RESIDUAL EFFECTS OF TWO NONSELECTIVE HERBICIDES ON SOIL MICROFLORA AND NITROGEN TRANSFORMATIONS

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INTRODUCTION

Control of weeds in intermittently-filled irrigation ditches is essential for the optimum flow of water and the maintenance of high irrigation efficiency in central and southwestern Saskatchewan. During the last 15 years irrigation engineers and herbicide chemists have jointly conducted a series of experiments designed to test and compare chemical vs. cultural methods for the control of weed growth in irrigation ditches. These studies have shown that at high application rates, simazine (22.4 kg/ha), atrazine (22.4 kg/ha), monuron (35.8 kg/ha) and bromacil (15.2 kg/ha) can provide a simple and economic means for weed-free maintenance of irrigation ditches for several years with a single soil treatment (Grover et al. 1980). The order of persistence for these four herbicides and their efficacy for weed control over a 3-yr period was simazine > atrazine > monuron > bromacil (Korven 1975, Smith et al. 1975). However, the transport of the soil sterilants in the initial water flush and their leaching into the soil profile were shown to be potential hazards for contamination and the rating for damage to crops from irrigation and erosion in the ditches was also of the same order. In irrigation waters, simazine concentrations up to 1000 ppb were found in the initial ponding and up to 200, 130 and 15 ppb during the first, second and third irrigation, respectively.

In response to concerns about the obvious soil pollution potential associated with chemical weed control of irrigation ditches H.C. Korven established a long-term field experiment at Swift Current designed to determine the effect of various concentrations of monuron [3-(4-chlorophenyl)-1,1-dimethyl-urea] and simazine [2-chloro-4,6-bis (ethylamino)-S-triazine] in irrigation water on alfalfa production and soil fertility. The marked treatment differences observed during the first seven years of this field experiment caused us to initiate an assessment of the effects of these two herbicides on soil microbes and nitrogen transformations.

MATERIALS AND METHODS

The field experiment consisted of seven different treatments, viz., irrigation with water containing 0, 10, 100 and 1000 ppb of monuron and simazine, respectively, with four replications per treatment, arranged in a randomized block design for a total of 28 plots on a Haverhill loam soil. Each plot was 2 m X 2 m in size and was enclosed by a shallow dam to prevent lateral movement of irrigation water into the surrounding dryland. In 1972 the area was seeded to 'Roamer' alfalfa and irrigation commenced in 1973. Each plot received 400 mm irrigation water annually, distributed over four irrigations of 100 mm each in May, June, July and August. Thus the total amount of monuron and simazine applied annually by irrigation was 4.06, 0.41 and 0.04 kg active ingredient/ha at the 1000, 100 and 10 ppb concentration level, respectively. Two cuts of alfalfa were taken annually for dry matter, tissue-N and tissue-P determinations. Soils were sampled at the 0-15, 15-30, 30-60, 60-90 and 90-120 cm depths in the fall of 1976, 1978 and 1979 for analyses of plant available N and P. In October 1979 all plots were sampled at the same depth segments plus the 120-150 cm depth for herbicide residue analyses by R. Grover at the Regina Research Station.

The plots were irrigated for a total of eight years from 1973 to 1980. In the fall of 1980 the alfalfa was worked under and in spring 1981 the entire area was seeded to 'Harmon' oats as a sensitive bioassay crop and was no longer irrigated. The oats germinated well in all plots but at the 3-leaf stage all plants in the previously 1000 ppb simazine-irrigated plots died off.

For microbiological analyses four core samples to a depth of 15 cm were taken with a 2-cm diameter tube and composited for each of the four replicates from the untreated control and the 1000 ppb herbicide-treated plots. Numbers of aerobic heterotrophic bacteria, actinomycetes, filamentous fungi and yeasts in freshly sampled soil were estimated by the dilution plate count method using soil extract agar and rose bengal streptomycin agar (Biederbeck and Nicholaichuk 1976). Rhizobia were enumerated by a modified MPN plant infection technique (Biederbeck and Waker 1981). The number of autotrophic nitrifying bacteria was estimated by a simplified MPN method (Sarathchandra 1979). Soil microbial biomass carbon and nitrogen was measured by the chloroform fumigation-incubation technique developed by Jenkinson and Powlson (1976) as modified by Voroney (1979). Soil nitrogen mineralization rates were determined by incubation with sand at 25°C and intermittent leaching (Campbell et al. 1981).

