SURFACE pH OF SASKATCHEWAN SOILS

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Soil pH is an important factor in crop growth. Many biological and chemical activities in the soil are greatly influenced by pH.

Importance of Soil pH

Soils with a pH ranging from 6.5 to 7.5 provide the best environment for crop growth. Below a pH of 6.0 yields of sweet clover and alfalfa are reduced. A pH less than 5.5 may reduce yields of these crops by as much as one-half. The effect of low pH on sweet clover and alfalfa is due to a decrease in the ability of Rhizobium bacteria to form nodules on the roots (Hoyt et al., 1974). These nodules take up atmospheric nitrogen and convert it for use by the legumes so that fertilizer nitrogen is not needed.

Yields of grain and oil seeds may also be reduced below a pH of 5.0. Acid soils (pH less than 5.0) may contain toxic amounts of Al or Mn that will restrict root growth and the uptake of phosphate. Calcium and Mg may also be at very low levels in acid soils which will show up as deficiencies in the crop.

In alkaline soils (pH greater than 7.5) copper, zinc and Mn availability may be reduced to deficient levels. Phosphate fixation by calcium is also increased at a high pH reducing available soil phosphate levels.

How the Data was Collected

During the summer of 1982 surface soil samples from the agricultural portion of Saskatchewan were collected and pH measured. The soil pH was measured in 0.01 M $CaCl_2$ in a 1:1 soil-solution mixture. Most soil testing laboratories measure pH in 1:1 soil-water mixtures. The $CaCl_2$ method will give pH values slightly lower (0.2-0.5 pH units) than those measured in water. The pH values indicated are for the surface layer of soil which ranges in thickness from 10 cm in the southwest to 20 cm in the east-central portion of the province. Most subsoils in Saskatchewan are more alkaline than the surface soil.

The soil samples were collected on a modified grid sampling system. This system allowed for most soil associations to be sampled. On the average, sampling sites were approximately 10 km apart. At each sampling site 3 or 4 samples were collected, each representing a position on the landscape (crest, upper, mid, and lower slope positions). The soil association and series of each sample was recorded in the field for later statistical analysis. The total number of samples collected in this manner was about 8,000. In addition, some rural municipalities were sampled during the course of routine soil survey activities.

After all the collection and individual analysis was complete, average pH and frequency distributions were calculated for each soil series and each rural municipality with the help of a computer.

The pH values were grouped into the following classes: A - acid, pH less than 5.5; B - slightly acid, pH 5.6 - 6.5; C - neutral, pH 6.6 - 7.5; and, D - alkaline, pH greater than 7.5.

Variation of Soil pH

Soil pH will vary from field to field and even within fields; but in the short term, very little change will occur from year to year.

