Pestic. Sci. 1979, **10**, 207–215

Effects of Sodium Azide and Methyl Bromide on Soil Bacterial Populations, Enzymic Activities and Other Biological Variables

Walter D. Kelley and Rodriguez Rodriguez-Kabana

Department of Botany and Microbiology, Auburn University Agricultural Experiment Station, Auburn, Alabama 36830, USA

(*Manuscript received 17 July 1978*)

The application of a granular formulation of sodium azide (Smite 8G), to pine nursery beds at rates of 0, 22.4, 67.2 and 134.5 kg active ingredient ha^{-1} under water seal or plastic seal, was compared over a 1-year period with methyl bromide, applied at a rate of 650 kg ha^{-1}, to determine the effects of soil bacterial populations, soil enzymic activities, development of mycorrhizal roots, weed population and incidence of root diseases. Bacterial populations at 24 days after treatment had increased in proportion to the amount of sodium azide added; however, highest numbers of bacteria were recorded from the methyl bromide-treated plot. At the last sampling date no significant differences were observed in bacterial populations among treatments. Neither sodium azide nor methyl bromide caused permanent changes in soil enzymic activities or adversely affected mycorrhizal root development on pine seedlings. Sodium azide at 134.5 kg ha^{-1} and methyl bromide both significantly decreased the number of *Cyperus* spp. plants in plots; however, the number of pine seedlings exhibiting a root disease was highest in plots receiving these treatments.

1. Introduction

Sodium and potassium azides have been proposed for use in field and nursery crops for control of fungal pathogens,[1–7] nematodes[8,9] and weeds.[3,10] As these compounds can be added easily to the soil in granular formulations, their use as substitutes for conventional fumigants such as methyl bromide has received considerable attention. Short-term effects of potassium azide on some soil enzymic activities and bacterial flora have been reported.[11] However, comparisons of the effects of azides and methyl bromide on such variables are lacking. Of particular importance would be comparison of the relative effects over extended periods. This paper presents results obtained from a 1-year comparative study on the effects of sodium azide and methyl bromide on soil bacterial populations, enzymic activities and other biological variables.

2. Materials and methods

2.1. Establishment of plots

A split plot experimental design with four replications per treatment was used in the study; plot size was 1.5 × 9.1 m. A granular formulation of sodium azide containing 8% active ingredient (Smite 8G) at rates of 0, 22.4, 67.2 and 134.5 kg ha^{-1} was applied to the plots in April with a calibrated fertiliser spreader (Gandy) and incorporated into the top 15 cm of soil with a Roto-tiller. One half of each plot (1.5 × 4.6 m) was covered with a polyethylene sheet and sealed around the edges with soil; the other half of each plot was water sealed by wetting the soil surface with an overhead irrigation system. Untreated control plots were prepared in a like manner. Methyl bromide plots were tillered, entire plots were covered with polyethylene, and methyl bromide (MC-2)

0031-613X/79/0600-0207 $02.00

was applied at a rate of 650 kg ha^{-1}. Plastic sheets were removed after 10 days. All plots were planted with slash pine seed (*Pinus elliotii* Engelm.) 2 weeks after removal of plastic sheets.

2.2. Soil sample collections

Initial soil samples were collected 10 days after treatment and subsequent samples were taken 2, 6, 10, 22, 32 and 44 weeks later. Soil samples consisted of 25 randomly collected soil cores taken from the top 15 cm of each half-plot with a soil tube and composited. The 25 cores for the methyl bromide-treated plot were from the entire plot. Each composite sample was thoroughly mixed and screened through a 4.75 mm mesh sieve prior to processing.

2.3. Laboratory analyses

2.3.1. Bacterial populations

For determining bacterial populations, soil (5 g fresh weight, approximately 8% moisture) was placed in a 500-ml Erlenmeyer flask containing sterile water (225 ml) and a magnetic stirring bar. After 2 min of stirring on a magnetic stirrer, a portion of the soil–water suspension (5 ml) was transferred to another flask containing sterile water (495 ml) and a magnetic stirring bar. After stirring on a magnetic stirrer (2 min) a Pasteur pipette with a rubber bulb attached was used to transfer one drop of the soil-water suspension to a sterile Petri dish. The suspension was stirred continuously while the drops were being removed with the pipette. A total of ten Petri dishes were prepared for each soil sample. Thornton's Standardized agar medium[12] (approximately 15 ml) was added to each of the dishes which were then incubated at room temperature (about 26 °C) for 6 days before the colonies were counted.

