

## SOIL MICROORGANISMS AND THE HERBICIDE TRIFLURALIN

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### INTRODUCTION

In Saskatchewan, trifluralin is used for weed control in a variety of crops such as canola (Brassica napus) and wheat (Triticum aestivum). Because this herbicide is soil incorporated, the interaction with non-target organisms such as the soil microflora must be considered. Various studies have examined the effects of trifluralin and/or other herbicides on soil microorganisms. Because of the conflicting nature of these results, no clear-cut effect can be attributed to trifluralin. For example, in soil receiving repeated applications of trifluralin the bacterial population level was inhibited whereas the fungal and actinomycete population levels were stimulated (Carter and Camper, 1973). Other studies have detected either no effect or else a short term reduction of the above microbial groups in trifluralin treated soils (Helmecki et al., 1978; Tolkachev and Solyanova, 1979; Kondratenko et al., 1981). Parameters of microbial activity such as respiration and soil enzymatic activity also exhibit variable responses towards trifluralin (Liu and Cibes-Viade, 1972; Davies and Marsh, 1977; Lewis et al., 1978; Tag El-Din et al., 1978; Bakalivanov et al., 1979; Tolkachev and Solyanova, 1979; Davies and Greaves, 1981).

Although major groups of soil microorganisms exhibit variable responses to trifluralin, this chemical demonstrated fungicidal activity against Rhizoctonia solani, Fusarium oxysporum (Eshel and Katan, 1972), Sclerotium rolfsii (Rodriguez-Kabana et al., 1969), and Phycomycetes mycotypha (Liu and Cibes-Viade, 1972). In contrast, populations of Azotobacter in a cotton field soil were stimulated by trifluralin incorporation (Ulanov and Voevodin, 1976).

Recently, Olson (1982) examined the adverse effects of trifluralin on the development of wheat root systems in a growth chamber study. Comparable field rate concentrations (0.8 ppm) of trifluralin severely inhibited root growth, while low concentrations (0.4 ppm) caused only slight damage to roots. Marked changes in root morphology, size, and weight were noted at a concentration of 0.8 ppm. Based on the above, it was apparent that information was needed on the effect of trifluralin on microbial populations in the rhizosphere of damaged wheat roots. The objective of this study, therefore, was to examine the response (or lack of response) of microbial populations in the rhizosphere and surrounding soil of wheat roots subjected to trifluralin incorporation. Wheat plants grown in a growth chamber were chosen as the main object of study as they allowed easy assay of roots and soil. For comparisons, field studies were conducted in an attempt to evaluate the herbicide under natural conditions. Also, information is presented on the effects of trifluralin on the growth of soil microorganisms in pure culture.

## MATERIALS AND METHODS

### Field Study

Field plots were established at the University of Saskatchewan Kernen Research Farm and at the Agriculture Canada Experimental Farm in Scott, Saskatchewan (Table 1) to examine the response of Neepawa wheat (*Triticum aestivum*) to soil incorporated trifluralin (Treflan E.C.). Both plot sites were completely randomized in a block design. At the Scott site trifluralin was applied and incorporated (0.1 m) on May 10, 1982, and wheat was sown the following day at 67 kg ha<sup>-1</sup> along with 45 kg ha<sup>-1</sup> of 11-51-0 fertilizer. At the Kernen site, trifluralin was applied, incorporated (0.1 m), and wheat seeded (84 kg ha<sup>-1</sup>) on May 25, 1982. Fertilizer (11-55-0) was applied at 56 kg ha<sup>-1</sup>.

Bulk surface soil samples were taken throughout the growing season from the control and high trifluralin treated (Scott: 1.0 kg ha<sup>-1</sup>, Kernen 1.12 kg ha<sup>-1</sup>; or approximately 0.77 and 0.93 ppm, respectively) plots at various time intervals; once near the time of herbicide application, two weeks later and once a month thereafter for two months. The samples were sealed in plastic bags and stored at 5°C until processed for microbial analysis (see below) which was always within two days. Subsamples were taken for moisture content determinations and for microbial analysis. Samples were analyzed for fungal, bacterial, actinomycete, nitrifying (i.e., *Nitrobacter*) and denitrifying populations.

Table 1. Soil characteristics.<sup>1</sup>

Soil association	Site in Saskatchewan	Organic <sup>2</sup> matter	Lime <sup>2</sup>	Texture <sup>2</sup>	pH	Electrical conductivity (mmhos cm <sup>-1</sup> )	Nutrient levels (ppm)			
							NO <sub>3</sub> -N	P	K	SO <sub>4</sub> -S
Asquith	Grandora	low	absent	fine sand	7.9	0.2	24	8	224	5
Bradwell	Flora	medium	absent	loam	7.2	0.2	12	24	504+	10
Scott	Scott	low	absent	loam	5.9	0.1	2	8	504+	5
Sutherland	Kernen Farm	low	trace	clay loam	7.7	0.2	9	13	504+	4

<sup>1</sup> Data determined by the Saskatchewan Soil Testing Laboratory, Saskatoon.