The eastern portion of Saskatchewan was found to have a higher average pH as compared with western Saskatchewan (Table 1). The main reason for this difference is due to higher carbonate contents of eastern parent materials (St. Arnaud, 1976).
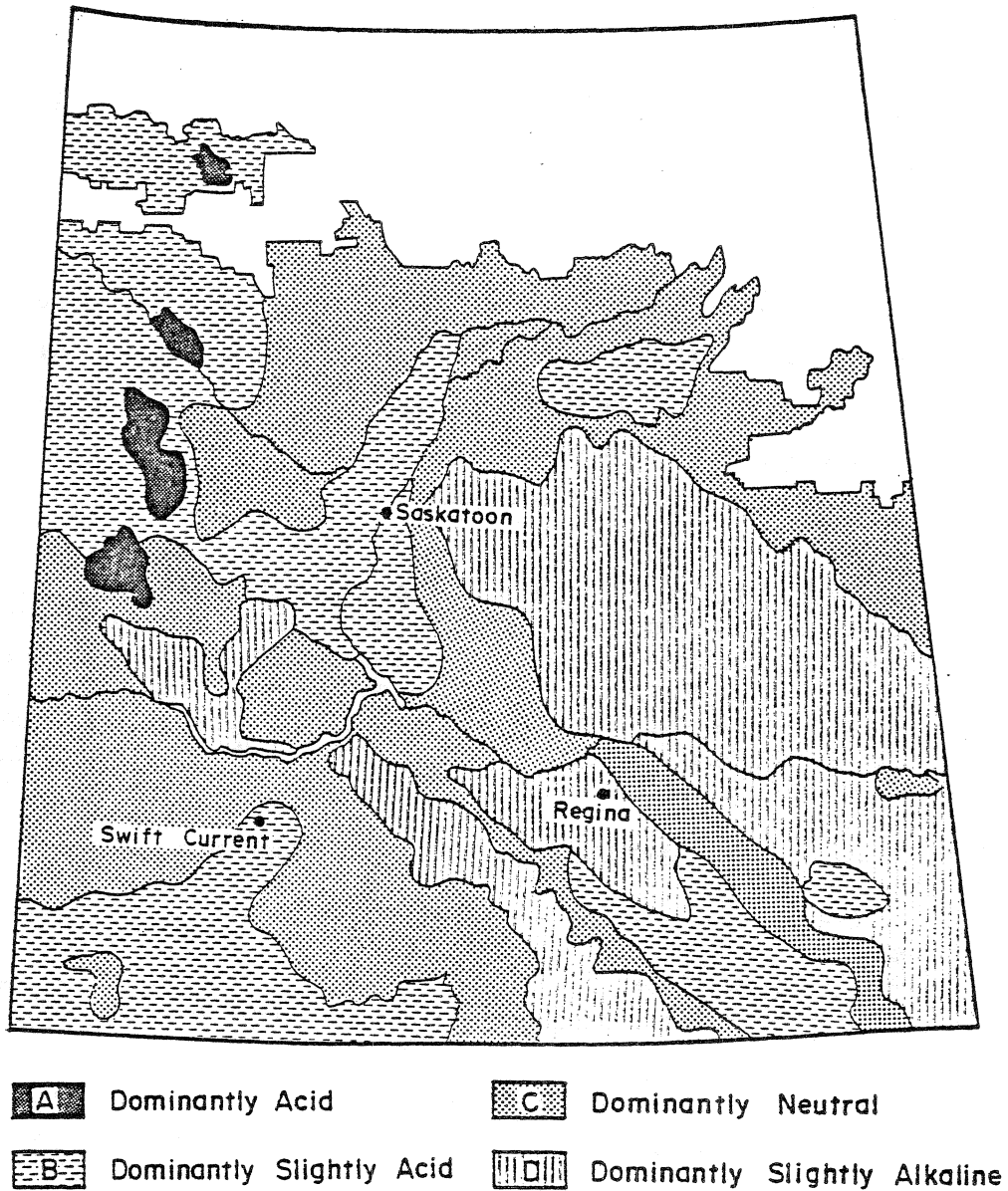
Table 1. pH Comparison of soils from East and West Saskatchewan.

Soil Type	East			West		
	n	Mean	pH Distribution	n	Mean	pH Distribution
Weyburn Orthic Dark Brown	175	7.1	$C^7D^2B^1$	107	6.9	$C^7B^2D^1$
Weyburn Calcareous Dark Brown	144	7.6	D^6C^4	59	7.3	$C^5D^4B^1$
Oxbow Orthic Black	251	7.5	C^5D^5	256	6.3	$B^5C^4A^1$
Oxbow Calcareous Black	231	7.7	D^8C^2	27	7.3	$C^6D^3B^1$
Waitville Orthic Gray Luvisol	24	7.0	$C^5B^3D^2$	96	6.3	B^6C^4

* East refers to soils sampled east of the 3rd meridian and west refers to soils sampled west of the 3rd meridian.

There are also pH differences between soil orders. Solonetzic soils such as Echo, Robsart, Rosemae and Waseca generally have a lower average pH than associated Chernozemic Soils (Table 2). This trend is independent of whether the soils are located in the east or the west. The average pH of Luvisolic Soils (Loon River and Waitville) does not differ greatly from those of associated Chernozemic soils.

Surface pH of Saskatchewan Soils

A Dominantly Acid		**C** Dominantly Neutral	
B Dominantly Slightly Acid		Dominantly Slightly Alkaline	

A more detailed map and brochure may be obtained from the Saskatchewan Institute of Pedology, University of Saskatchewan, Saskatoon, S7N 0W0.

