EFFECTS OF LONG-TERM USE OF A COMMON PHENOXY HERBICIDE ON THE MICROBIOTA OF A BLACK CHERNOZEM

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INTRODUCTION

The development of the phenoxy herbicides during the early 1940's heralded the advent and rapid adoption of chemical weed control in Europe and North America. Ester and amine formulations of 2,4-D [(2,4-dichlorophenoxy) acetic acid] and MCPA [(4-chloro-2-methylphenoxy) acetic acid] were first tested in Canada during 1945, and because they selectively killed broad-leaved weeds while allowing the cereals to grow relatively unharmed, by 1950 almost 15 million acres of western Canadian cereal crops were being sprayed annually (Smith, 1982a). In recent years, more than 50% of prairie cropland is routinely treated with phenoxy herbicides each year (Smith et al., 1981).

The present and near future trend toward less soil tillage and extended crop rotations will necessitate an even greater use of chemical weed control (Campbell and Biederbeck, 1980). The increasing use of herbicides may eventually result in alteration of the biological equilibrium over the short- or long-run depending on the persistence of the herbicides or their metabolites. Although MCPA, like 2,4-D, is readily degraded by some soil bacteria under favorable moisture and temperature conditions and although field studies in Saskatchewan have confirmed that applications made in May or June are not carried over to the next crop year (Smith, 1982a and 1982b) some environmentalists are still concerned about the possibility of long-term detrimental effects on the soil biosphere due to application of these herbicides for almost 40 consecutive years. Numerous studies have been conducted on the effect of herbicides, including the phenoxies, on soil microbial populations and activity, but most have been done in the laboratory or greenhouse, many using unrealistic herbicide rates and when field studies were carried out they were mostly short-term (Simon-Sylvestre and Fournier, 1979).

The existence of long-term 2,4-D and MCPA plot experiments at Indian Head, Saskatchewan, presented an ideal opportunity to check for latent soil biological effects of these two very commonly used phenoxy herbicides. The rather minor effects of long-term use of amine- and ester-forms of 2,4-D at Indian Head have already been reported at a herbicide workshop (Biederbeck and Campbell, 1984). Thus the objective of the investigation, reported here, was to determine whether many consecutive years of MCPA application at normal field rates have affected soil microbial populations and biochemical activities that are essential to the maintenance of soil fertility.

MATERIALS AND METHODS

The field experiment at the Experimental Farm was established on Indian Head clay (Orthic Black Chernozem) in 1947 with a 3-year rotation of wheat, wheat, summerfallow. Originally, the herbicide treatments consisted of applications of amine, ester and sodium salt formulations of 2,4-D only. In 1953, the sodium salt of 2,4-D was dropped from the experiment and replaced with MCPA (McCurdy and Molberg, 1974). The herbicide was applied at two rates: a low rate of 0.42 kg a.i./ha for the control of annual broad-leaved weeds, and a high rate of 1.12 kg a.i./ha designed to control perennial weeds. The MCPA formulation used varied: during the first 7 years only an ester was used, then the amine form was used in some years and an ester in others, but since 1966 only the amine form has been used. Fallow plots were sprayed only once each year at the first flush of weed growth in spring. The weeds in wheat were sprayed at recommended stages of the crop. The treatments were arranged so that there was always an untreated check plot between two treated plots. All treatments were made in duplicate on plots 3 x 21 m, separated by pathways 0.9 m wide.

