Effect of Trifluralin Soil Metabolites on Cotton Boll Components and Fiber and Seed Properties

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Greenhouse experiments were conducted to determine the effect of twelve soil metabolites of trifluralin (α,α,α-trifluoro-2,6-dinitro-*N*,*N*-dipropyl-*p*-toluidine) on cotton (*Gossypium hirsutum* L.), cv. Stoneville 213, boll components and fiber and seed properties. The metabolites included oxidative dealkylated, reduced nitro group, benzimidazole, azoxy, azo, oxidized, and hydroxylated derivatives of trifluralin. When applied at rates equivalent to at least a 14-year accumulation of metabolite, no individual metabolite significantly affected boll components, fiber properties, or seed properties, including boll size, lint index and percent, fiber fineness (micronaire), span length and strength, and seed oil and protein content. Potential accumulation of individual metabolites from the long-term use of trifluralin does not appear to adversely affect cotton quality.

INTRODUCTION

Cotton yield in the United States has declined slightly over the last 20 years (Starbird and Hazera, 1982). Concern and speculation have been increasing that the yield decline is a soil related problem, which possibly has resulted from the widespread use of dinitroaniline (DNA) herbicides, particularly trifluralin, since their introduction 20 years ago (Brown, 1982). Cotton fiber quality and seed oil content, in addition to lint yield, are important determinants of a cotton crop's value. For this reason, further concern has been expressed that these cotton quality factors may be affected by the possible accumulation of parent compounds or metabolites as a result of long-term use of DNA herbicides.

Sublethal doses of numerous herbicides, including trifluralin on cotton (Parka and Soper, 1977), have been shown to affect different plant species in a variety of ways (Ries, 1976). Herbicides have also been reported in some instances to affect boll components and fiber properties (Everson and Arle, 1956; Hamilton and Arle, 1979; Santelmann et al., 1966; Scifres and Santelmann, 1966), whereas in other instances no effect was observed (Foy and Miller, 1963; Hamilton and Arle, 1970; Hamilton and Arle, 1971; Hayes et al., 1981). Hamilton and Arle (1970, 1971) showed no differences in effects of various herbicides on boll components and fiber properties in comparisons between herbicides, but they did not have an untreated control.

There are also a variety of reports on the effect of herbicides on the oil and protein content of crops. The oil content of flax (*Linum usitatissimum* L.) (Dunham, 1951) and cotton seed (Epps, 1953) was reduced by 2,4-D applications in amounts that did not affect yield. Wilkinson and Hardcastle (1972) found isolated variations in composition of the fatty acids of cotton seed oils due to different herbicides. Ries (1976) reviewed numerous effects of herbicides on the protein content of crops.

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Although trifluralin has been reported not to affect fiber properties (Hayes et al., 1981), there are no reports on the effect of its soil metabolites on cotton fiber and seed quality. Major trifluralin metabolites do not affect cotton growth or yield (Koskinen et al., 1984), but, in theory, cotton plants may be able to absorb the metabolites from the soil in sufficient quantity to affect fiber and seed quality of cotton. Probst et al. (1967) found ^{14}C activity in all parts of cotton plants grown in [^{14}C] trifluralin treated soil. It was not clear whether the ^{14}C activity came from plant metabolism of absorbed trifluralin or from soil degradation and subsequent absorption of trifluralin metabolites by the plant.

Because of concern over potential adverse effects from long-term use of trifluralin on cotton quality, this study was conducted to determine whether trifluralin soil metabolites affect cotton boll components and fiber and seed properties.

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 individual 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 followed for 3 years before soil collection. The herbicide history prior to following is unknown. Some soil properties were 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 or 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 the same manner except that 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 metabolite and 0.56 kg ha^{-1} (7.5-cm depth)$^{-1}$ (0.5 ppmw) for trifluralin.

Because of space limitations, the low and high rates of metabolites were placed in separate greenhouses along with a trifluralin treatment and control. The experiment in each greenhouse used a randomized complete block design with 10 replicates per treatment. Five cotton seeds, cv.