To determine the dilution factor for each soil–water suspension, 20 drops of the initial suspension were placed in a previously tared aluminium weighing dish, oven-dried overnight (approximately 85 °C), and the oven-dry weight of the soil/drop was calculated. The final dilution factor was then calculated on the basis of the 1:100 dilution used in the Petri dish.

2.3.2. Soil enzyme analysis

Portions of the soil samples at each sampling date were air-dried and stored at 5 °C for later use in analyses of soil enzymes. Although activities of soil enzymes were decreased by the drying process, such losses are proportional and the residual activities in the air-dried soil remain stable for long periods of time.[13]

To determine saccharase activity, air-dried soil (5 g) was placed in a 50-ml bottle and 20 ml of either water (control) or a 5% sucrose solution was added. The bottle was stoppered and placed in a 37 °C incubator for 3 h. After incubation, water (40 ml) was added, the bottle was shaken and a portion (10 ml) of the suspension was centrifuged at 5000 *g* for 20 min. One ml of the clear supernatant layer was then analysed for reducing groups by a modified Somoggi method as developed by Nelson.[14] Saccharase activity was expressed as reducing groups released (in micrograms of glucose equivalents) per gram of air-dried soil after 3 h incubation at 37 °C.

Amylase activity was determined on 5 g of air-dried soil. The substrate used was a solution of soluble potato starch (10 ml; 20 g $litre^{-1}$) and incubation time was 16 h, otherwise the procedure was as described for saccharase activity. Amylase activity was expressed as reducing groups released (in micrograms of glucose equivalents) per gram of air-dried soil after 16 h incubation at 37 °C.

To determine catalase activity, portions of the air-dried samples were pulverised with a mortar and pestle. The pulverised soil was passed through a 425 μm mesh sieve, and 100-mg portions were used to determine catalase activity as described by Rodriguez-Kabana and Truelove.[15] Catalase activity was expressed as the value of tan θ of the initial slope.

2.3.3. Seedling root evaluation

Ten randomly selected slash pine seedlings were collected from each plot just prior to harvest (February 1974) and used to evaluate development of mycorrhizal roots. Intact root systems were excised at the root collar and each root system was rated individually on a scale of 0 to 6, with 0

representing the poorest development and 6 representing the best. Three observers individually evaluated each root system; these evaluations were then averaged to obtain a final value for each seedling. Values thus obtained for the ten seedlings from each plot were averaged to obtain the final plot value.

2.4. Field observations

2.4.1. Weed populations

To determine the effect of the various treatments on weed populations in the plots, total counts of nutsedge (*Cyperus* spp.) plants were made for each plot. Nutsedge was chosen for evaluation because it was the most uniformly distributed weed in the study areas.

2.4.2. Disease occurrence

The total number of diseased and dead slash pine seedlings within each plot were counted just prior to harvest. Selected dead and diseased seedlings were taken to the laboratory and portions of these were used to isolate pathogenic fungi by standard laboratory procedures.

2.5. Statistical analysis

All data were analysed for analysis of variance; means were compared for significance by Duncan's multiple range test. All differences referred to in this paper were significant at the 1% level of probability.

3. Results

3.1. Laboratory analyses

3.1.1. Bacterial populations

By the first three sampling dates numbers of bacteria had increased in response to the amount of sodium azide added [Figures 1(a) and (b)]; this pattern was independent of the type of seal used. Plots treated with methyl bromide had the highest number of bacteria at the second sampling date [Figure 1(a)]; at this time the sodium azide-treated plots showed a near linear response to the amount added [(Figure 1(c)]. Bacterial numbers in sodium azide-treated plots and in methyl bromide-treated plots were significantly higher than those for untreated control plots at the second and third sampling dates. Bacterial numbers in plastic-sealed plots for each rate of sodium azide were generally higher than those in corresponding water-sealed plots. After 3 months there were no significant differences in bacterial numbers among the treatments.

3.1.2. Enzymic activities

Catalase activity generally increased with time throughout the study (Figure 2); however, there were no significant differences among the treatments.

Saccharase activity [Figure 3(a)] in methyl bromide-treated plots was lower than in the control plots on the first four sampling dates but these differences were not evident late in the season. In plots treated with sodium azide, saccharase activity generally followed the same trend as that in the methyl bromide-treated plot [Figure 3(b–d)].