RESULTS AND DISCUSSION

Effect on Yield of Alfalfa

No significant yield reductions were observed in any of the eight years of irrigation with water containing 10 ppb or 100 ppb monuron or simazine. However, irrigation with 1000 ppb herbicide enriched water caused marked yield reductions (Table 1). The crop damage effected by both herbicides was definitely cumulative as the yield reductions at the 1000 ppb level increased steadily from 10% with monuron and 25% with simazine in 1974 (data not shown) to 22% and 55%, respectively, in 1979 (Table 1).

These results indicate that the initial flush water from soil

Table 1. Effect of irrigation with monuron and simazine-enriched water on yield of alfalfa in 1979

Treatment & Herbicide Concentration ppb	Monuron			Simazine			Control
	10	100	1000	10	100	1000	0
Yield, t/ha	10.0	9.5	7.5	10.4	9.6	4.3	9.6
Yield as % of control	104	98	78	108	100	45	100

sterilant-treated ditches should not be used for irrigation because it normally contains herbicides well in excess of 100 ppb.

Residue Distribution in Soil

Each year a high proportion of these herbicides was degraded in the soil because after seven years of irrigation with water containing monuron and simazine only 4% of the total monuron applied and 11% of the simazine remained in the soil profile. The residues were not uniformly distributed throughout the profile (Table 2). At the 1000 ppb application rate 62% of the residual monuron and 69% of the residual simazine were concentrated in the top 15 cm of the profile to yield a herbicide level of 0.31 and 1.07 ppm in the surface soil (Table 2). Considerably less monuron remained in the surface soil because of its greater biodegradability and water solubility than that of simazine.

Table 2. Residual concentration of monuron and simazine in soil profile in 1979 - after 7 years of irrigation

Soil depth (cm)	Monuron (ppb)			Simazine (ppb)		
	10	100	1000	10	100	1000
0-15	5*	6±3	309±52	12±5	106±22	1074±267
15-30	5	12±12	61±30	10	40±16	238±79
30-60	5	10±12	30±36	10	10	83±28
60-90	5	5±6	35±40	10	10	43±13

* Limits of detection

Effect on Soil Microorganisms

In semiarid areas such as southwestern Saskatchewan irrigation causes large increases in microbial numbers and activities in surface soils (Biederbeck and Nicholaichuk 1976). A comparison of microbial populations in the irrigated and the dryland control plots (Table 3) indicates that all populations were increased, although not to the same extent, in response to the improved soil moisture conditions and the greater frequency of wetting and drying cycles prevailing under irrigation. While irrigation resulted in a fourfold increase in the number of total bacteria and of Rhizobium meliloti and a threefold increase in yeasts it barely doubled the populations of the more drought-tolerant organisms such as the actinomycetes and filamentous fungi.

Table 3. Microbial populations in soil of irrigated and dryland control plots

Type of organism	Irrigated alfalfa June, 1980	Dryland oats June, 1981
	— Number of organisms/g O.D. soil* —	
Bacteria X 10^6	403±14	94±9
Actinomycetes X 10^6	50±7	28±2
Rhizobium mel. X 10^4	101±18	27±3
Fil. fungi X 10^3	571±32	329±22
Yeasts X 10^3	57±3	21±1

* Mean ± standard error for 4 replicate samples from 0-15 cm depth

It was assumed that the irrigated surface soil would provide an ideal medium for the objective assessment of herbicide side effects on the microflora because it supports a highly active and diversified microbial community that should enhance herbicide degradation and provide the soil with a well-developed biological buffering capacity. To differentiate between short- and long-term effects of irrigation with monuron- and simazine-enriched water soils were sampled for microbial population estimates at different times during the irrigation season and also after herbicide additions were discontinued and the area reverted to dryland. For easy recognition of the extent of herbicide-effected population changes the population levels in the treated soils are always expressed as a percentage of the population level present in the untreated control soil at each sampling period. The hatched line across all bargraphs in subsequent figures provides a rough gauge for the ecological significance of the chemical stress since population deficits greater than 50% are reported to be indicative of a potentially 'critical' disturbance of microbial equilibria in soils (Greaves et al. 1980).