Table 2. pH Comparison of Soil Orders.

Soil Type	n	Mean	pH Distribution
Haverhill Orthic Brown	340	6.7	$C^5B^4D^1$
Echo Solonetz	27	5.8	$A^4B^4C^2$
Robsart Solod	32	5.7	$B^5A^4C^1$
Regina Orthic Dark Brown	39	7.1	$C^4D^4B^2$
Weyburn Orthic Dark Brown	107	6.9	$C^7B^2D^1$
Rosemae Solonetzic	21	6.1	$C^7B^2A^1$
Oxbow Orthic Black	256	6.3	$B^5C^4A^1$
Waseca Solonetzic	41	5.6	B^6A^4
Whitewood Orthic Dark Gray	119	6.6	$C^5B^4D^1$
Loon River Orthic Gray Luvisol	26	5.8	B^8A^2
Waitville Orthic Gray Luvisol	96	6.3	B^6C^4

Variability of soil pH within a field will also be affected by the type of landscape. Hummocky and rolling landscapes will have larger areas of Calcareous soils on the upper slope positions. These soils have a high pH due to incorporation of alkaline parent material with the thin surface soil (Table 3). Soils on the mid and lower slopes (Orthic and Eluviated soils) will have lower pHs as the surface horizons are much thicker.

Table 3. pH Comparisons of various slope positions

Slope Position	Soil Series	n	Mean	pH Distribution
Upper	Weyburn Calcareous	144	7.6	D^6C^4
Middle	Weyburn Orthic	175	7.1	$C^7D^2B^1$
Lower	Weyburn Eluviated	18	6.8	C^7B^3
Upper	Oxbow Calcareous	231	7.7	D^8C^2
Middle	Oxbow Orthic	251	7.5	C^5D^5
Lower	Oxbow Eluviated	25	7.4	$D^6C^3B^1$

Acid Soils

Soil acidity may only be corrected by addition of liming materials that will neutralize the acidic ions in the soil. The most common liming material is ground limestone or calcium carbonate ($CaCO_3$). Depending on how acid a soil is, it may require from 2 - 10 tonnes per hectare to raise the soil pH to an acceptable value (pH 6.5). An application of lime will probably last 20 years or more under climatic conditions present in acidic areas of Western Saskatchewan. Areas on the map having dominantly A (pH < 5.5) soils may require liming if sensitive crops such as alfalfa or sweet clover are to be grown. Within the "A" group of soils, very limited areas may require liming for maximum yields of barley, wheat or canola (Ukrainetz, 1973). Detailed soil testing, by a soil testing laboratory, would be necessary to determine the lime requirement to raise the soil pH.

Table 4. Areal Extent of pH Classes.

Class	pH Range		Hectares
A	less than	5.5	1,143,000
B	5.6 -	6.5	6,637,000
C	6.6 -	7.5	12,427,000
D	greater than	7.5	7,181,000
Variable River Valleys			969,000
Total			28,357,000

Alkaline soils

Alkaline soils are soils with a pH greater than 7.5. Many of the alkaline soils (D group) are among the most productive soils in Saskatchewan. The reason for this is because they generally occur in the more humid part of the province and have higher organic matter contents. Less productive alkaline soils occur on eroded knolls where soil erosion has removed fertile topsoil and exposed the underlying alkaline parent material. These soils often occupy 20% to 40% of the area on hummocky or rolling landscapes in Central or Eastern Saskatchewan. The degradation of some herbicides may be prolonged in soils with alkaline pH values thereby causing damage to sensitive crops in subsequent years.

Acidification of Soils

Natural activities such as leaching by rain water and microbial activity are lowering the pH of most soils very slowly. Some of man's activities can also add to the acidification process. Addition of ammonium and/or certain sulfur fertilizers will add small amounts of acid to the topsoil. Atmospheric emissions of sulfur and nitrogen from the burning of fossil fuels also add small amounts of acid in the form of acid precipitation (Hammer, 1980). Most soils in Saskatchewan are able to absorb some amounts of an acid without showing any change in soil pH. Alkaline soils with high clay and/or organic matter are very resistant to acidification (Wang and Coote, 1981). Soils which are slightly acid, low in organic matter, and/or clay are most sensitive to additions of acid.