Amylase activity in methyl bromide-treated plots [Figure 4(a)] was not significantly different from the controls until the fourth sampling date, but there was a significant decline to levels below those of the controls at the last three sampling dates. Amylase activity in plastic-sealed plots treated with 134.5 kg sodium azide ha^{-1} was significantly higher than for all other treatments under plastic on the first three sampling dates (Figure 4); no significant differences were observed between this treatment and any other sodium azide treatment on the last three sampling dates. Differences in amylase activity between plastic- and water-sealed plots treated with sodium azide were not significant. At the second sampling date (May 1973) amylase activity was increased with increasing amounts of sodium azide applied to the plots [Figure 5(a)]; however, by February 1974, there were no significant differences in amylase activity among plots receiving the various rates of sodium azide.

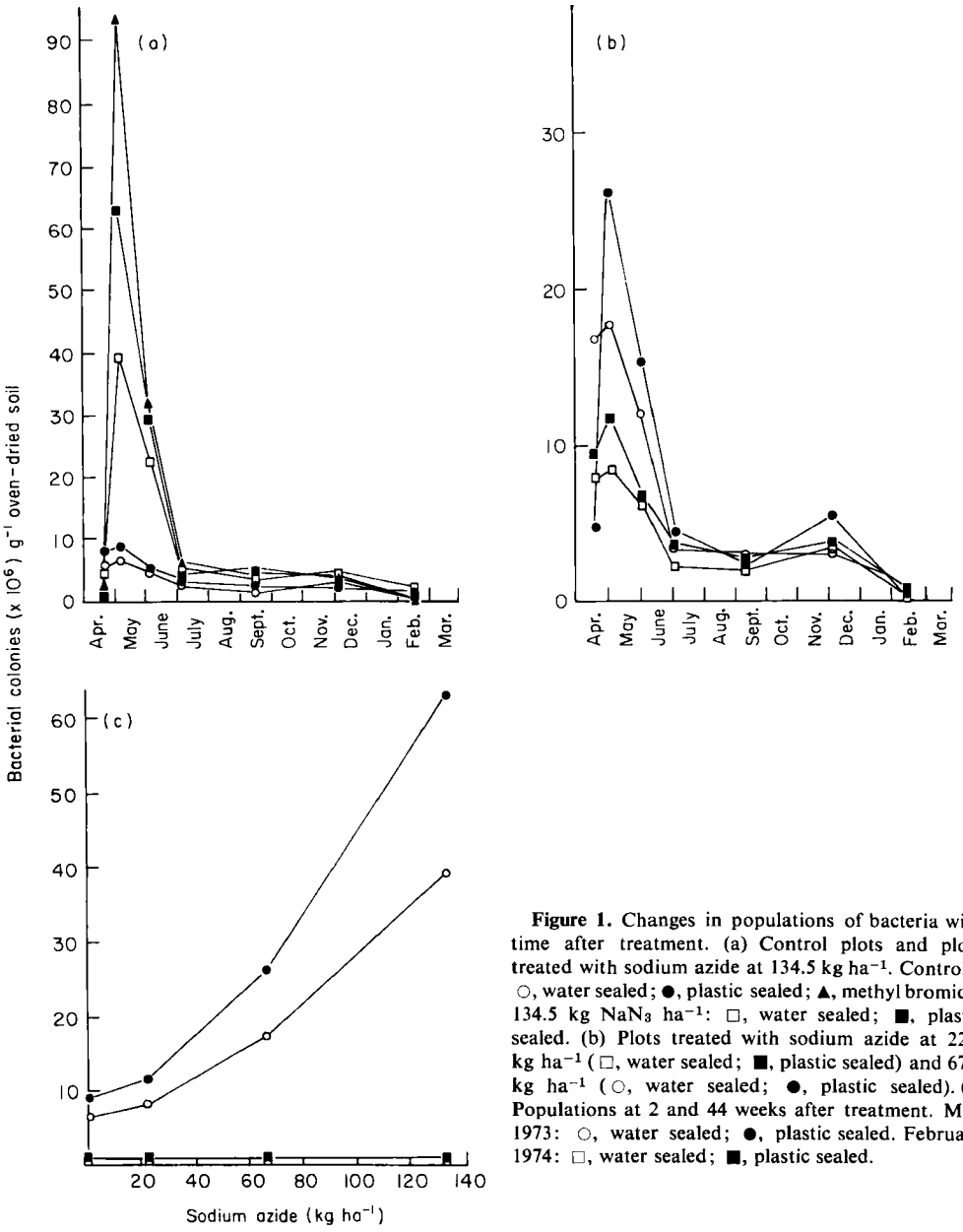

Figure 1. Changes in populations of bacteria with time after treatment. (a) Control plots and plots treated with sodium azide at 134.5 kg ha^{-1}. Controls: ○, water sealed; ●, plastic sealed; ▲, methyl bromide. 134.5 kg NaN_3 ha^{-1}: □, water sealed; ■, plastic sealed. (b) Plots treated with sodium azide at 22.4 kg ha^{-1} (□, water sealed; ■, plastic sealed) and 67.2 kg ha^{-1} (○, water sealed; ●, plastic sealed). (c) Populations at 2 and 44 weeks after treatment. May 1973: ○, water sealed; ●, plastic sealed. February 1974: □, water sealed; ■, plastic sealed.

3.1.3. Seedling root evaluations

Neither sodium azide nor methyl bromide hindered colonisation of pine roots by mycorrhizal fungi [Figure 5(b)].

3.2. Field observations

3.2.1. Weed populations

Weed counts taken 2 months after initiation of the experiment showed a decline in the number of *Cyperus* spp. in response to increasing levels of sodium azide [Figure 5(c)]. Sodium azide applied at