<sup>2</sup> Qualitative estimates.

### Growth Chamber and Laboratory Study

#### EXPERIMENT 1: FIELD RATE CONCENTRATIONS

The soils used in this study are described in Table 1. Two dark brown chernozemic soils, an Asquith and a Bradwell, were selected for the growth chamber study. For herbicide incorporation, the soils were air-dried and passed through a 2 mm sieve. A commercial source of trifluralin (Treflan E.C.) was used and added to soil samples to provide herbicide concentrations of 0.0, 0.09, and 0.18 ppm or 0.0, 0.4, and 0.8 ppm in the Asquith and Bradwell soils respectively. The herbicide rates for both soils were determined in a preliminary experiment such that sub-lethal trifluralin injury to wheat seedlings occurred at the highest

concentration. After trifluralin treatment, the soils were brought to field capacity, thoroughly mixed, and allowed to equilibrate for 10 to 12 hours before use.

Black plastic tubes, 0.051 m inside diameter by 0.4 m long were used as plant growth containers. These tubes have been described previously and were used as they allowed recovery of intact root systems (Olson, 1982). The herbicide treated and control soils (at field capacity) were poured into the tubes and packed to  $1300 \text{ kg m}^{-3}$  and  $1100 \text{ kg m}^{-3}$  bulk densities for the Asquith and Bradwell soils, respectively, by dropping the tubes on the closed end from 0.03 m above a cement floor. Three Neepawa caryopses were planted per tube at a seeding depth of 0.02 m. When more than one plant emerged they were thinned to one plant per tube. Each treatment was replicated three times and placed randomly in a growth chamber with a 16 hour,  $22^{\circ}\text{C}$  day and 8 hour,  $17^{\circ}\text{C}$  night cycle under lights balanced to about 5600 K and about 12-14 Klux. The relative humidity was approximately 50%.

Plants were grown for nine days after seeding at which time the soil cores were removed from the tubes and the root systems recovered. Soil from the cores was divided into two fractions. Soil clinging to the roots was designated the rhizosphere soil fraction and the remaining soil from the tube was designated the bulk soil fraction. The latter soil fraction was sub-sampled for moisture content determinations and for microbial analysis (see below). The root system with clinging rhizosphere soil was placed into a sterile glass bottle containing 100 ml water and glass beads, shaken on a wrist action shaker (15 minutes, Asquith, or 25 minutes, Bradwell) and the slurries were analyzed for fungal, bacterial, and actinomycete populations (see below).

#### **EXPERIMENT 2: HIGH RATE CONCENTRATION**

Soil preparation, incubation, and assay conditions were as in Experiment 1 except for the following changes: (1) the trifluralin concentration was 16 ppm in both soil types, and (2) wheat caryopses were not sown since trifluralin at this concentration would be lethal.

#### **EXPERIMENT 3: MICROBIAL $\text{CO}_2$ RESPIRATION**

Asquith and Bradwell soils were also used for respiration studies. Before use the soils (maintained at field capacity) were passed through a 0.2 mm sieve and were not allowed to air-dry. Treatments were soil or soil amended with coarsely ground wheat straw ( $1650 \text{ mg kg}^{-1}$ , based on  $3300 \text{ kg straw ha}^{-1}$ ) with and without added trifluralin. Trifluralin concentrations used were 0.0, 0.18, 0.36, and 3.6 ppm, or 0.0, 0.8, 1.6, and 16 ppm for the Asquith and Bradwell soils, respectively. The amended soils were brought to field capacity, thoroughly mixed, and 0.1 kg (oven dry basis) of the treated soils placed into plastic vials which were incubated in 2.26 L screw cap glass jars. A plastic vial containing 30 ml of 0.5 M NaOH solution was placed into each jar to serve as a  $\text{CO}_2$  trap, and a few ml of water were added to the jars to create a saturated atmosphere. The jars were sealed and kept in the dark at a day temperature of  $22^{\circ}\text{C}$  and a night temperature of  $17^{\circ}\text{C}$ . The jars were recharged with air and fresh NaOH approximately every week, at which time the NaOH samples were titrated with 0.1 M HCl using the carbonic anhydrase method (Underwood, 1961; Tiessen et al., 1981). All treatments were replicated twice.