Table I. Trifluralin Soil Metabolites

code[a]	name[b]	origin[c]	quantity,[d] 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*-propionotoluidide	LRL	>40	>400

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

Stoneville 213, that had been treated with pentachloronitrobenzene and 5-ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole were planted in each container. Plants were thinned for uniformity to one plant/container at the three- to four-leaf stage. From the time of thinning until the cessation of flowering, all plants received weekly applications of water-soluble complete fertilizer. Insects were controlled when needed by applications of aldicarb [2-methyl-2-(methylthio)propionaldehyde *O*-(methylcarbamoyl)oxime], chlorpyrifos [*O*,*O*-diethyl *O*-(3,5,6-trichloro-2-pyridyl)phosphorothioate], dicofol [4,4′-dichloro-α-(trichloromethyl)benzhydrol], or diazinon [*O*,*O*-diethyl *O*-(6-methyl-2-(1-methylethyl)-4-pyrimidinyl)-phosphorothioate]. All plants in both greenhouses received identical applications of fertilizer and insecticides. 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.

Span length, strength, and micronaire of the cotton fibers were determined by Starlab, Inc., Knoxville, TN. After seeds were delinted in concentrated sulfuric acid and then neutralized in a dilute solution of sodium bicarbonate, they were analyzed for physical and chemical characteristics. Subsamples of 100 seeds were used to determine seed weights and volumes by gravimetric and volumetric displacement procedures, respectively. Oil, protein, and gossypol contents of the seeds were measured by near-infrared reflectance spectroscopy with a Neotec GQA-31EL analyzer (Leffler and Williams, 1983). Concentrations were adjusted to dry basis by correction for meal moisture levels and then used with the seed weights to determine the content of each component per seed.

Data were disproportionate and statistical analyses were conducted by using least-squares procedures. The linear model assumed was one of a randomized complete block. Comparisons of treatment means to the control mean were done by using 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 those found in the highest concentrations and were principal products in each of the possible transformation pathways. Metabolites not studied were those found in the smallest amounts and were intermediates between the metabolites studied in the various degradative pathways. Golab et al. (1979) found no single metabolite to exceed 3% of the trifluralin initially applied. On the basis of amounts they measured, 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 metabolites we used would represent approximately a 1.4 to >40 and a 14 to >400 year accumulation, respectively. These estimates depend on the metabolite (see Table I) and assume no further degradation of the metabolite. The rate of trifluralin [0.56 kg ha^{-1} (7.5-cm depth)$^{-1}$] is the labeled rate for use on a Dundee silt loam soil with 0.7% organic matter.

Boll Components. Boll components were largely unaffected by the incorporation of single metabolites into the soil (Table II). At the low level of incorporation, no metabolite affected any boll component, and at the high level of incorporation only one significant difference was found in any boll component. The lint percentage was significantly lower in seed cotton harvested from plants grown in the presence of the high level of metabolite TR-40. TR-40 is a minor metabolite that was applied at a rate equivalent to approximately a 400-year accumulation. Although statistically significant, the decrease does not appear to be biologically important because it represents only a 3.3% difference from the control value; and the related boll components boll size, seed number, and seed weight (see Table IV) were not affected significantly by TR-40.

Fiber Properties. Fiber properties also were largely independent of any influence of trifluralin metabolites (Table III). At the low rate of applied metabolite, no fiber property was affected significantly by any treatment, whereas at the high rate of applied metabolite only fiber fineness (micronaire) was affected and then only by metabolite TR-32. Micronaire is an index value that is often associated with fiber maturity, with lower values suggesting less well-developed fibers. Even with a significantly lower value than the control, however, the fiber fineness reading of the sample from TR-32 is well within the range of micronaire values produced in the field by this cultivar at this location; therefore, this statistical significance is probably of little consequence. Fiber span length and strength are more important than fineness in determining fiber quality, but neither property was affected by the metabolite treatments.

Seed Properties. Environmental and seasonal factors often affect seed composition (Gipson and Joham, 1969; Leffler et al., 1977; Elmore et al., 1979; Kohel and Cherry, 1983). In our study, however, no measure of cotton seed physical or chemical composition was affected significantly by any of the trifluralin metabolites studied (Table IV). The ratio of oil to protein in the seed was calculated because Leffler and Williams (1983) and Leffler and Hunter

Table II. Effect of Trifluralin Soil Metabolites at 0.022 and 0.22 kg ha^{-1} (7.5-cm Depth)$^{-1}$ on Cotton Boll Components