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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 (Helmeczi 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^{-1} along with 45 kg ha^{-1} of 11-51-0 fertilizer. At the Kernen site, trifluralin was applied, incorporated (0.1 m), and wheat seeded (84 kg ha^{-1}) on May 25, 1982. Fertilizer (11-55-0) was applied at 56 kg ha^{-1}.

Bulk surface soil samples were taken throughout the growing season from the control and high trifluralin treated (Scott: 1.0 kg ha^{-1}, Kernan 1.12 kg ha^{-1}; 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.[1]

Soil association	Site in Saskatchewan	Organic[2] matter	Lime[2]	Texture[2]	pH	Electrical conductivity (mmhos cm^{-1})	Nutrient levels (ppm)			
							NO_3-N	P	K	SO_4-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

[1] Data determined by the Saskatchewan Soil Testing Laboratory, Saskatoon.
[2] 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 kg m^{-3} and 1100 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° C day and 8 hour, 17° 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 15 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 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 mg kg^{-1}, based on 3300 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 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° C and a night temperature of 17° 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 mg L^{-1}) and tetracycline (5.0 mg L^{-1}) for actinomycetes. All media were incubated at 25° C and counts taken after four to nine days. All media components were Difco products (Difco Laboratories, Detroit, Mi.).

Nitrifying (Nitrobacter sp.) and denitrifing 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° C using nitrite medium in test tubes and the reagents described by Alexander and Francis (1965).

Counts of fungi, bacateria and actinomvcetes 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 um 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 H_2O 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° 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 and 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 1.39×10^4, 3.86×10^6, and 7.96×10^5 g^{-1} soil (oven dry wt) as compared to counts of 1.15×10^4, 9.61×10^6, and 9.21×10^5 g^{-1} soil, respectively, for the Kernen 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 Kernen 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.
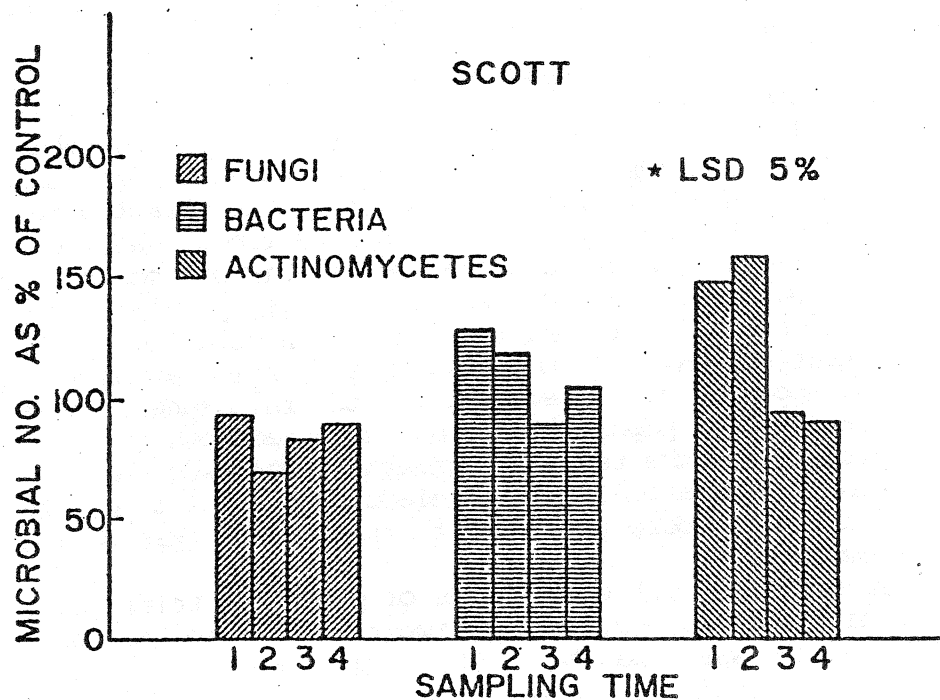

Fig. 1. Effect of trifluralin on microbial populations in the Scott bulk field soil; sampling time 1, day of herbicide incorporation; 2, two weeks; 3, six weeks; 4, ten weeks post herbicide incorporation.