## Microbial Analysis

The spread-plate technique was used to obtain total counts of fungi, bacteria, and actinomycetes. The media employed were : Czapek-Dox Agar (pH 3.5) for fungi, 0.3% Trypticase-Soy-Agar for bacteria (Martin, 1975), and Actinomycete Isolation Agar with added actidione ( $50 \text{ mg L}^{-1}$ ) and tetracycline ( $5.0 \text{ mg L}^{-1}$ ) for actinomycetes. All media were incubated at  $25^{\circ} \text{C}$  and counts taken after four to nine days. All media components were Difco products (Difco Laboratories, Detroit, Mi.).

Nitrifying (*Nitrobacter* sp.) and denitrifying populations were determined using the most probable number (MPN) technique as described by Alexander (1965a, 1965b). Nitrite nutrient broth (Volz, 1977) in microtiter plates was used for the denitrifying bacteria. These plates were incubated at room temperature for 7 days under anaerobic conditions in a vacuum dessicator filled with 100% He (Staley and Griffin, 1981). The absence of nitrite upon the addition of NEDD reagent (Volz, 1977) was used to identify the presence of denitrifying bacteria. *Nitrobacter* populations were determined after 21 days incubation at  $25^{\circ} \text{C}$  using nitrite medium in test tubes and the reagents described by Alexander and Francis (1965).

Counts of fungi, bacteria and actinomycetes populations represent the average of three replicate plates for three soil sample replicates (i.e., 9 plates total for a soil sample). The MPN results are the average of one MPN test per soil replicate (i.e., 3 MPN tests for a soil sample). All microbial counts were expressed as number of organisms per gram of oven-dried soil.

## Pure Culture

A sensitivity test was used to assess the minimum inhibitory concentration (M.I.C.) of trifluralin on microbial growth. The soil microorganisms and media used in this study are listed in Table 2 (see Results and Discussion). Prepared media plates were inoculated (i.e., complete surface coverage) with a 72 h broth stock culture of the appropriate microorganisms. Trifluralin (Treflan E.C.) was passed through a sterile  $0.22 \text{ } \mu\text{m}$  nucleopore filter and stock suspensions were prepared in sterile water with concentrations of 0.4, 4, 16, 400, 4000, 16000, 25000, 50000, and 100000 ppm trifluralin. Sterile paper discs (Schleicher & Schuell, Inc., Keene, N.H.) 12.7 mm in diameter were treated with 3 or 4 drops of the filtered trifluralin stock suspensions. Sterile distilled water was used as the control solution. One  $\text{H}_2\text{O}$  control disc and three trifluralin discs, each with a different concentration, were aseptically transferred onto the preinoculated surface of each plate. Treatments were replicated twice and the plates incubated at  $25^{\circ} \text{C}$ . After 2 to 3 days of incubation, the plate were examined for zones of inhibition (or stimulation) around each disc.

## RESULTS AND DISCUSSION

### Field Study

The two field sites (Table 1; Scott and Kernen) selected for this study were approximately 150 km apart and roughly same latitude. The microbial population levels in the bulk soil from the control plots were very similar. For example, at the first sampling time the fungal,

bacterial and actinomycete counts for the Scott site were  $1.39 \times 10^4$ ,  $3.86 \times 10^6$ , and  $7.96 \times 10^5 \text{ g}^{-1}$  soil (oven dry wt) as compared to counts of  $1.15 \times 10^4$ ,  $9.61 \times 10^6$ , and  $9.21 \times 10^5 \text{ g}^{-1}$  soil, respectively, for the Kern site. The nitrifying and denitrifying populations in these soils were also similar, and this trend (i.e. comparable population levels) continued for all microbial groups throughout the entire sampling period.

Wheat plants in the trifluralin treated plots exhibited reduced plant density and definite signs of trifluralin injury such as tip damage of primary leaves, reduced root growth, and clubbing of root tips. The effects of trifluralin on the fungal, bacterial and actinomycete populations in the bulk soil from the Scott site are shown in Figure 1. In the Scott bulk soil, slight inhibition (fungal populations) and an initial period of stimulation (bacteria and actinomycete populations) occurred, but no significant or consistent trend was evident. Little, if any, trifluralin effect on nitrifying and denitrifying populations (data not presented) was detected. Rhizosphere soil could not be assessed because of the difficulty in removing entire root systems from the field soil. The picture for the Kern bulk soil was approximately the same. Unfortunately, we do not have any information on residual trifluralin concentrations in either field soil over the sampling period.