For the assessment of possible MCPA effects on soil microbial characteristics only the plots of wheat on stubble were sampled. As the experiment contained only two replicates per treatment each plot was sampled at three equi-distant points along the length of the plot. At each sampling point soil from the 0- to 2.5-cm and the 2.5- to 7.5-cm depth was taken with a narrow trowel to maintain minimal crop disturbance and these samples were kept separate. Samples from the 7.5- to 15-cm depth were also taken at each of the three points, but these samples were bulked into one representative sample per plot. To facilitate differentiation between short-, medium- and long-term effects of MCPA the same plots were sampled at three different dates in 1985, the year of the 33rd consecutive annual application of MCPA. They were first sampled June 11, i.e., 1 year after the 32nd annual application and 1 week before the current season's application. This sampling was timed to detect possible long-term effects. Then the plots were sampled again July 3, i.e., 2 weeks after the 33rd annual application, to assess possible short-term MCPA effects. The final sampling was conducted October 10, i.e., 16 weeks after the annual application, to assess possible medium-term herbicide effects. All samples were placed in plastic bags and after returning to Swift Current they were sieved (< 2 mm) and stored field moist at 0°C until used for the various soil microbiological and biochemical analyses.

Soil microbial biomass was determined by the chloroform fumigation-incubation technique (Jenkinson and Powlson, 1976) with minor modifications as described by Biederbeck et al. (1984). Respiration was determined by measuring CO_2 evolution from 50 g subsamples wetted to field capacity (-0.03 MPa) and incubated in biometer flasks at 21°C for a total of 14 days. Numbers of aerobic heterotrophic bacteria and actinomycetes were determined by the dilution plate count method using soil extract agar and a 14-day incubation at 20°C. Filamentous fungi and yeasts were enumerated by dilution plate counts on rose bengal-streptomycin agar after 7 days at 20°C. The number of autotrophic nitrifying bacteria was estimated by a simplified MPN method (Sarathchandra, 1979) and denitrifying bacteria were enumerated by the standard MPN method (Alexander, 1965) but with the inclusion of Durham tubes for catching and scoring gaseous evolution.

RESULTS AND DISCUSSION

Herbicides with biocidal properties can produce a limited number of negative responses in reactive biological systems. In assessing the relevance of observed herbicide effects we have followed the system for evaluation of side effects of pesticides on the soil microflora, that has been developed by a group of European workers (Greaves et al., 1980) because this system appears to be rather objective and has proven very useful. According to this system one must first differentiate beteween 'reversible' and 'irreversible' negative responses occurring within the monitoring period. If the effects are reversible, as with 2,4-D and MCPA, then there are two main criteria that can be applied to all reversible negative responses:

(i) the amplitude of the 'maximal depression', and

(ii) the phase shift due to a retarded process, expressed as the 'maximal time-deficit'.

The developers of this system emphasize that from an ecological point of view, irrespective of the magnitude of depression, the delay in the re-establishment of a negatively influenced microbial population or function ranks higher in the hierarchy of evaluation criteria.

A decision on which effects might be ecologically insignificant, tolerable or even critical can only be made after the magnitude of response to the herbicide-induced stress is compared to that of naturally occurring stress situations, such as drought, flooding, freezing, etc. From studies in microbial ecology and on the consequences of naturally occurring 'catastrophes' in soil microhabitats it can be concluded that depressions of 50% or more frequently do occur under natural conditions. With regard to herbicide-induced stress a maximal depression of well over 50% coupled with a delay of up to 30 days is suggested to be of 'no ecological significance'. A similar depression with a delay of 31 to 60 days can still be grouped as 'tolerable' while delays of more than 60 days indicate 'critical' situations.

In the present study, our analyses to date are limited to the 0- to 2.5-cm soil depth as this is generally not only the site of initial herbicide contact but also the prime site of soil microbial activity (Campbell and Biederbeck, 1982).

The size of the microbial biomass was monitored because the biomass functions as a major nutrient sink during microbial growth and as a source during microbial decay. Thus changes in biomass have a direct impact on soil fertility. Just before the annual MCPA application microbial biomass in low- and in high-rate plots was only 11% and 9% lower, respectively, than that of the control plots (Fig. 1). Consequently, there was no ecologically significant long-term effect of MCPA on soil microbial biomass. Although the biomass in all plots increased by an average 40% over the next 3 weeks, despite a soil moisture decrease from 19% on June 11 to about 7% on July 3, there was also no significant short-term treatment effect from MCPA. At 16 weeks after the MCPA application there was again no significant treatment effect.