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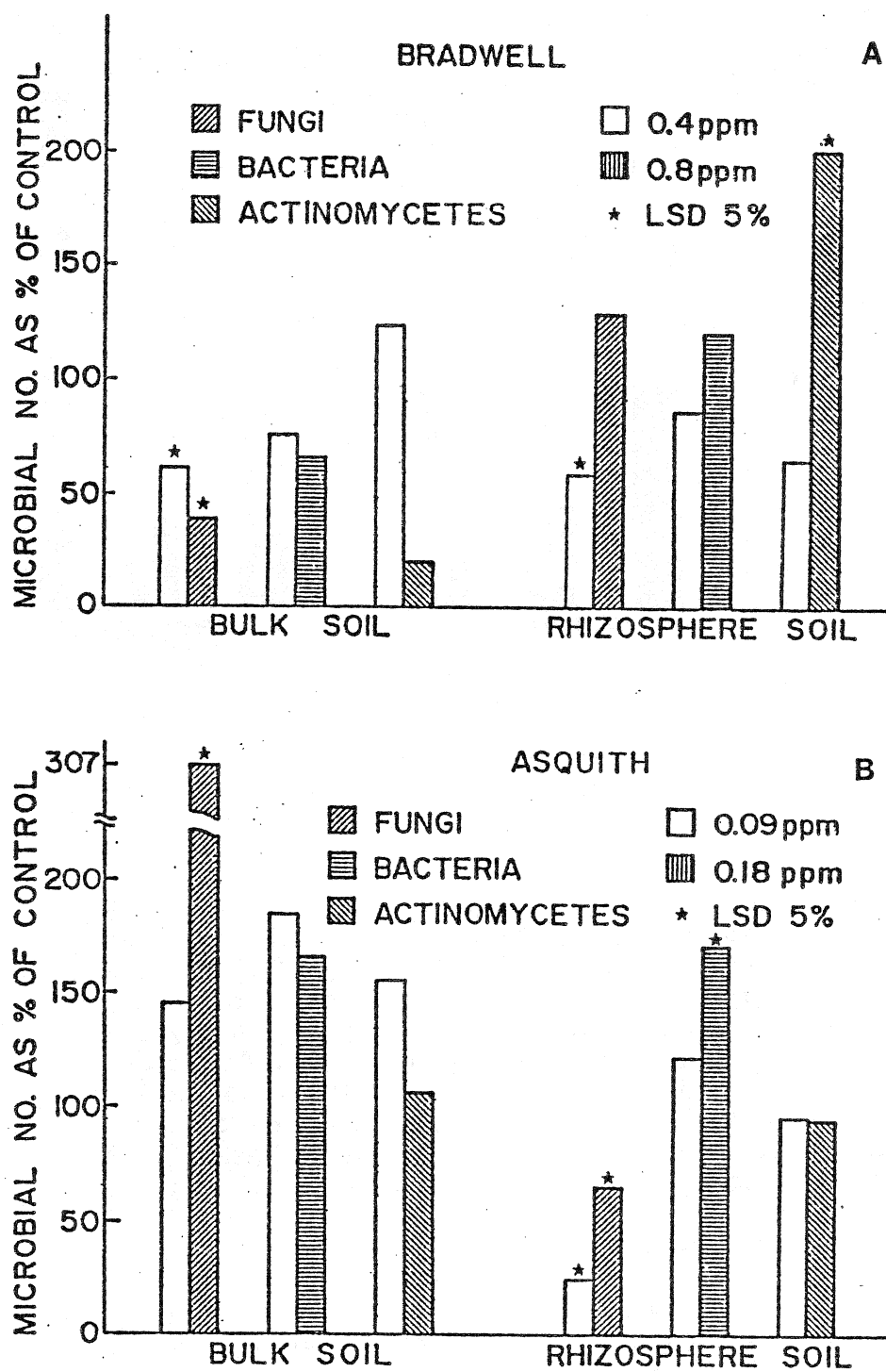
Fig. 2. Effect of trifluralin on microbial populations in the bulk and rhizosphere soil of 9 day old wheat plants grown in a growth chamber.