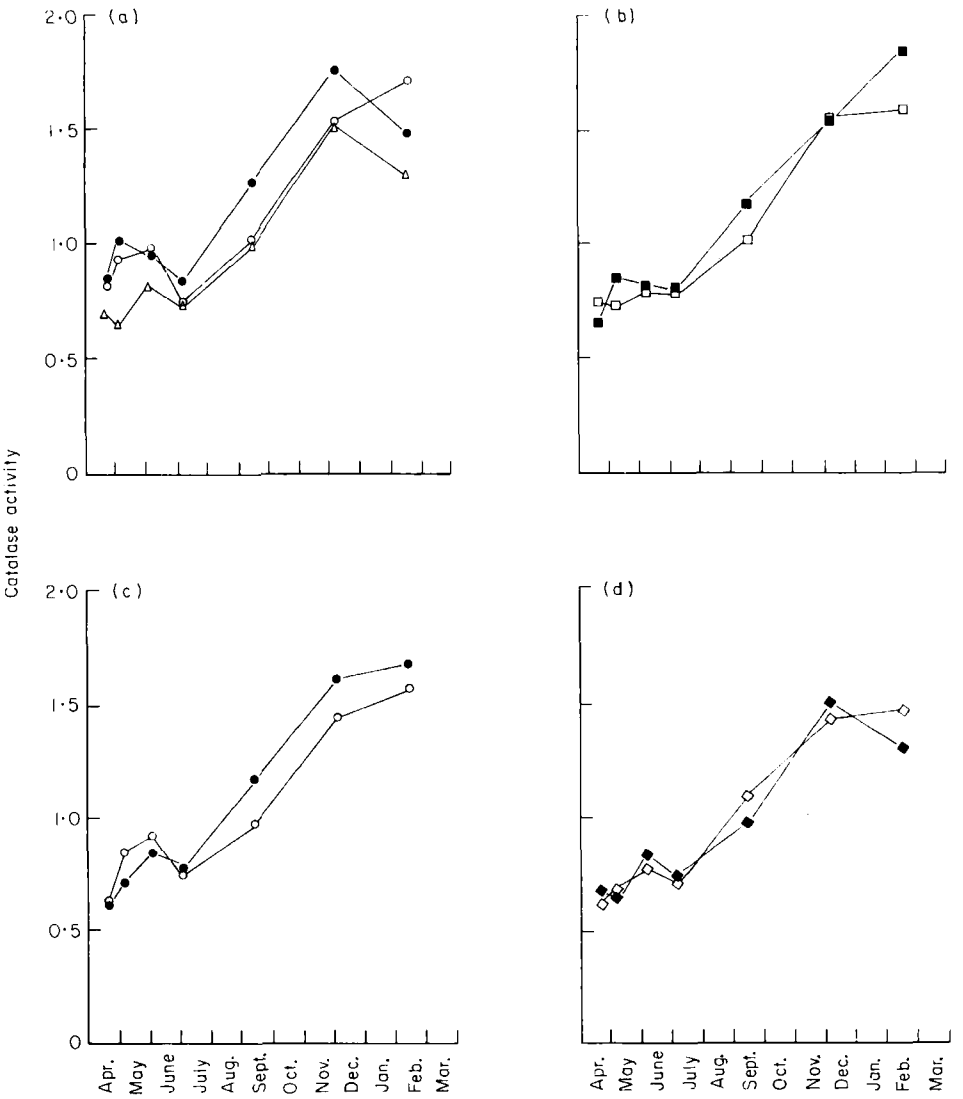

Figure 2. Soil catalase activity *vs* time after treatment. Catalase activity is expressed as the value of tan θ of the initial slope.[15] (a) Control and methyl bromide-treated plots (○, water sealed; ●, plastic sealed; △, methyl bromide). (b) Plots treated with sodium azide at 22.4 kg ha^{-1} (□, water sealed; ■, plastic sealed). (c) Plots treated with sodium azide at 67.2 kg ha^{-1} (○, water sealed; ●, plastic sealed). (d) Plots treated with sodium azide at 134 kg ha^{-1} (◇, water sealed; ◆, plastic sealed).

134.5 kg ha^{-1} almost eliminated the sedge from the plots; no significant differences in effects were observed between treatment at this rate and the methyl bromide treatment.

3.2.2. Disease occurrence

A number of pine seedlings were found just prior to harvest time dying from a vascular disease. Isolations from these seedlings yielded *Fusarium solani*. There was a significantly higher incidence of diseased seedlings in plots treated with methyl bromide, or with sodium azide at 134.5 kg ha^{-1}, than in other plots [Figure 5(d)]. The lowest incidence of disease was recorded in plots that received 22.4 kg of sodium azide ha^{-1}.