Irrigation with 1000 ppb monuron-containing water caused only minor reductions in the number of aerobic heterotrophic bacteria (Fig. 1). During the last year of irrigation the population reduction was statistically significant and increased from 25% in May to 33% in September but after irrigation was discontinued the bacteria readily regained normal population levels in the dryland soil. At the same application rate, simazine caused much larger and longer lasting reductions in the number of viable bacteria (Fig. 1). Between the third irrigation in 1979 and just prior to the first irrigation in 1980 the bacterial

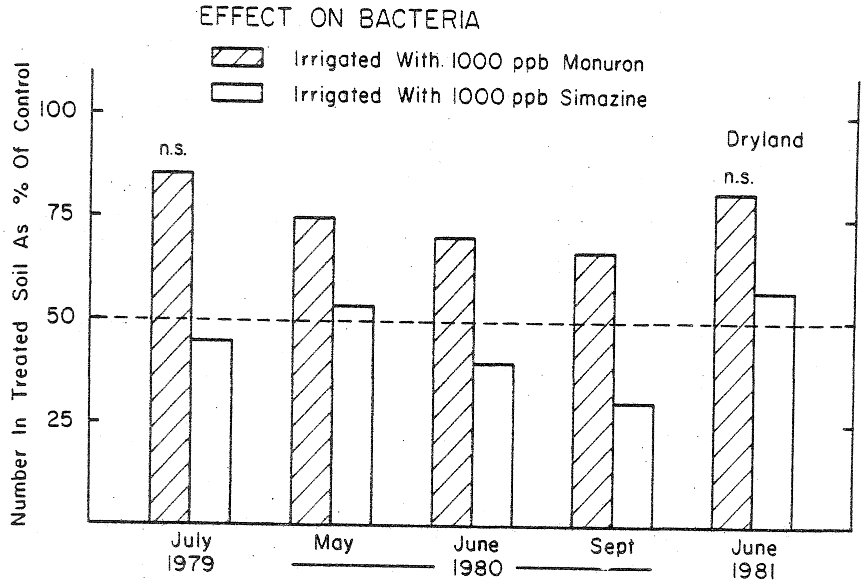

Fig. 1. Short- and long-term effects of irrigation with herbicide-enriched water on aerobic heterotrophic bacteria in soil

population deficit decreased only from 55 to 46% indicating very little recovery between irrigation seasons. During the irrigation season the chemical stress exerted by simazine was definitely of a cumulative nature as the depression of bacteria increased from 46% in May to 60% in June (i.e., after two irrigations) and 70% in September 1980 (i.e., after four irrigations). Upon discontinuance of applications the bacterial population did recover somewhat, but even nine months later it was still reduced by 42% compared to the control. This attests to the great persistence and bactericidal effects of simazine residues in soil. These drastic bacterial depressions resulting from annual applications of 4.1 kg/ha simazine via irrigation were rather unexpected as other workers have reported that simazine at field rates of 1.1, 1.5 and 4.5 kg/ha has had no effect on the number of soil bacteria (Corke and Robinson 1960, Singh 1971). Yet, the effects observed in the present field study should be of ecological significance not only because the bacterial population deficit frequently exceeded 50% but also because the time-deficit of the population depressions greatly exceeded the 60-day duration that Domsch (cf. Greaves et al. 1980) suggests is indicative of 'critical' situations.

The actinomycete population was considerably less affected by irrigation with monuron-and simazine-enriched water (Fig. 2). Monuron failed to produce any statistically significant reductions in the number of actinomycetes at any time under irrigation. But there was an unexpected 22% population depression under dryland conditions nine months after the last irrigation. This may have been due to upward