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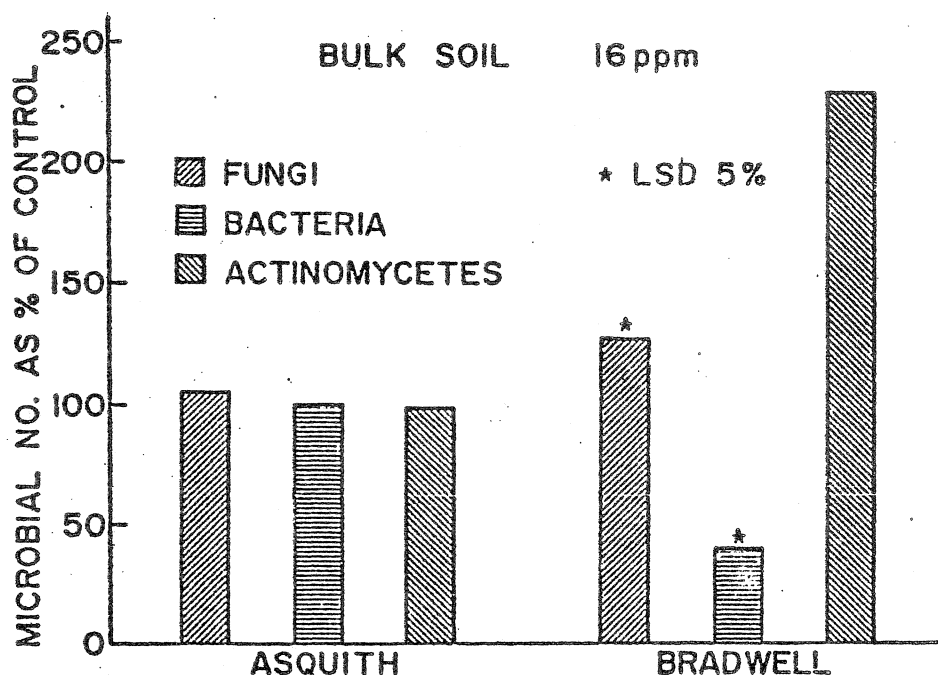

Fig. 3. Effect of high trifluralin rate (16ppm) on microbial populations in growth chamber soil after 9 days.

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_2 RESPIRATION:

The overall microbial activity as measured by CO_2 evolution was not affected by high rates of trifluralin in the Bradwell soil (Fig. 4). Added wheat straw caused a large increase in CO_2 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_2 evolution and N-mineralization. Similar results for

CO_2 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.
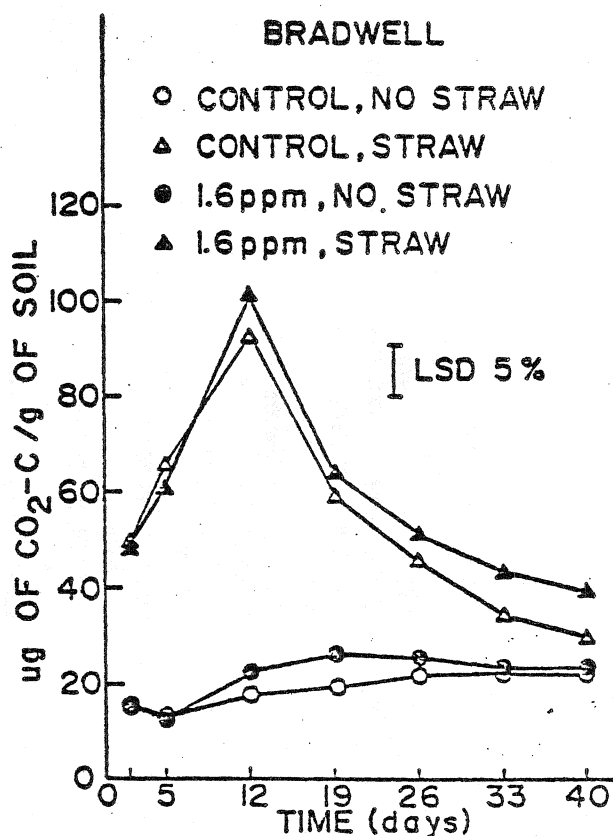

Fig. 4. Effect of trifluralin on microbial respiration in straw amended and non-amended soil.