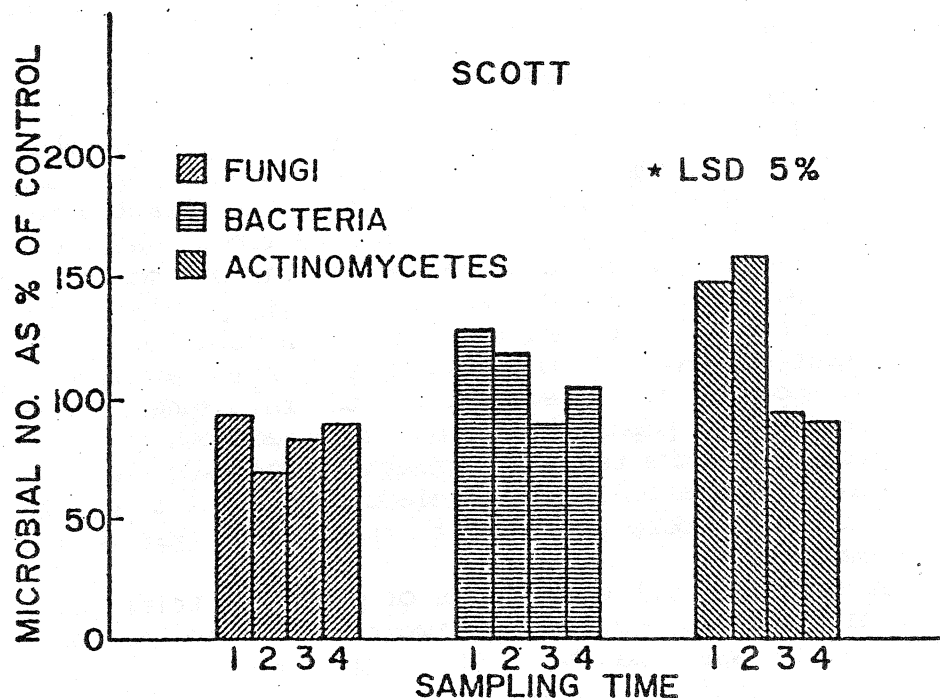


Fig. 1. Effect of trifluralin on microbial populations in the Scott bulk field soil; sampling time 1, day of herbicide incorporation; 2, two weeks; 3, six weeks; 4, ten weeks post herbicide incorporation.

Our results indicate that trifluralin did not significantly affect microbial populations in the bulk field soil. Similar observations on

microbial populations of trifluralin treated soils have been made by and Helrmeczi et al. (1978). In contrast, one study found that trifluralin inhibited the bacterial and actinomycete populations (Tolkachev and Solyanova, 1979), while Carter and Camper (1973) observed stimulation of soil fungi and actinomycetes but inhibition of soil bacteria. Because of the different soil types, field crops, and other environmental conditions, our results cannot be directly compared to these studies. In order to examine the microbial populations in the rhizosphere of trifluralin-damaged wheat plants and to verify bulk field soil results, we initiated a series of growth chamber studies.