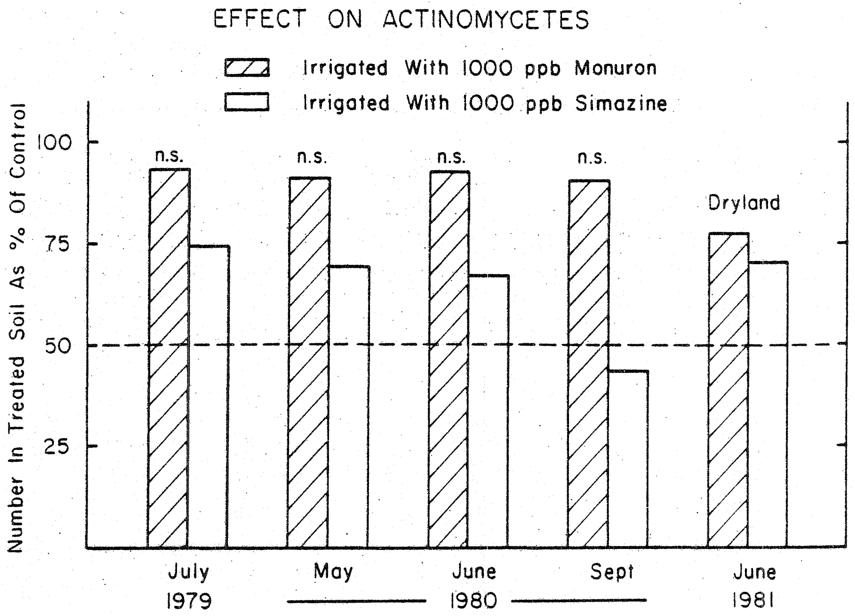

Fig. 2. Short- and long-term effects of irrigation with herbicide-enriched water on actinomycetes in soil

movement of the more soluble monuron residues within the soil profile and their accumulation near the soil surface. Simazine generally reduced the actinomycete population by one-quarter to one-third of that in the control soil except for a 56% deficit shortly after the fourth irrigation in 1980. Although the magnitude of these reductions appears to be ecologically insignificant the population remained in a repressed state for a rather long time. These effects are certainly greater than expected on the basis of reports by others that simazine, at rates of 1.0 to 7.5 kg/ha, did not inhibit the growth of soil actinomycetes (Koltcheva and Markova 1964).

Both herbicides caused drastic and lasting reductions in the number of Rhizobium meliloti in the irrigated soils (Fig. 3). Monuron depressed the rhizobia population by 53 to 68% and simazine by 86 to 94%. Obviously, the rhizobia are highly susceptible to chemical stress and their recovery from this stress was rather weak as indicated by a population deficit of > 50% even nine months after the last herbicide application. These sharp reductions in the number of viable and infective rhizobia were not unexpected since many herbicides, including phenylureas and s-triazines, are known to be harmful to rhizobia (Kecskes 1972). Decreased nodule formation and a severe reduction of symbiotic N_2-fixation would be a logical consequence of the herbicide-induced large population deficits of R. meliloti. A cursory survey of the extent of root nodulation, conducted in June 1980, did indicate that nodulation of the alfalfa plants growing in the control, 1000 ppb monuron- and 1000 ppb simazine-treated plots was generally good, poor and very poor, respectively. Thus the repression of rhizobial growth and nodule formation

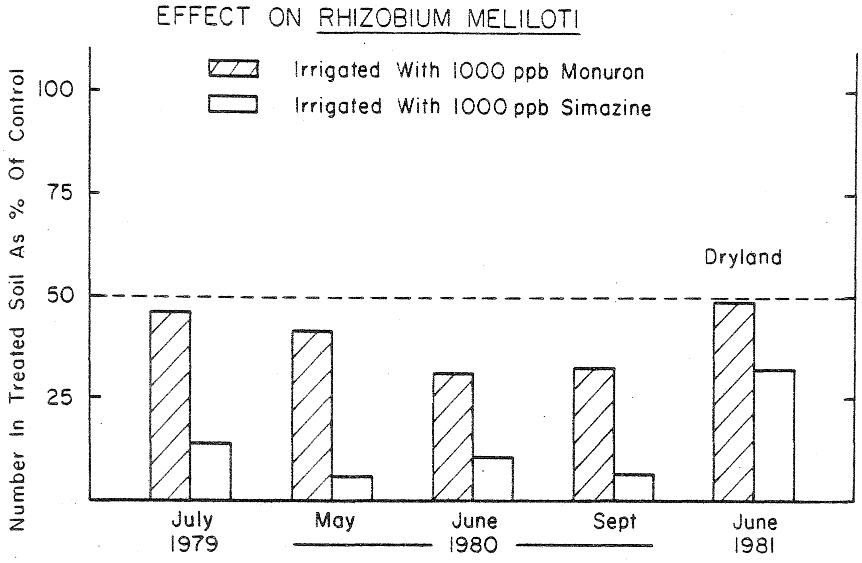

Fig. 3. Short- and long-term effects of irrigation with herbicide-enriched water on <u>Rhizobium meliloti</u> in soil

can markedly affect plant nutrition and soil fertility in an irrigated legume crop.