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 medium[1]	Trifluralin Concentration[2] (ppm)					
		400	4,000	16,000	25,000	50,000	100,000
Fungi							
Absida glauca	CZD	-[3]	-	+	NT[4]	NT	NT
Aspergillus fumigates	CZD	-	-	-	-	+	++
Chrysosporium pannorum	CZD	-	-	-	+	+	++
Chrysosporium sp.	CZD	-	-	-	+	+	++
Cladosporium herbarum	CZD	-	-	-	+	+	+
Fusarium culmorum	CZD	-	-	+	+	+	++
Fusarium epishaeria	CZD	-	-	-	-	+	+
Fusarium roseum	CZD	-	-	-	+	+	++
Gliocladium roseum	CZD	-	-	-	-	+	++
Mortierella isabellina	CZD	-	-	-	-	-	-
Mortierella nana	CZD	-	-	+	++	++++	+++++
Mucor silvaticus	CZD	-	-	-	+	+	++
Paecilomyces carneus	CZD	-	-	-	+	+	+
Paecilomyces marquandii	CZD	-	-	+	+	+	++
Penicillium frequentans	CZD	-	-	-	-	-	-
Penicillium nalgiouensis	CZD	-	-	-	-	-	-
Sporotrichum carnis	CZD	-	-	+	+	++	++++
Trichoderma hamatum	CZD	-	-	+	+	+	++
Trichoderma viride	CZD	-	-	-	-	+	++
Zygorrhynchus vuilleminii	CZD	-	+	++	+	+++	+++
Bacteria							
Agromyces ramosus ATCC 25173	HI	+++	+++++	+++++	+++++	+++++	+++++
Arthrobacter globiformis ATCC 8010	NA	-	+	++	NT	NT	NT
Arthrobacter oxydans ATCC 14358	NA	-	+	+++	NT	NT	NT
Azospirillum brasilense ATCC 29145	CCM	-	-	-	-	-	-
Azotobacter chrococcum ATCC 9043	BA	-	-	-	-	-	-
Azotobacter vinelandii ATCC 12837	BA	-	-	-	-	-	-
Bacillus megaterium	NA	+	+	+	++++	+++++	+++++
Ensifer adhaerens ATCC 33212	NA	s	ss	ss	sss	ssss	sssss
Ensifer adhaerens Strain SA	NA	-	-	-	-	-	-
Ensifer adhaerens ATCC 33499	NA	s	s	ss	ss	ssss	sssss
Enterobacter aerogenes	NA	-	-	-	-	-	+
Micrococcus flavis	NA	-	-	+	NT	NT	NT
Pseudomonas aeruginosa	NA	-	-	-	-	-	-
Pseudomonas flourescens	NA	-	-	-	-	-	-
Rhizobium leguminosarum 128C30	TY	-	-	-	+	++	+++
Rhizobium leguminosarum 128C52	TY	-	-	-	+	++	+++
Rhizobium leguminosarum 128C79	TY	-	-	-	+	+	++
Rhizobium meliloti NRG185	TY	-	+	++	+++	+++	+++
Rhizobium meliloti NRG9930	TY	-	-	-	++	+	+
Rhizobium trifolii 14480	TY	-	-	-	-	+	++
Rhizobium trifolii TAI	TY	+	+	++	++	++	+++
Actinomycetes							
Streptomyces sp.	NA	-	+	++	++	+++	+++
Streptomyces sp. A-12	NA	-	+	+	NT	NT	NT
Streptomyces sp. A-16	NA	-	-	+	++	+++++	+++++
Streptomyces sp. S-34	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 < 20 mm; +++++, > 20 mm; s.

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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