 2 kg per hectare rate significantly affected mycorrhizal development (Tables 1 & 2). A significant reduction

in root colonization by mycorrhizal fungi was observed for 25 and 60 day old plants grown in soil treated with 4 kg/ha alachlor. This rate is more than twice the normal commercial rate for soybeans. Trifluralin and diazinon had no significant effect on mycorrhizal development at any rate.

Alachlor and trifluralin, applied at 4 kg/ha, caused a stunting of roots and resulted in a significant reduction in shoot weights of 25 day old plants (Table 1). Shoot weights of 60 day old plants were not significantly reduced (Table 2) and roots had recovered from the initial stunting. Diazinon had no significant effect on shoot weight at either sampling period.

The percent P content of leaves was consistently greater in plants grown in soil having more available P. Pesticide application did not appear to affect P accumulation. No pesticide treatment resulted in a significant interaction for phosphorus accumulation. (Tables 1 & 2).

The majority of spores observed were larger than 50u in diameter and possessed funnel-shaped bases indicative of the fungal species Glomus mosseae Gerd. & Trap. (GERDEMANN and TRAPPE, 1974).

DISCUSSION

The herbicides alachlor and trifluralin when applied at registered label rates (2 kg/ha), and incorporated into the soil prior to planting, did not significantly affect mycorrhizal development, shoot weight, or phosphorus accumulation in soybeans. These data, we believe, indicate that alachlor and trifluralin use does not affect soybean growth or phosphorus nutrition under commercial usage if correct application rates are used. However, with higher rates the picture becomes less clear. Inhibition of root growth, observed in this study, at 4 kg/ha for both herbicides, has also been noted to occur in a number of other plant species (ESHEL and KATAN, 1972, DEMORANVILLE and DEVLIN, 1971). With alachlor this root inhibition also resulted in a reduction in root colonization by the mycorrhizal fungi. We feel that the reduced colonization may be a direct effect of treatment with alachlor. The continued suppression of mycorrhizal development in plants grown in 4 kg/ha alachlor treated soil after roots had recovered from the initial period of growth inhibition support this notion. We have observed that other pesticides, when applied to the

TABLE 1

The effect of alachlor, trifluralin, and diazinon on mycorrhizal development, shoot weight, and leaf P content of 25 day old soybean plants grown under two levels of available phosphorus.[a]

Treatment	Rate (kg/ha)	Soil Mean P[b] (ppm)	Colonization (%)	Vesicle Formation (%)	Shoot Wt.[c] (g)	Leaf P Content[c] (%)
	0	18	34.94 A	9.14 A	0.286 A	0.31
	0	34	27.54 AB	9.67 A	0.249 BC	0.27
	2	18	33.68 A	9.61 A	0.263 AB	0.30
Alachlor	2	34	31.42 A	8.74 A	0.291 A	0.33
	4	18	15.16 BC	9.90 A	0.188 C	0.26
	4	34	13.61 C	9.88 A	0.225 BC	0.30
	0	18	29.31 A	6.51 A	0.240 A	0.22
	0	34	25.35 A	7.32 A	0.238 AB	0.33
	2	18	26.41 A	9.43 A	0.221 AB	0.27
Trifluralin	2	34	26.22 A	8.61 A	0.234 AB	0.30
	4	18	26.12 A	9.49 A	0.206 B	0.26
	4	34	25.97 A	8.53 A	0.207 AB	0.26
	0	18	35.57 A	9.53 A	0.221 B	0.25
	0	34	31.45 A	8.68 A	0.368 A	0.32
	2	18	32.33 A	8.89 A	0.271 AB	0.27
Diazinon	2	34	30.67 A	8.11 A	0.346 AB	0.30
	4	18	39.38 A	10.65 A	0.254 AB	0.24
	4	34	32.38 A	9.82 A	0.325 AB	0.36

[a]For each treatment, the numbers within a column, followed by the same letter, do not differ significantly from each other at the .05 level.
[b]Extracted with 0.03 N NH_4F in 0.025 N HCl.
[c]Dry Weight

TABLE 2

The effect of alachlor, trifluralin, and diazinon on mycorrhizal development, shoot weight, and leaf P content of 60 day old soybean plants grown under two levels of available phosphorus.[a]

Treatment	Rate (kg/ha)	Soil Mean P[b] (ppm)	Colonization (%)	Vesicle Formation (%)	Shoot Wt.[c] (g)	Leaf P Content[c] (%)
	0	18	46.63 A	28.81 A	3.95 A	0.33
	0	34	44.88 AB	27.21 A	3.23 A	0.42
	2	18	44.11 AB	21.63 A	3.91 A	0.29
Alachlor	2	34	45.68 AB	27.38 A	3.73 A	0.39
	4	18	38.85 BC	21.88 A	3.26 A	0.35
	4	34	30.53 C	33.54 A	3.51 A	0.45
	0	18	54.69 A	32.47 A	3.73 A	0.31
	0	34	49.70 A	23.71 A	3.60 A	0.37
	2	18	50.22 A	31.71 A	3.79 A	0.29
Trifluralin	2	34	49.61 A	28.13 A	3.86 A	0.41
	4	18	52.98 A	28.94 A	2.88 A	0.30
	4	34	52.78 A	26.04 A	3.93 A	0.34
	0	18	50.57 A	22.36 A	3.81 A	0.34
	0	34	51.46 A	20.90 A	3.67 A	0.44
	2	18	50.19 A	21.33 A	3.73 A	0.28
Diazinon	2	34	50.88 A	20.81 A	3.88 A	0.33
	4	18	53.62 A	24.01 A	3.86 A	0.29
	4	34	54.72 A	22.16 A	3.44 A	0.36

[a]For each treatment, the numbers within a column, followed by the same letter, do not differ significantly from each other at the .05 level.
[b]Extracted with 0.03 N NH_4F in 0.025 N HCl.
[c]Dry Weight

soil at higher than recommended rates, considerably reduce root development yet do not have a significant effect on colonization by mycorrhizal fungi (BURPEE and COLE, unpublished data).

Vesicular-arbuscular mycorrhizae increase the phosphorus absorbing capacity of a number of plant species, especially in soils low in available phosphorus (GERDEMANN, 1968, KLEINSCHMIDT and GERDEMANN, 1972). Root colonization by mycorrhizal fungi has been shown to be reduced in plants grown in soils having optimal or supraoptimal levels of available P. This appears to be caused by a phosphorus induced resistance mechanism within the root (MOSSE, 1973b). Although differences were not statistically significant ($P = 0.05$), in the majority of treatments in this study, there was a consistent trend toward reduced mycorrhizal development in plants grown in soil containing added phosphorus. It was anticipated that the added phosphorus would increase the available supply to such a level that root colonization would be significantly reduced. However, this did not occur, possibly due to a high phosphorus 'fixing' capacity of the soil in the plot area.

SUMMARY

Preplant incorporated treatments of 2 and 4 kg/ha of trifluralin and diazinon had no significant effect on growth, P accumulation or root colonization by mycorrhizal fungi in soybeans planted in an Andover clay loam. At 4 kg/ha, alachlor and trifluralin inhibited root development of 25 day-old plants. The 4 kg/ha alachlor treatment reduced shoot weight of 25 day old plants significantly and suppressed mycorrhizal development of 25 to 60 day old plants. At currently used commercial rates neither alachlor, trifluralin, nor diazinon affected mycorrhizal development under the conditions of the experiment.

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