Effect of Trifluralin Soil Metabolites on Cotton Growth and Yield

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Greenhouse experiments were conducted to determine the effect of 12 different soil metabolites of trifluralin on cotton growth and yield. The metabolites included oxidative dealkylated, reduced nitro group, benzimidazole, azoxy, azo, oxidized, and hydroxylated derivatives of trifluralin. When applied to soil at rates that would be equal to at least a 14-year accumulative residual of the metabolite, individual metabolites had no significant effect on cotton growth or yield. The growth and yield factors examined included seedling emergence, plant height and weight, number of flowers and bolls, and seed cotton and lint yields. The results do not support the view that metabolite accumulation from long-term usage of trifluralin is a significant contributing factor in the cotton yield decline problem.

Despite genetic improvement of cotton varieties, the yield of cotton in the United States seemingly has reached a plateau or has declined slightly over the last 20 years (Starbird and Hazera, 1982). There has been increasing concern that the yield decline is a soil-related problem resulting from the long-term use of herbicides (Brown, 1982). The widespread use of dinitroaniline (DNA) herbicides, particularly trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine), began about 20 years ago. It has been therefore speculated that the parent compound or a recalcitrant metabolite of these DNA herbicides could have accumulated and caused phytotoxicity to the cotton plants. It has been further speculated that accumulation of the parent compound and (or) metabolite is involved, because a linear decline suggests a cumulative effect.

Causes of yield reduction by DNA herbicides and (or) possibly their metabolites have been suggested to be due to their effect on plant growth and nutrient biochemistry. Numerous studies (Standifer and Thomas, 1965; Anderson et al., 1967; Oliver and Frans, 1968) have reported that trifluralin adversely affects root and shoot growth of cotton seedlings. Inhibition of root growth in seedlings results in reduced phosphorus and sulfate uptake (Cathey and Sabbe, 1972; Bucholtz and Lavy, 1979); however, correlations between these seedling effects and subsequent yield have not been reported. Additional biochemical responses have been reviewed by Parka and Soper (1977).

If DNA herbicides are involved directly in cotton yield decline, the cause of the decline must be the result of long-term accumulation of phytotoxic metabolites. Trifluralin did not affect cotton yield in short-term (3–5 year) field studies (Miller et al., 1975; Hayes et al., 1981). Also, trifluralin did not accumulate in soils that had repeated applications over a 4–5-year period (Parka and Tepe, 1969; Savage, 1973; Burnside, 1974).

Because of the growing concern over the possible role of DNA herbicides, used in the long term, in the cotton yield decline, it is important to determine whether trifluralin soil metabolites affect cotton growth and yield.

MATERIALS AND METHODS

Twelve major soil metabolites of trifluralin (listed in Table I) were synthesized by Lilly Research Laboratories and the USDA-ARS Pesticide Degradation Laboratory. They were used as received, without purification; the minimum purity of the metabolites and trifluralin was 95%. Dundee silt loam (Aeric Ochraqualf) surface soil (0–7.5-cm depth) was collected from a field that had been fallowed for 3 years before soil collection. The herbicide history prior to fallowing is unknown. Some soil properties are as follows: pH 6.4; 0.7% OM, CEC 17 mequiv/100 g; 16% clay; 56% silt.

Air-dried soil (17 kg) was added to 18.9-L plastic containers, watered to saturation, and allowed to stand for 1 week. The appropriate amount of metabolite (0.1 and 1.0 mg) or trifluralin (2.5 mg), dissolved in 10 mL of methanol, was sprayed onto an additional 5 kg of soil. The soil was thoroughly mixed and placed on top of the soil in the container. The control soil was prepared in same manner;