### Growth Chamber Study

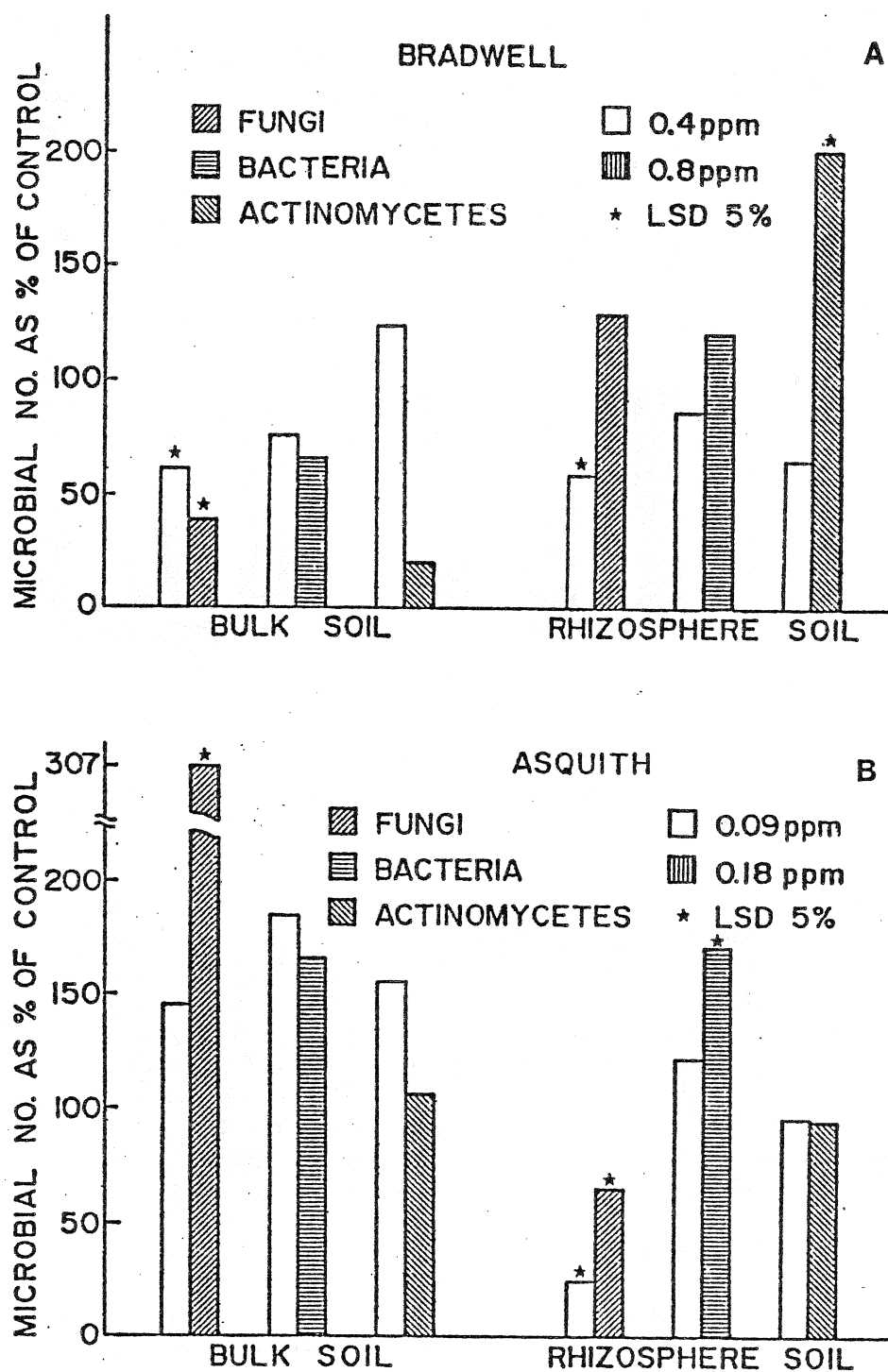
Trifluralin concentrations of 0.09 and 0.8 ppm caused considerable damage to wheat seedlings in the Asquith and Bradwell soils, respectively. This damage was similar to that described for our field study and by Olson (1982).

The microbial population levels (i.e., fungi, bacteria, actinomycetes) of the Bradwell bulk (control) soil were 5- to 10-fold greater than in the Asquith bulk (control) soil. This difference was probably due to the higher organic matter level in the Bradwell soil (Table 1). Interestingly, the microbial population levels of the rhizosphere (control) soil were approximately the same for both soils; although these population levels were, in most cases, statistically higher than the levels detected in the respective bulk soil. Root exudates could be responsible for this rhizosphere effect.

The effects of trifluralin amendments on the microbial populations in the bulk and rhizosphere soil are shown in Figure 2a (Bradwell) and Figure 2b (Asquith). Trifluralin amendments affected the fungal populations in both bulk and rhizosphere fractions of each soil. Unfortunately, both stimulation and inhibition occurred and no clear-cut response was observed. For example, fungal populations in the Bradwell bulk soil exhibited increasing inhibition with increasing trifluralin concentration, while in the Asquith soil stimulation was observed. The picture appeared to be reversed for the rhizosphere soils. The bacterial and actinomycete populations also exhibited both stimulation and inhibition, and the responses generally followed the trend observed for the fungal population. Nevertheless, our results seem to indicate that soil fungi were more sensitive to trifluralin than soil bacteria and actinomycetes.

In order to test the effects of excessive trifluralin rates on microbial populations, an experiment similar to the above but without wheat plants was conducted. The fungal, bacterial, and actinomycete population levels were similar for both the Asquith and Bradwell control soils. The population counts were lower than the control population levels reported for the above experiment with wheat plants present, and this might be explained by the absence of an actively growing root system in the soil columns.

The relatively high trifluralin rate (i.e., 16 ppm) in this study, as compared to the approximate field rates used in the growth chamber study, did not affect the three microbial population levels in the Asquith soil (Fig. 3). In contrast, in the Bradwell soil with added trifluralin the bacterial population decreased 60%, while the fungal and actinomycete populations increased 27% and 100%, respectively. Even though the



**Fig. 2.** Effect of trifluralin on microbial populations in the bulk and rhizosphere soil of 9 day old wheat plants grown in a growth chamber.



actinomycetes appeared to be greatly stimulated, the variability was large enough to make it non-significant.