Numbers of filamentous fungi were 20% to 35% reduced by monuron but only during the irrigation seasons (Fig. 4). Between irrigation

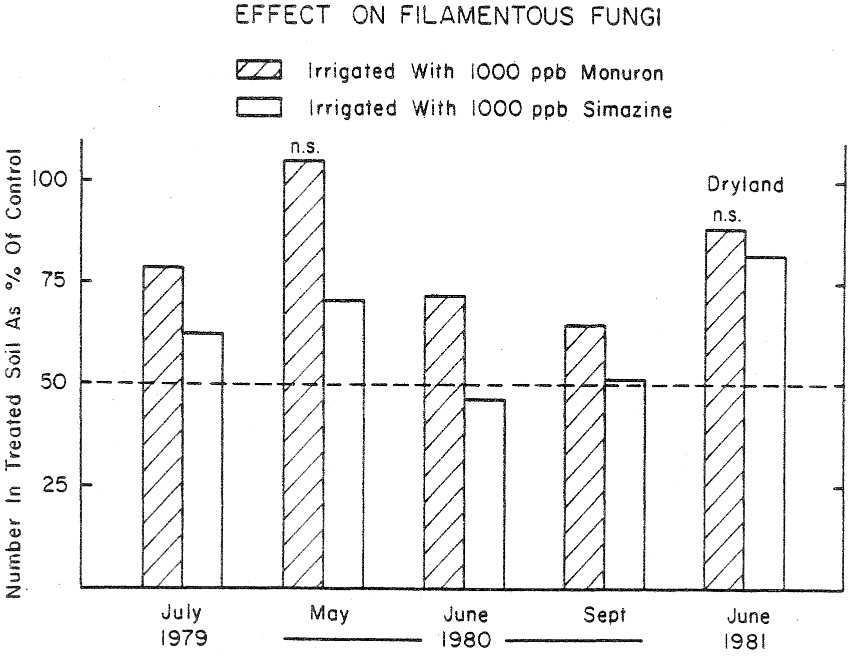

Fig. 4. Short- and long-term effects of irrigation with herbicide-enriched water on filamentous fungi in soil

seasons and after discontinuance of monuron additions they readily recovered to normal population levels. Simazine caused considerably larger population reductions, ranging from 37% to 54% during the irrigation season, and caused significant residual inhibition between irrigation seasons and after discontinuance of simazine application. It may well be that the observed reductions in the number of fungi severely underestimate the actual extent of damage to the massive mycelial growth in surface soil because our enumerations were based on soil dilution plate counts, a method that is known to estimate primarily the presence of fungal spores rather than metabolically active mycelia. Fungal spores, like other inactive forms of soil microbes, are naturally much more resistant to chemical stress than young, physiologically active cells. Our suggestion of a possible underestimate of fungal repression is supported by reports that the vegetative growth and the respiration of many soil fungi is inhibited by several triazine herbicides at normal field rates (Bakalinov 1972) and that simazine, at a soil concentration of 5 ppm, effected a strong inhibition of <u>Penicillium</u> species (Vhlahov et al. 1972) which frequently dominate the fungus flora of temperate soils.

The yeast population in the irrigated soil was more severely affected by both herbicides than previously indicated by the 'apparent' reductions of filamentous fungi (Fig. 5). Again, simazine caused significantly greater and more lasting repression. The deleterious effect of both herbicides on the yeasts was clearly cumulative as the population levels decreased progressively between May and September (Fig. 5). Although simazine did produce population deficits of a magnitude and duration sufficient to indicate a herbicide effect of ecological significance, it is suggested that this, albeit severe, repression of yeasts is of very little agricultural importance because the yeasts constitute