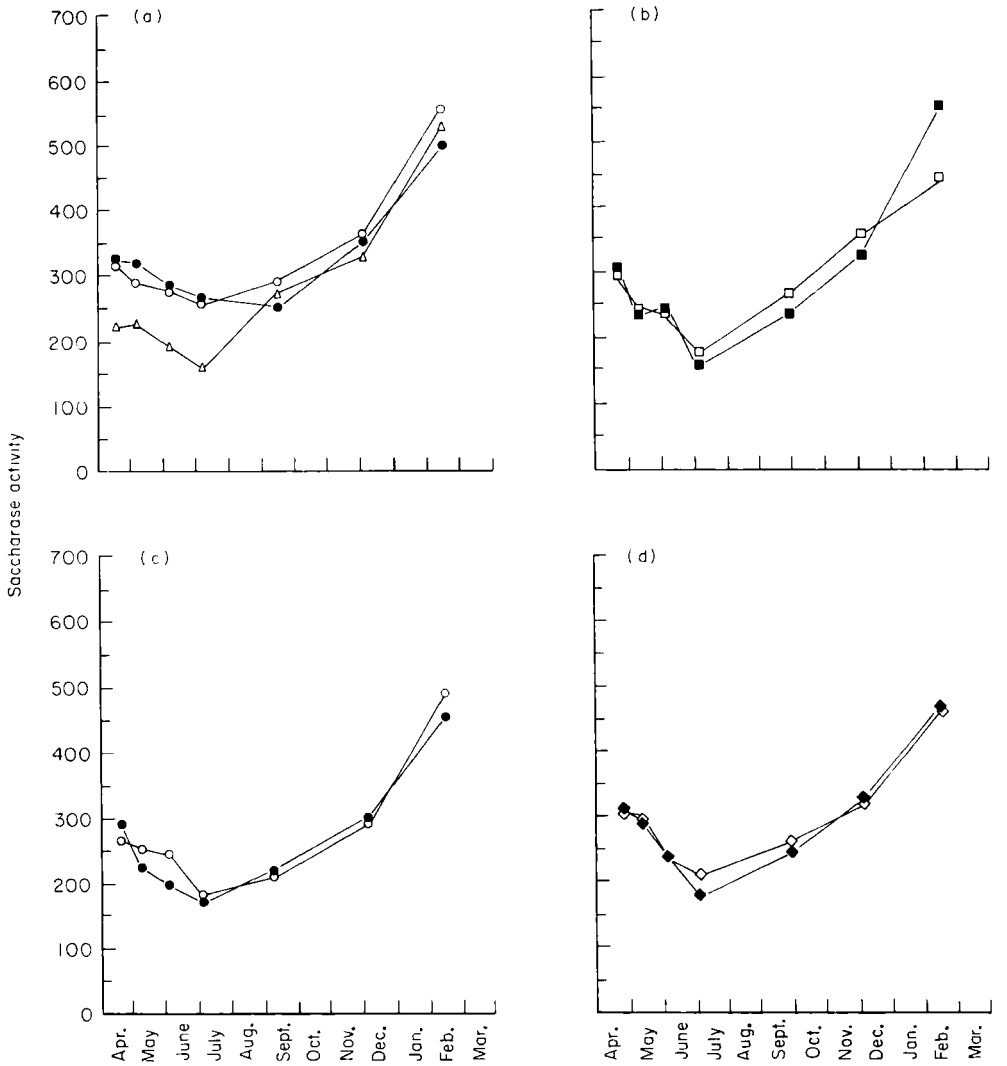

Figure 3. Soil saccharase activity *vs* time after treatment. Saccharase activity is expressed as reducing groups released (in μg glucose equivalents) per gram of air-dried soil after 3 h of incubation at 37 °C. (a) Control (○, water sealed; ●, plastic sealed) and methyl bromide-treated (△) plots. (b) Plots treated with sodium azide at 22.4 kg ha^{-1} (□, water sealed; ■, plastic sealed). (c) Plots treated with sodium azide at 67.2 kg ha^{-1} (○, water sealed; ●, plastic sealed). (d) Plots treated with sodium azide at 134.5 kg ha^{-1} (◇, water sealed; ◆, plastic sealed).

4. Discussion

The biocidal activity of sodium azide depends on the liberation of gaseous hydrogen azide (HN_3) under acidic conditions,[11,16,17] such as were present in the plots used in this study (pH 5.3). In these conditions, sodium azide can be considered as a fumigant precursor. The results obtained here show that the action of sodium azide resembled that of methyl bromide, particularly as the concentration increased, and when applied under plastic seal to minimise loss of hydrogen azide. Differences in effects of the biocides were quantitative rather than qualitative. These differences can probably be attributed to the fact that hydrogen azide is more soluble than methyl bromide in water; this property would slow the movement of gaseous hydrogen azide throughout the soil under the soil moisture conditions maintained in this study. However, when sodium azide was applied at 134.5