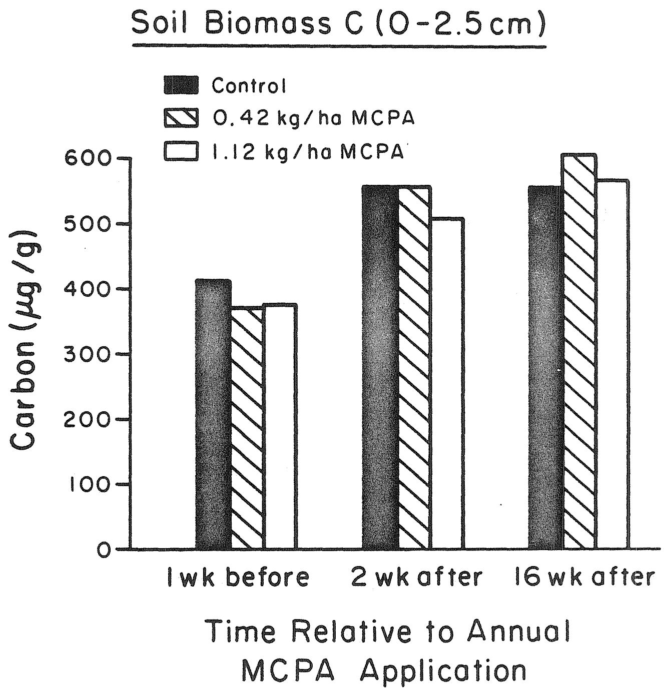

Fig. 1. Effect of long-term use of MCPA on microbial biomass in Indian Head clay.

Soil respiration was measured because it is generally considered to be a reliable index of the overall microbial activity and the rate of C-turnover in soils. At 1 week before and at 2 weeks after the annual MCPA application the respiration in low-rate treated soil was about 15% higher and in high-rate treated soil it was about 30% higher than in the untreated control soil (Fig. 2). Although these increases in CO_2-evolution rates were statistically significant they are not considered to be ecologically or agronomically relevant. Furthermore, at 16 weeks after the spraying with MCPA there was no statistically significant treatment effect at either rate.

Just before the herbicide application the population of bacteria and that of actinomycetes was about 30% and 16% lower, respectively, in MCPA-treated than in untreated soil (Fig. 3). Consequently, the bacteria/

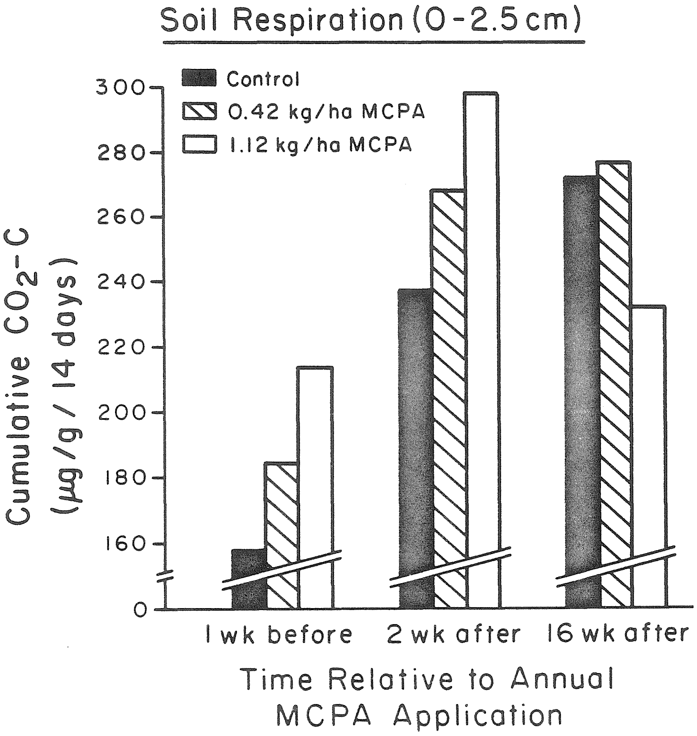

Fig. 2. Effect of long-term use of MCPA on respiration in Indian Head clay.