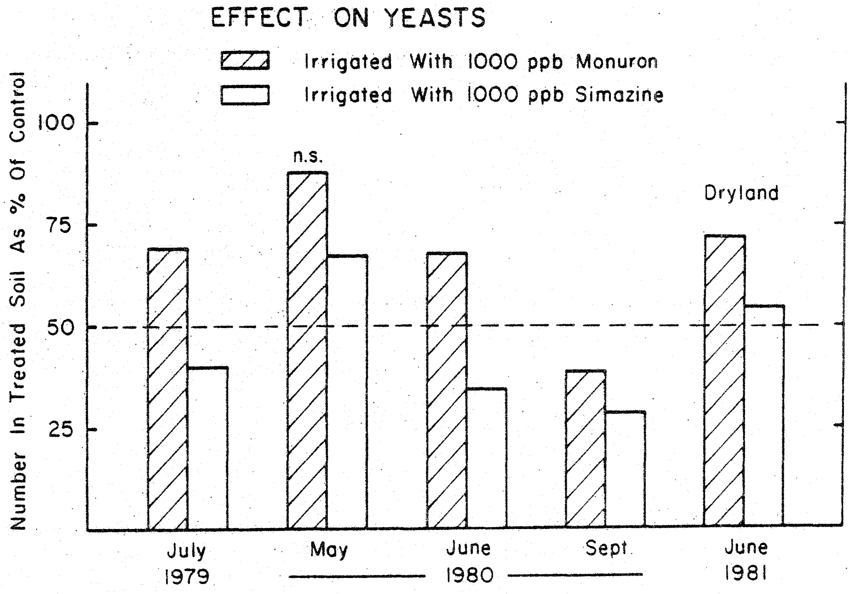

Fig. 5. Short- and long-term effects of irrigation with herbicide-enriched water on yeasts in soil

a very minor portion (usually < 10%) of the total fungi in soil and their role in soil fertility is still rather ill-defined.

The separate enumeration of different soil organisms by selective cultural methods provides estimates that can be used to gauge and compare the relative sensitivity of widely different types of microorganisms to chemical stress induced by a herbicide but they are inadequate for an assessment of the overall effect of a herbicide on the total soil microflora <u>in situ</u> at a given time. To assess the overall effect of herbicide enrichment of irrigation water the total microbial biomass, present in surface soil after the eighth irrigation season, was measured by means of a fumigation-respiration technique. The results indicate that monuron caused only a very slight reduction in biomass-C while simazine had effected a highly significant reduction of 26% (Table 4). In contrast to the changes in biomass-C, the amount of nitrogen, present in the form of biomass, was the same in all three treatments. Consequently, the biomass C/N ratio decreased noticeably from the control to the monuron-treated and particularly in the simazine-treated soil (Table 4). This narrowing of the biomass C/N ratio suggests that the vegetative growth of soil organisms with typically wide C/N ratios, such as the fungi, was inhibited considerably more by simazine than was the growth of the more N-rich bacterial cells. It also supports our earlier contention that soil dilution plate counts may have grossly underestimated the deleterious effect of simazine on mycelial growth in soil.

Table 4. Effect of 8 years irrigation with herbicide-enriched water on soil microbial biomass

	Soil biomass		
Treatment	Carbon kg/ha	Nitrogen kg/ha	Carbon/Nitrogen ratio
	Mean* ± S.E.M.		
Control	986±59	251±30	4.0±0.3
Monuron, 1000 ppb	913±32	260±15	3.5±0.1
Simazine, 1000 ppb	730±41	250±4	2.9±0.2

* Mean of 4 replicates, 0-15 cm depth, sampled September 1980

Effect on Nitrogen Transformations

Maintenance of adequate soil fertility depends largely on the ability of the microflora to continually release nutrients, primarily nitrogen, in a plant-available form from the soil organic reservoir of biomass, plant residues and humus. The results of our N-mineralization tests conducted in the laboratory indicate that the simazine-treated soil mineralized 25% less N than the control soil and 38% less N than the monuron-treated soil (Table 5). However, under field conditions simazine probably inhibited N-mineralization to a greater extent and

Table 5. Effect of 8 years irrigation with herbicide-enriched water on rate of nitrogen mineralization

Treatment	Cumulative total N mineralized during 20 weeks incubation at 25°C mean* ppm ± S.E.M.
Control	54.2±11.7
Monuron, 1000 ppb	65.5± 9.1
Simazine, 1000 ppb	40.8± 2.4

* Mean of 4 replicates, 0-15 cm depth, sampled September 1980 and leached after 2, 4, 6, 8, 12, 16 and 20 weeks of incubation

even the monuron treatment may have effected lower rates of mineralization than those in the untreated control soil because the herbicide residue would not have been leached from the soil as extensively as they were by the seven consecutive leachings during the laboratory incubation. The laboratory tests do, nevertheless, demonstrate that potential interferences in ammonification by monuron can be readily reversed while the inhibitory effects of simazine are much more persistent. The observed inhibition of ammonification by simazine is contrary to some published reports (Singh 1971), indicating that field rates of simazine had no effect on N-mineralization.