Table I. Trifluralin Soil Metabolites

code no.[a]	name	origin[b]	quantity,[c] years of accumulation 0.02 kg/ha	0.2 kg/ha
TR-2	α,α,α-trifluoro-2,6-dinitro-*N*-propyl-*p*-toluidine	LRL	1.4	14
TR-3	α,α,α-trifluoro-2,6-dinitro-*p*-toluidine	LRL	20	200
TR-6	α,α,α-trifluoro-5-nitrotoluene-3,4-diamine	LRL	>40	>400
TR-9	α,α,α-trifluorotoluene-3,4,5-triamine	PDL	>40	>400
TR-13	2-ethyl-7-nitro-1-propyl-5-(trifluoromethyl)-benzimidazole	PDL	2	20
TR-15	2-ethyl-7-nitro-5-(trifluoromethyl)-benzimidazole	PDL	2.7	27
TR-17	7-nitro-1-propyl-5-(trifluoromethyl)-benzimidazole	PDL	4	40
TR-21	4-(dipropylamino)-3,5-dinitrobenzoic acid	LRL	>40	>400
TR-28	2,2′-azoxybis(α,α,α-trifluoro-6-nitro-*N*-propyl-*p*-toluidine	PDL	6.7	67
TR-32	2,2′-azobis(α,α,α-trifluoro-6-nitro-*N*-propyl-*p*-toluidine)	PDL	40	400
TR-36M	2,6-dinitro-*N*,*N*-dipropyl-4-(trifluoromethyl)-*m*-anisidine	LRL	>40	>400
TR-40	α,α,α-trifluoro-2′,6′-dinitro-*N*-propyl-*p*-propionotoluidine	LRL	>40	>400

[a] Code numbers according to Golab et al. (1979). [b] LRL = Lilly Research Laboratories, Greenfield, IN.; PDL = USDA-ARS-Pesticide Degradation Laboratory, Beltsville, MD. [c] Based on the amount found after one application of trifluralin by Golab et al. (1979) assuming no further degradation.

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Table II. Effect of Trifluralin Soil Metabolites at 0.022 kg ha^{-1} (7.5-cm Depth)$^{-1}$ on Cotton Growth and Yield

treatment	seedling emergence, %	plant ht at 20 days, cm	stalk wt, g	root wt, g	no. of flowers	no. of bolls	seed cotton wt, g	lint wt, g	mean maturity date, days	production rate index, g/day
control	94	21.8	60.7		34.0	20.1	72.8	28.3	142	0.199
trifluralin (0.5 mg/kg)	90	21.3	61.2		32.5	20.8	75.8	29.4	140	0.211
TR-2	96	24.5*[a]	60.6		30.0*	20.0	71.8	28.1	139	0.203
TR-3	90	23.6	63.2		34.3	21.1	74.9	29.2	139	0.210
TR-6	92	25.1**	61.0		32.4	20.5	74.6	29.2	140	0.209
TR-9	94	23.8	59.8		32.4	20.1	74.5	29.5	139	0.213
TR-13	90	22.9	62.3		30.6	20.7	69.6	27.1	140	0.193
TR-15	88	23.4	56.2		30.8	19.2	68.8	26.2	139	0.189
TR-17	86	22.3	59.8		32.0	19.3	73.2	28.2	138*	0.203
TR-21	94	21.8	62.5		32.6	21.1	73.0	28.8	139	0.206
TR-28	92	23.1	65.6		34.3	21.1	77.1	29.8	141	0.211
TR-32	86	22.9	62.0		33.4	20.8	73.7	29.0	142	0.204
TR-36M	92	21.6	59.8		32.6	20.5	73.0	28.8	141	0.205
TR-40	82	23.3	63.8		34.0	22.0	76.7	30.4	143	0.217
mean	90	23.0	61.3		32.6	20.5	73.5	28.7	140	0.205
SE	4	0.8	2.2		1.4	0.8	2.6	1.1	3	0.008

[a] * and ** indicate the value is significantly different from the control at the 5 and 1% level, respectively.

an equal volume of solvent only was sprayed on the soil. The final rates of chemical were 0.022 and 0.22 kg ha^{-1} (7.5-cm depth)$^{-1}$ (0.020 and 0.20 ppmw, respectively) for each metabolite and 0.56 kg ha^{-1} (7.5-cm depth)$^{-1}$ (0.5 ppmw) for trifluralin.