actinomycetes ratio was also lower in treated soil suggesting a minor qualitative shift within the large population of procaryotic soil organisms. These small changes would indicate a slight depressive long-term effect of MCPA on procaryotes, particularly some bacteria. However, by 2 weeks after the MCPA application this trend had been reversed as the bacterial populations were now 10% and 24% greater in low- and in high-rate treated soil, respectively, than in untreated soil. At this time, the actinomycete population was also increased by 22% over the control but only in the soil sprayed at the high rate of MCPA. By 16 weeks after the annual spraying bacterial numbers were essentially the same in all plots while the actinomycete population was about 18% lower in MCPA-treated soils (Fig. 3). The large increase in the populations of both types of organisms at the soil surface in all

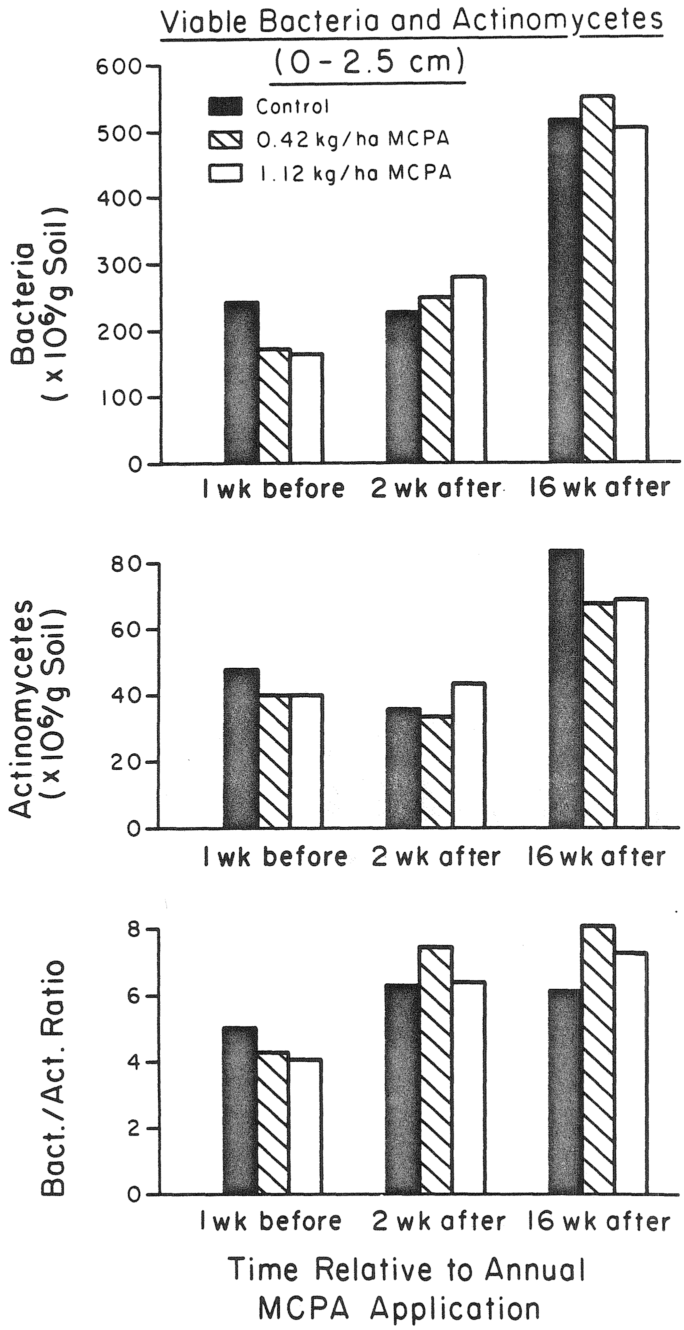

Fig. 3. Effect of long-term use of MCPA on bacteria and actinomycetes in Indian Head clay.