	0.022 kg ha^{-1} (7.5-cm depth)$^{-1}$				0.22 kg ha^{-1} (7.5-cm depth)$^{-1}$			
treatment	boll size, g	seeds per boll	lint index, mg/seed	lint, %	boll size, g	seeds per boll	lint index, mg/seed	lint, %
control	3.62	22.9	61.7	39.7	3.88	23.7	67.7	42.1
trifluralin[a]	3.65	22.8	62.3	39.4	3.88	23.4	67.8	41.9
TR-2	3.64	22.7	63.0	40.1	3.75	22.9	67.4	41.8
TR-3	3.58	22.1	63.0	39.7	3.63	22.4	66.9	41.8
TR-6	3.65	22.7	62.9	39.9	3.94	24.3	67.1	42.2
TR-9	3.74	23.6	62.8	40.1	3.64	22.1	68.0	41.7
TR-13	3.39	21.6	61.0	39.5	3.83	23.0	68.2	41.5
TR-15	3.59	23.2	58.9	38.7	4.00	24.2	68.7	41.8
TR-17	3.83	23.6	62.6	39.2	3.82	23.7	65.9	41.7
TR-21	3.47	21.6	63.4	40.1	3.78	22.6	69.5	41.9
TR-28	3.67	22.8	62.1	39.2	3.84	23.2	69.9	42.3
TR-32	3.56	22.8	61.6	40.1	3.80	23.0	66.5	41.1
TR-36M	3.59	22.3	63.6	41.1	3.81	23.6	65.7	41.1
TR-40	3.49	21.8	63.6	40.2	3.78	23.6	64.2	40.7[b]
mean	3.61	22.6	62.3	39.7	3.81	23.3	67.4	41.7
std error	0.10	0.7	1.1	0.5	0.10	0.7	0.4	0.4

[a] Trifluralin applied at 0.56 kg ha^{-1} (7.5-cm depth)$^{-1}$. [b] The value is significantly different from the control at the 5% level.

Table III. Effect of Trifluralin Soil Metabolites at 0.022 and 0.22 kg ha^{-1} (7.5-cm Depth)$^{-1}$ on Cotton Fiber Properties

	0.022 kg ha^{-1} (7.5-cm depth)$^{-1}$					0.22 kg ha^{-1} (7.5-cm depth)$^{-1}$				
	fineness micronaire,	span length, index		strength		fineness micronaire,	span length, index		strength	
treatment	index	50% SL	2.5% SL	E_1, %	T_1, g/tex	index	50% SL	2.5% SL	E_1, %	T_1, g/tex
control	4.4	0.62	1.20	8.33	20.4	4.5	0.64	1.21	10.0	19.2
trifluralin[a]	3.9	0.63	1.21	8.67	20.6	4.5	0.61	1.20	9.2	19.7
TR-2	4.2	0.63	1.22	9.00	20.2	4.4	0.63	1.19	11.3	18.7
TR-3	4.2	0.64	1.22	8.67	19.9	4.7	0.63	1.22	9.3	19.8
TR-6	4.5	0.59	1.18	9.00	20.6	4.5	0.61	1.20	10.3	18.2
TR-9	4.3	0.63	1.23	8.67	21.3	4.6	0.64	1.23	10.0	18.3
TR-13	4.6	0.63	1.20	9.50	20.1	4.4	0.62	1.22	10.5	19.7
TR-15	4.0	0.64	1.22	8.50	20.4	4.5	0.62	1.20	9.7	18.4
TR-17	4.6	0.61	1.21	8.67	19.9	4.3	0.63	1.20	10.3	18.8
TR-21	4.8	0.63	1.21	9.00	20.0	4.4	0.65	1.19	9.7	19.2
TR-28	4.3	0.63	1.22	8.50	21.1	4.1	0.64	1.22	9.5	19.0
TR-32	4.2	0.61	1.18	8.67	20.4	3.9[b]	0.64	1.21	10.5	19.0
TR-36M	4.5	0.64	1.22	8.17	19.8	4.3	0.64	1.20	9.7	19.1
TR-40	4.6	0.60	1.19	9.00	19.8	4.4	0.64	1.22	9.7	19.3
mean	4.4	0.62	1.21	8.74	20.3	4.4	0.63	1.21	10.0	19.0
std error	0.2	0.02	0.01	0.47	0.5	0.1	0.01	0.01	0.5	0.4

[a] Trifluralin applied at 0.56 kg ha^{-1} (7.5-cm depth)$^{-1}$. [b] The value is significantly different from the control at the 1% level.