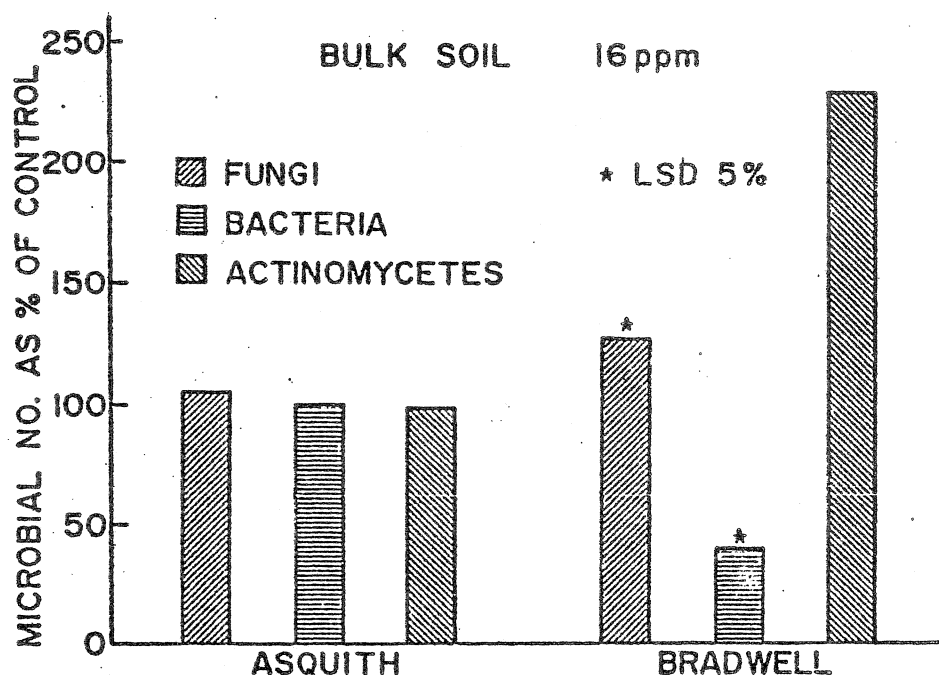


Fig. 3. Effect of high trifluralin rate (16ppm) on microbial populations in growth chamber soil after 9 days.

Greaves et al. (1980) stated that soil microbial population changes due to herbicides should be compared to the population variation that occurs under natural conditions. They proposed that a population would have to experience at least a 50% decrease to be considered ecologically significant. These guidelines should also apply to any increase in microbial population levels. Our results show most of the microbial population levels fall within these limits and nearly half that were outside these limits were statistically non-significant. Taking into account the above guidelines, most of the effects of trifluralin on the microbial populations we studied were not ecologically significant.

#### MICROBIAL CO<sub>2</sub> RESPIRATION:

The overall microbial activity as measured by CO<sub>2</sub> evolution was not affected by high rates of trifluralin in the Bradwell soil (Fig. 4). Added wheat straw caused a large increase in CO<sub>2</sub> evolution and the presence of trifluralin neither stimulated or inhibited straw decomposition. The results for the Asquith soil (data not presented) were similar. Davies and Marsh (1977) observed no effect of trifluralin (100 ppm) on CO<sub>2</sub> evolution and N-mineralization. Similar results for



CO<sub>2</sub> evolution were obtained by Lewis et al. (1978); and other studies indicate that trifluralin does not affect enzymatic activity in soil (Davies and Greaves, 1981; Lewis et al., 1978). It should be pointed out, however, that some workers have observed either stimulation (Bakalivanov et al., 1979; Rodriguez-Kabana et al., 1969) or inhibition (Kondratenko et al., 1981) of soil respiration.

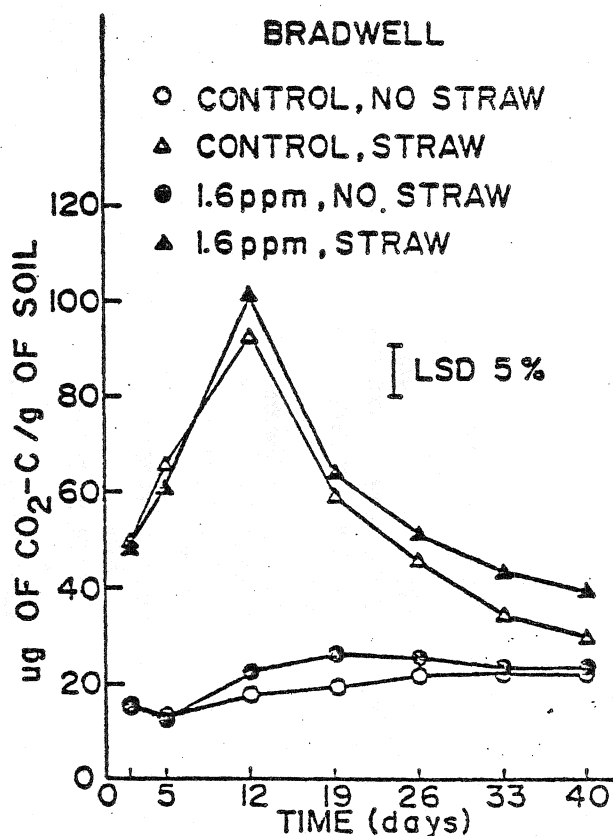


Fig. 4. Effect of trifluralin on microbial respiration in straw amended and non-amended soil.