treatments between July 3 and October 10 should be attributed to the high substrate availability from wheat roots and straw residues during fall and is consistent with population dynamics observed in earlier studies with a Brown

Chernozem (Campbell and Biederbeck, 1982). Compared to the magnitude of these natural population changes the small depressions and stimulations of procaryotic organisms in response to MCPA application, at any of the three sampling dates, are obviously not of any ecological significance.

At all three sampling times, numbers of filamentous fungi, which refer primarily to fungal spores in the soil as they were determined by the dilution plate count technique (Parkinson et al., 1971), did not differ statistically between MCPA-treated and untreated soil (Fig. 4). However, the yeast population, which is a small but metabolically rather active component of the fungal flora near the soil surface, was statistically always significantly lower in MCPA-treated soil. This herbicide-induced reduction in the number of yeasts was on average 29%, 41% and 40% at 1 week before, 2 weeks after and 16 weeks after the annual MCPA application, respectively. But it must also be noted that the numerical reduction of yeasts was essentially the same in soil receiving the low rate and in that receiving the high rate of MCPA (Fig. 4). Due to the differential response by eucaryotic organisms to this herbicide the MCPA applications did effect a marked increase in the filamentous fungi/yeasts ratio in the short-, medium-, and long-term. However, the ecological significance of this qualitative shift in the soil fungal flora appears to be poorly defined and must be assumed to be rather minimal because major activities such as soil respiration (cf. Fig. 2) were not depressed by MCPA applications.

The nitrifiers are a very small group of chemo-autotrophic soil bacteria that are highly susceptible to environmental stress conditions and are known to be severely, albeit temporarily reduced by many herbicides including the 'phenoxies' (Chandra, 1964). Our MPN estimates show that there was no statistically significant reduction (which would require > 3.3-fold population