Table IV. Effect of Trifluralin Soil Metabolites at 0.022 and 0.22 kg ha^{-1} (7.5-cm Depth)$^{-1}$ on Cotton Seed Properties

	0.022 kg ha^{-1} (7.5-cm depth)$^{-1}$						0.22 kg ha^{-1} (7.5-cm depth)$^{-1}$					
	wt, mg/seed	density, mg/m^3	oil, % dry wt	protein, % dry wt	gossypol, % dry wt	oil/ protein	wt, mg/seed	density, mg/m^3	oil, % dry wt	protein, % dry wt	gossypol, % dry wt	oil/ protein
control	85.9	1.001	27.4	26.6	1.07	1.05	81.9	0.965	27.6	25.5	0.883	1.10
trifluralin[a]	82.9	1.022	27.9	26.3	1.09	1.08	85.6	0.953	26.9	25.4	0.903	1.07
TR-2	83.4	1.053	27.0	25.9	1.08	1.05	83.4	0.965	27.6	25.5	0.896	1.10
TR-3	85.3	1.005	26.8	26.7	1.06	1.01	82.2	0.960	27.9	25.3	0.901	1.12
TR-6	85.1	1.002	26.6	27.1	1.06	1.00	81.9	0.963	28.1	24.8	0.929	1.16
TR-9	82.2	1.016	27.3	26.5	1.09	1.04	83.8	0.968	27.7	25.7	0.887	1.10
TR-13	81.8	0.998	26.9	26.1	1.08	1.04	81.6	0.960	27.4	25.6	0.901	1.09
TR-15	82.6	1.002	28.3	25.7	1.11	1.12	81.9	0.962	27.9	26.0	0.918	1.09
TR-17	84.7	0.998	27.6	25.9	1.09	1.08	82.1	0.983	28.4	25.2	0.919	1.15
TR-21	86.0	0.986	27.3	26.2	1.18	1.05	82.4	0.960	27.9	25.8	0.874	1.10
TR-28	84.7	0.984	27.2	25.6	1.11	1.08	83.4	0.967	27.9	24.9	0.913	1.15
TR-32	81.3	0.998	27.6	25.5	1.14	1.10	85.1	0.948	27.3	25.6	0.876	1.09
TR-36M	85.2	0.991	28.0	25.9	1.10	1.09	83.3	0.970	27.9	25.2	0.907	1.12
TR-40	83.9	0.978	28.0	25.5	1.10	1.11	83.1	0.968	27.5	26.3	0.899	1.06
mean	83.7	1.002	27.4	26.1	1.10	1.06	83.0	0.964	27.7	25.5	0.900	1.11
std error	3.6	0.021	0.7	0.7	0.04	0.05	1.9	0.009	0.6	0.8	0.031	0.06

[a] Trifluralin applied at 0.56 kg ha^{-1} (7.5-cm depth)$^{-1}$.

(1985) found that ratio to be associated with the biological performance of cotton seeds and because its response might be different from that of either principal storage reserve alone. This ratio and seed density are interrelated (Leffler and Williams, 1983), and neither was influenced by the treatments in this study. These data indicate that the seed value of the crop was not affected by the metabolites, either as a source of commercial vegetable oil and

protein meal or as planting seeds.

SUMMARY AND CONCLUSIONS

Our present data indicate that the 12 trifluralin metabolites we studied did not influence cotton quality, even when the metabolites were applied at levels that greatly exceeded those that reasonably could be expected to accumulate in agricultural situations. It is highly probable that the trifluralin metabolites would continue to degrade under the conditions of the experiment. Thus, the use of exaggerated levels in the study further supports lack of accumulation of any one metabolite that would exhibit detrimental effects on cotton. In all of the quality measurements that were evaluated, only two statistically significant effects were detected, and neither of these appeared to be of a major biological consequence. Thus, it appears that these differences were due to random variation and may not reflect true differences.

We did not evaluate potential synergism or antagonism between metabolites because of the restrictions in the experimental resources that were available to us. We feel, however, that such interactions between or among metabolites are unlikely because no true differences were detected in numerous quality measurements on materials produced in the presence of relatively massive amounts of individual metabolites. These quantities normally would not be found in field situations, even after many years of herbicide application. Our study, within the limitations of any greenhouse study, does not support the concept that the accumulation of trifluralin metabolites has contributed to any loss of cotton quality or to the deterioration in cotton yields in recent years (Koskinen et al., 1984).

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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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