#### PURE CULTURE STUDIES:

Even though the field rates and higher trifluralin concentrations used in the previous experiments did not significantly alter microbial populations, we considered it important to determine at what concentrations trifluralin would affect pure cultures grown under laboratory conditions. This information would be vital for waste disposal, spill sites, and improper application where the trifluralin concentration might be excessive.

The effects of trifluralin on the growth of various soil microorganisms in pure culture are shown in Table 2. None of the organisms tested were sensitive to low (i.e., 0.4-16 ppm) levels of trifluralin. The majority of the fungal isolates tested were not affected by trifluralin at concentrations less than 16,000 ppm. Most

Table 2. Effect of trifluralin on growth of various soil microorganisms in pure culture.

Organism	Growth <sup>1</sup> medium	Trifluralin Concentration <sup>2</sup> (ppm)					
		400	4,000	16,000	25,000	50,000	100,000
<u>Fungi</u>							
<u>Absidia glauca</u>	CZD	- <sup>3</sup>	-	+	NT <sup>4</sup>	NT	NT
<u>Aspergillus fumigates</u>	CZD	-	-	-	-	+	++
<u>Chrysosporium pannorum</u>	CZD	-	-	-	+	+	++
<u>Chrysosporium sp.</u>	CZD	-	-	-	+	+	++
<u>Cladosporium herbarum</u>	CZD	-	-	-	+	+	+
<u>Fusarium culmorum</u>	CZD	-	-	+	+	+	++
<u>Fusarium epishaeria</u>	CZD	-	-	-	-	+	+
<u>Fusarium roseum</u>	CZD	-	-	-	+	+	++
<u>Gliocladium roseum</u>	CZD	-	-	-	-	+	++
<u>Mortierella isabellina</u>	CZD	-	-	-	-	-	-
<u>Mortierella nana</u>	CZD	-	-	+	++	++++	+++++
<u>Mucor silvaticus</u>	CZD	-	-	-	+	+	++
<u>Paecilomyces carneus</u>	CZD	-	-	-	+	+	+
<u>Paecilomyces marquandii</u>	CZD	-	-	+	+	+	++
<u>Penicillium frequentans</u>	CZD	-	-	-	-	-	-
<u>Penicillium nalgiovensis</u>	CZD	-	-	-	-	-	-
<u>Sporotrichum carnis</u>	CZD	-	-	+	+	++	++++
<u>Trichoderma hamatum</u>	CZD	-	-	+	+	+	++
<u>Trichoderma viride</u>	CZD	-	-	-	-	+	++
<u>Zygorrhynchus vuilleminii</u>	CZD	-	+	++	+	+++	+++
<u>Bacteria</u>							
<u>Agromyces ramosus</u> ATCC 25173	HI	+++	++++	++++	++++	++++	++++
<u>Arthrobacter globiformis</u>							
ATCC 8010	NA	-	+	++	NT	NT	NT
<u>Arthrobacter oxydans</u>							
ATCC 14358	NA	-	+	+++	NT	NT	NT

1. CZD = Czapek Dox Agar; HI = Heart Infusion Agar; NA = Nutrient Agar; CCM = Combined Carbon Medium (Rennie, 1981); BA = Burks Agar (Burk, 1930).
2. Trifluralin concentrations of 0.4 - 16 ppm tested but no effect observed.
3. Size of inhibition zone: -, no effect; +, > 0 to < 5 mm; ++, > 5 to < 10 mm; +++, > 10 to < 15 mm; +++, > 15 to < 20 mm; +++, > 20 mm; s.