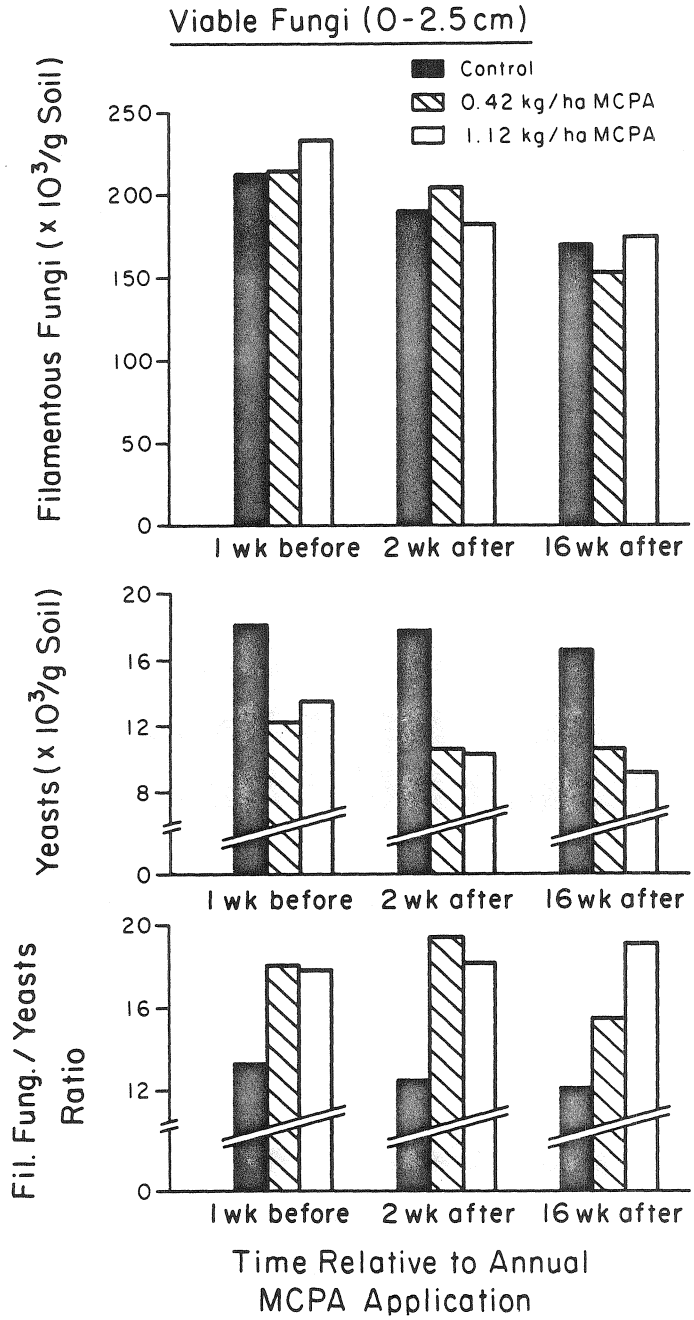

Fig. 4. Effect of long-term use of MCPA on filamentous fungi and yeasts in Indian Head clay.

decrease) of nitrifiers in low- or in high-rate treated surface soil neither at 2 weeks after the annual MCPA application nor at any of the other two sampling dates (Table 1). These results confirm that 30 or more years of consecutive treatments with MCPA have provided ample time for the indigenous nitrifier population to fully adapt physiologically to the frequent presence of this synthetic organic herbicide. The progressive adaptation of soil microorganisms to repeat applications of herbicides is a phenomenon that has been known for many years (Chandra, 1964; Teater et al., 1958).

Table 1. Effect of long-term use of MCPA on MPN estimates of nitrifiers in Indian Head clay (0-2.5 cm)

Sampling date & time relative to MCPA application	Annual rate of application kg a.i./ha	Nitrifiers, No. x 10^3/g soil*
June 11, 1985	0 (control)	1.12
i.e., 1 week before	0.42	0.65
	1.12	1.68
July 3, 1985	0	7.96
i.e., 2 weeks after	0.42	5.95
	1.12	7.93
October 10, 1985	0	8.77
i.e., 16 weeks after	0.42	11.82
	1.12	8.53

* Mean of analyses of 6 soil samples/treatment.

The denitrifiers, a larger group of heterotrophic soil bacteria were also enumerated by a selective MPN method at each of the three sampling dates. These MPN estimates (data not shown) again indicated the absence of any ecologically significant effects due to annual applications of MCPA.

CONCLUSIONS

The soil microbiological analyses conducted to date with the very surface layer of the Indian Head long-term plots show that MCPA applications at field rates have produced some statistically significant depressive effects (e.g., yeasts) and some stimulatory effects (e.g., respiration). However, all changes in microbial populations and activities were relatively small (i.e., < 40% of control) and not nearly as persistent as those observed earlier with long-term use of 2,4-D at Indian Head, particularly with the ester formulation (Biederbeck and Campbell, 1984). In addition to minor quantitative and qualitative changes we have also found evidence of complete adaptation by some groups of soil organisms (e.g., nitrifiers) to the long-term use of MCPA. Thus the general conclusion drawn from the results obtained to date is simply that 33 years of consecutive applications of MCPA at low and high field rates have not produced changes in soil microbial biomass, populations or major activities that could be considered to be ecologically or agronomically significant. This conclusion should help to alleviate the concerns of some environmentalists about the possibility of permanent damage or disturbance of the soil biosphere as a result of long-term use of readily degradable phenoxy herbicides, such as MCPA.

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