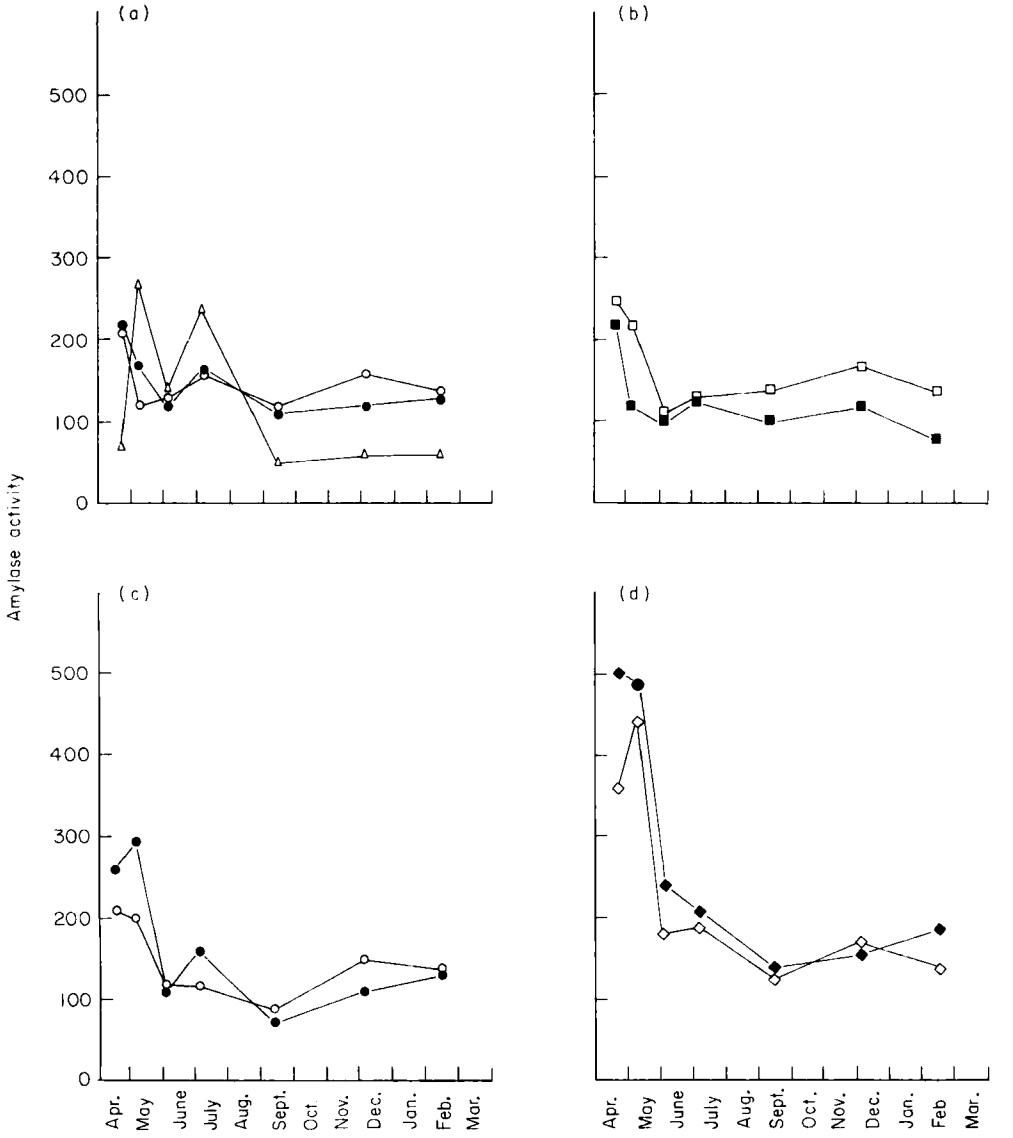

Figure 4. Soil amylase activity *vs* time after treatment. Amylase activity is expressed as reducing groups released (in μg glucose equivalent) per gram of air-dried soil after 16 h of incubation at 37 °C. (a) Control (○, water sealed; ●, plastic sealed) and methyl bromide-treated (△) plots. (b) Plots treated with sodium azide at 22.4 kg ha^{-1} (□, water sealed; ■, plastic sealed). (c) Plots treated with sodium azide at 67.2 kg ha^{-1} (○, water sealed; ●, plastic sealed). (d) Plots treated with sodium azide at 134.5 kg ha^{-1} (◇, water sealed; ◆, plastic sealed).

kg ha^{-1}, the effects of sodium azide and methyl bromide began to agree more closely. The results obtained showed that sodium azide, like methyl bromide, caused no permanent changes in soil enzymic activities or bacterial numbers. Changes in response to sodium azide additions to soil were limited to the first 3–4 months after application. This agrees with previous results obtained with potassium azide in a more limited study.[11]

Soil enzymic activities can be divided into two classes on the basis of the results. Amylase activity followed closely the changes in bacterial numbers, whereas saccharase and catalase activities did not.