Because of space limitations, the two rates of metabolites, as well as a trifluralin treatment and control, were placed in separate greenhouses. In each greenhouse, the experiment used a randomized complete block design with 10 replicates per treatment. Five cotton seeds, var. "Stoneville 213", that had been treated with pentachloronitrobenzene and 5-ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole were planted in each of the containers on Jan 20, 1983. Plants were thinned for uniformity to one plant per container at the three- to four-leaf stage. From time of thinning until termination of flowering, the plants received weekly applications of water-soluble complete fertilizer. Insects were controlled with timely applications of aldicarb [2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime], chloropyrifos [*O*,*O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl)phosphorothioate], dicofol [4,4′-dichloro-α-(trichloromethyl)benzhydrol], or diazinon [*O*,*O*-diethyl *O*-(2-isopropyl-6-methyl-4-pyrimidinyl) phosphorothioate]. All plants in both greenhouses received identical applications of fertilizer and insecticides. During the experiment the plants did not receive supplementary lighting. The range in temperature during the experiment was 16–24 °C at night and 29–38 °C during the day, which was normal for cotton production. Flowers were tagged daily from March 21 to provide records of flowering, boll retention, and boll opening. Seed cotton was harvested by 10-day increments of flowering.

To measure maturity, mean maturity date (MMD) and production rate index (PRI) were calculated by Thomas' method (1975) as follows:

$$\text{MMD} = \frac{W_1H_1 + W_2H_2 + \ldots + W_nH_n}{W_1 + W_2 + \ldots + W_n} \quad (1)$$

where W = lint weight from each period of flowering, H = number of days from planting to complete opening of all bolls from each period of flowering, and 1, 2, ..., n = consecutive periods of flowering. PRI = total lint weight/MMD.

Data were disproportionate and statistical analyses were done by least-squares procedures. The linear model assumed was one of a randomized complete block. Treatment means were compared to the control mean by appropriate t tests.

RESULTS AND DISCUSSION

Soil Metabolites and Application Rates. The metabolites used in this study were 12 of 28 degradation products isolated and identified by Golab et al. (1979). These metabolites included the ones found in the highest concentrations and were principle products in all the possible transformation pathways. The metabolites not studied were those found in the smallest amount and were intermediates between the metabolites studied in the various pathways. In the Golab study, no single degradation product exceeded 3% of the trifluralin initially applied. On the basis of amounts found by Golab et al. (1979), the low rate [0.022 kg ha^{-1} (7.5-cm depth)$^{-1}$] and the high rate [0.22 kg ha^{-1} (7.5-cm depth)$^{-1}$] of metabolite used in the present study would be equivalent to a 1.4 to >40 and a 14 to >400 year accumulation, respectively, depending on the metabolite (see Table I), assuming no further degradation of the metabolite occurred. The rate of trifluralin [0.56 kg ha^{-1} (7.5-cm depth)$^{-1}$] is the recommended rate for the Dundee silt loam soil with 0.7% organic matter.

Plant Growth. Data for the effect of the low and high rates of metabolites on cotton growth on a per plant basis are shown in Tables II and III. None of the metabolites affected seedling emergence at either the low or high rate.

At the low rate of chemical, TR-2 and TR-6 significantly increased the growth of cotton at 20 days. Similar growth stimulation has been reported for subtoxic levels of a number of herbicides (Ries, 1976). At the high rate of chemical, TR-2, TR-15, and TR-28 significantly decreased growth compared with the control. The decrease, although significant, is probably not important. The control plants in the high rate experiment were slightly, but not significantly, taller than the plants in the trifluralin treatment. If they were the same, as in the low-rate experiment, there would be no significant differences between the control and any treatment. The significant differences in the early growth are probably not important. The cotton plant seems to compensate for early growth effects, such as root growth inhibition (Oliver and Frans, 1968). In this study, by harvest time, the plant height (data not shown) and stalk and root weights were not significantly different for any of the treatments at either rate of any chemical.

Table III. Effect of Trifluralin Soil Metabolites at 0.22 kg ha^{-1} (7.5-cm Depth)$^{-1}$ on Cotton Growth and Yield