Nitrification, being a process completely dependent on the activity of a very small segment of the total soil microflora, is known to be readily inhibited by a wide variety of herbicides (Domsch 1972), including monuron and simazine (Singh 1971, Gaur and Misra 1977). However, the herbicide-induced repression of nitrification is generally reported to be rather transitory. To assess the longevity of residual effects of irrigation with monuron- and simazine-enriched water we enumerated the nitrifiers present in the dryland soil nine months after the last irrigation and herbicide application (Table 6). The results indicate that monuron residues were still causing a 42% repression of nitrifying bacteria while the residual simzaine exerted an even greater chemical stress to maintain the population deficit as high as 75%. The extent and persistence

Table 6. Residual effect of irrigation with herbicide-enriched water on nitrifiers in dryland soil

	Nitrifying bacteria	
Previous Treatment	No. X 10^3/g O.D. soil*	No. as % of control
Control	36	100
Monuron, 1000 ppb	21	58
Simazine, 1000 ppb	9	25

* Mean of 4 replicates sampled June 1981

of these nitrifier population deficits were unexpected because Gaur and Misra (1977) reported that the inhibitory effect of simazine at soil concentrations up to 20 ppm lasted only for three to four weeks. Based on the residual reduction of nitrifiers in the dryland soil (Table 6), we assumed that nitrification must have been severely inhibited during the previous irrigation seasons, which, in itself, should be considered as being beneficial rather than deleterious to soil fertility because it would reduce those N losses caused by denitrification and NO_3-N leaching.

However, the results of soil tests from 1978 and 1979 show that nitrate levels in the simazine-treated soil were always significantly higher than in monuron-treated or control soils, not only in the topsoil (Table 7), but right through the profile down to a depth of 120 cm (data not shown).

Table 7. Effect of irrigation with herbicide-enriched water on nitrate in surface soil

Sampling Time	Monuron --- 1000 ppb	Simazine ---	Control
	kg NO_3-N/ha*		
Fall 1978	16.8	19.7**	10.8
Fall 1979	10.1	22.8**	9.7

* Mean of 4 replicates from 0-15 cm depth
** Different from control at P = .05

At first glance, it certainly seems paradoxical that application of simazine, a herbicide shown to exert severe chemical stress on the microflora, including the nitrifiers, and causing a marked reduction in N-mineralization, should also produce consistently greater soil nitrate levels. At present, we have no satisfactory explanation for this discrepancy except to suggest that the severely reduced alfalfa yield, and consequently lower N-uptake, in the 1000 ppb simazine plots (Table 1) may have contributed, at least to some extent, to the distinctly higher soil nitrate levels.

Clarification of the effects of simazine on N-transformations and the sorting out of the observed contradictory phenomena requires that these field studies be followed up by model experiments under strictly controlled environmental conditions in the laboratory.

SUMMARY

Irrigation of alfalfa with water containing monuron and simazine, at concentrations similar to those normally present in the initial flushing of soil sterilant-treated ditches, resulted not only in large reductions of forage yields but also in agronomically significant disturbances of microbial equilibria and N-cycling in surface soil. The

susceptibility of soil organisms to chemical stress exerted by the herbicide residues varied widely with the extent of repression of different types of microorganisms, generally decreasing in the order: rhizobia > nitrifiers > yeasts > filamentous fungi > heterotrophic bacteria > actinomycetes. Metabolically active cells and tissues appeared to be more severely affected than spores and other resting structures. The cycling of nitrogen was disturbed indirectly by a reduction of the microbial biomass and its C/N ratio and more directly by severe inhibition of root nodulation and a marked decrease in the rates of ammonification and nitrification.

All soil biological parameters examined in this study indicated that the effects caused by simazine residues were considerably more deleterious and persisted much longer than those from residual monuron.

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