Organism	Growth <sup>1</sup> medium	Trifluralin Concentration <sup>2</sup> (ppm)					
		400	4,000	16,000	25,000	50,000	100,000
<u>Azospirillum brasilense</u>							
ATCC 29145	CCM	-	-	-	-	-	-
<u>Azotobacter chroococcum</u>							
ATCC 9043	BA	-	-	-	-	-	-
<u>Azotobacter vinelandii</u>							
ATCC 12837	BA	-	-	-	-	-	-
<u>Bacillus megaterium</u>	NA	+	+	+	++++	++++	++++
<u>Ensifer adhaerens</u> ATCC 33212	NA	S	SS	SS	SSS	SSSS	SSSSS
<u>Ensifer adhaerens</u> Strain SA	NA	-	-	-	-	-	-
<u>Ensifer adhaerens</u> ATCC 33499	NA	S	S	SS	SS	SSSS	SSSSS
<u>Enterobacter aerogenes</u>	NA	-	-	-	-	-	+
<u>Micrococcus flavis</u>	NA	-	-	+	NT	NT	NT
<u>Pseudomonas aeruginosa</u>	NA	-	-	-	-	-	-
<u>Pseudomonas fluorescens</u>	NA	-	-	-	-	-	-
<u>Rhizobium leguminosarum</u>							
128C30	TY	-	-	-	+	++	+++
<u>Rhizobium leguminosarum</u>							
128C52	TY	-	-	-	+	++	+++
<u>Rhizobium leguminosarum</u>							
128C79	TY	-	-	-	+	+	++
<u>Rhizobium meliloti</u> NRG185	TY	-	+	++	+++	+++	+++
<u>Rhizobium meliloti</u> NRG9930	TY	-	-	-	++	+	+
<u>Rhizobium trifolii</u> 14480	TY	-	-	-	-	+	++
<u>Rhizobium trifolii</u> TAI	TY	+	+	++	++	++	+++
<u>Actinomycetes</u>							
<u>Streptomyces</u> sp.							
<u>Streptomyces</u> sp. A-12	NA	-	+	++	++	+++	+++
<u>Streptomyces</u> sp. A-16	NA	-	-	+	++	++++	++++
<u>Streptomyces</u> sp. S-34	NA	-	-	+	NT	NT	NT

isolates, however, were inhibited by concentrations in excess of 25,000 ppm. In any case, the inhibition observed for fungi was not as extensive as that exhibited by the bacteria and actinomycetes tested. The trend for soil populations (see growth chamber studies) appeared to be reversed. Three fungal isolates were unaffected by any trifluralin concentrations tested (Table 2), and no case of stimulation was observed. Our results indicate the 85% of the fungal isolates tested were sensitive to high concentrations of trifluralin. Similar examples of soil fungi inhibition in pure culture have been reported (Eshel and Katan, 1972; Rodriguez-Kabana et al., 1969).

Most of the bacterial (67%) strains and the four actinomycetes tested were inhibited by trifluralin. Unlike the fungi, however, inhibition began to appear at lower trifluralin concentrations; i.e., 400, 4000, and 16,000 ppm. None of the free-living  $N_2$  fixing microorganisms (i.e., *Azotobacter*, *Azospirillum*,) or the two *Pseudomonas* spp. tested were inhibited by trifluralin even at 100,000 ppm. Similar observations have been made by Carter and Camper (1975) and Kondratenko et al. (1981). In contrast, all the *Rhizobium* species tested were sensitive to trifluralin. Two strains of *E. adhaerens* displayed stimulation by producing zones of slime around the trifluralin treated discs. This stimulatory response was observed with the 400 ppm and increased with increasing concentrations of trifluralin.

Based on our results, the growth of approximately 75% of the soil microorganisms tested was inhibited by trifluralin. However, little inhibition was observed at concentrations less than 4000 ppm. It would appear then, that trifluralin toxicity towards soil microflora should not be a problem in soil. However, calculations show that the trifluralin concentration at the surface of carrier particles used in the granular formulation of trifluralin (5% trifluralin by weight) is approximately 70,000 ppm. Therefore, until the trifluralin is released from the carrier material (i.e., under moist conditions), soil microbes in a microenvironment would be exposed to these extremely high concentrations, and growth might be inhibited as observed for pure cultures.

#### SUMMARY AND CONCLUSIONS

Our results shows that recommended trifluralin rates did not cause significant changes in the microbial populations in field soil. In the growth chamber study, however, some microbial population levels in the bulk and rhizosphere soil of wheat plants exhibited significant inhibition or stimulation. Unfortunately, no consistent trend was evident. Unlike the variability associated with microbial number determinations, trifluralin, even at excessive rates, did not affect microbial respiration or straw decomposition.

A pure culture study with various soil microorganisms demonstrated that over 75% of the isolates tested were inhibited by trifluralin. Inhibition occurred, only at very high trifluralin concentrations, ranging from 400 to 100,000 ppm. Surprisingly, some soil microorganisms were unaffected even by the highest trifluralin concentration tested. Those microorganisms that were unaffected included free-living nitrogen-fixing bacteria. In addition, the growth of one soil bacterium appeared to be stimulated by trifluralin, and this stimulation increased

with increasing concentrations. Our results indicate that trifluralin can be inhibitory to many soil microorganisms, but only at relatively high concentrations.

In summary, then, recommended trifluralin field rates are considerably lower than those found to inhibit microbial growth in pure culture.

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