treatment	seedling emergence, %	plant ht at 20 days, cm	stalk wt, g	root wt, g	no. of flowers	no. of bolls	seed cotton wt, g	lint wt, g	mean maturity date, days	production rate index, g/day
control	90	18.3	61.1	6.6	32.1	20.7	80.1	33.1	141	0.236
trifluralin (0.5 mg/kg)	92	16.6	56.8	7.6	29.3	19.9	76.9	31.4	145*	0.217
TR-2	96	16.4*[a]	61.3	9.9	30.8	20.8	78.0	32.0	142	0.226
TR-3	98	17.7	61.6	6.8	32.2	21.7	78.8	32.5	142	0.228
TR-6	92	17.4	59.5	6.8	30.2	20.5	80.7	33.3	141	0.236
TR-9	88	17.1	62.9	9.8	35.5	22.3	80.3	33.0	143	0.232
TR-13	92	17.8	59.2	7.8	32.3	21.3	81.7	33.3	143	0.233
TR-15	82	16.1*	58.8	7.9	30.7	19.2	76.1	31.2	143	0.219
TR-17	84	16.9	58.9	8.8	30.9	20.3	77.3	31.6	141	0.224
TR-21	96	16.7	57.8	8.2	30.8	20.2	76.2	31.4	143	0.220
TR-28	90	16.4*	62.2	8.7	29.0	20.0	76.4	31.7	143	0.222
TR-32	92	17.6	60.7	7.2	32.7	20.7	78.4	31.5	144	0.219
TR-36M	90	17.9	58.5	8.0	31.0	20.4	77.4	31.4	141	0.223
TR-40	90	17.3	56.8	6.6	30.2	19.6	73.5	29.4*	140	0.211*
mean	90	17.2	59.8	8.0	31.1	20.5	77.9	31.9	142	0.225
SE	4	0.6	2.2	1.1	1.3	0.8	2.8	1.1	3	0.008

[a] * indicates the value is significantly different from the control at the 5% level.

At the low rate of application, TR-2 significantly decreased the number of flowers per plant. This decrease apparently is a random occurrence and does not seem important, because the high rate of TR-2 did not significantly affect the number of flowers. Also, the number of bolls opened and boll retention were not significantly different for any of the metabolite treatments at either rate of chemical.

Yield. Seed cotton yield was not affected by any of the metabolites at either rate of chemical (Tables II and III). At the high rate, however, TR-40 significantly decreased lint yield, but the reason for the decrease in lint yield for TR-40 is not known. None of the other treatments affected lint yield. TR-40 is one of the minor metabolites, and the high rate of chemical applied is equivalent to at least a 400-year accumulation. Also, the low rate of TR-40, a 40-year accumulation, had no effect on lint yield.

Time to maturity can indirectly affect yield. A significant delay in maturity decreases the number of bolls opened at the end of the growing season especially if the season is shortened, and this may result in decreased yields. The only significant differences observed were for the low rate of TR-17, which shortened the time for maturity, and one of the two trifluralin treatments, which delayed maturity. The only significant effect on the production rate index was for the high rate of TR-40. This is due to the decreased lint yield observed as the mean maturity dates were equal for the treatment and the control.

SUMMARY AND CONCLUSIONS

Present data indicate that the trifluralin metabolites studied do not affect cotton growth or yield even at levels greatly exceeding those expected to be found in agricultural situations. The significant differences found did not seem to fit a logical or consistent pattern. If we accept the null hypothesis that the least-squares treatment means are equal to the control mean for all the comparisons made, we would expect to be wrong 5 and 1% of the time for comparisons made at the 0.05 and 0.01 level of probability, respectivity. We found that 4 and 0.4% of the comparisons between the control and treatment were significantly different, which is within the statistical probability. We therefore conclude that the significant differences that were found are due to random variation and are not truly different.

Although we did not evaluate potential synergistic or antagonistic effects on growth or yield of cotton due to interactions between metabolites, the results of this experiment corroborate and extend the results of Golab et al. (1979), who reported that selected metabolites of trifluralin were less phytotoxic to unnamed plants than the parent compound. Since the high rate of metabolite treatment represented a hypothetical accumulation of the metabolite over a minimum 14–400-year period (under the highly unlikely conditions wherein the metabolite was neither further metabolized or otherwise dissipated), our results do not support the view that metabolite formation from long-term usage of trifluralin is a significant contributing factor in the cotton yield decline problem.

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Registry No. TR-2, 2077-99-8; TR-3, 445-66-9; TR-6, 2078-01-5; TR-9, 17661-60-8; TR-13, 55702-44-8; TR-15, 51026-15-4; TR-17, 69145-23-9; TR-21, 2347-38-8; TR-28, 69145-29-5; TR-32, 69145-32-0; TR-36m, 36462-40-5; TR-40, 69236-56-2; trifluralin, 1582-09-8.

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