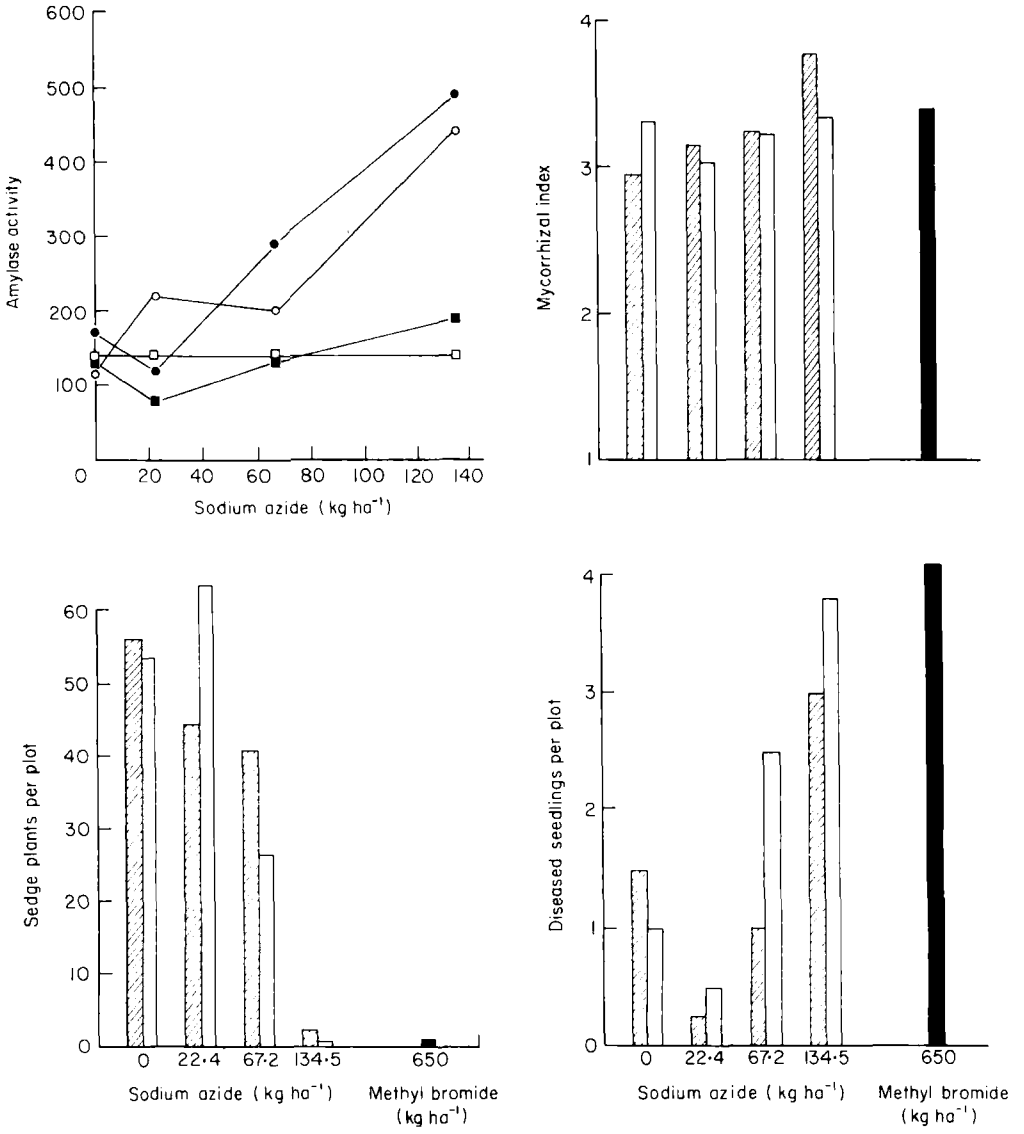

Figure 5. Effect of sodium azide and methyl bromide on: (a) amylase activity at 2 and 44 weeks after treatment. (May 1973: ○, water sealed; ●, plastic sealed. February 1974 □, water sealed; ■, plastic sealed). Amylase activity is expressed as reducing groups released (in μg glucose equivalents) per gram of air-dried soil after 16 h of incubation at 37°C. (b) Mycorrhizal formation on pine roots (▨, water sealed; □, plastic sealed; ■, methyl bromide). (c) Numbers of sedge (*Cyperus* spp.) plants in study plots (▨, water sealed; □, plastic sealed; ■, methyl bromide. (d) Numbers of diseased seedlings in the study plots (▨, water sealed; □, plastic sealed; ■, methyl bromide.

The lack of activity of sodium azide or methyl bromide on mycorrhizal development on pine roots may be attributed to the fast degree of recolonisation of the soil by ectomycorrhizal fungi after fumigation. Basidiospores of these fungi are produced in copious quantities and are easily disseminated by air, water, insects and movement of soil.[18]

The effect of sodium azide on the numbers of nutsedge agreed with what is known about the effect of this compound on other weed species;[3,10] at sufficiently high concentrations, azides have herbicidal activity comparable to that of standard herbicides.

The number of diseased seedlings in the treated plots was small in relation to the total seedling population (1–5 per 3000 seedlings per plot); however, the results are significant in one respect. Application of sodium azide or methyl bromide apparently caused an ecological shift such that either one or more pathogens was favoured, or the normal antagonists of such pathogens were reduced or eliminated. Conceivably, if this trend continued in nursery beds, where application of these compounds was routine, much more serious disease problems could develop. This aspect is receiving further attention.

5. Conclusion

Sodium azide applied under plastic-seal at 134.5 kg ha^{-1} appears to be a satisfactory substitute for methyl bromide as a soil fumigant in forest nursery beds.

References

1. Davis, J. R.; Callihan, R. H.; Groskopp, M. D. *Plant Dis. Rep.* 1972, **56**, 400.
2. Gay, J. D. *Plant Dis. Rep.* 1970, **54**, 600.
3. Rodriguez-Kabana, R.; Backman, P. A.; Ivey, H.; Farrar, L. L. *Plant Dis. Rep.* 1972, **56**, 362.
4. Rodriguez-Kabana, R.; Backman, P. A.; King, P. S. *Plant Dis. Rep.* 1975, **59**, 528.
5. Rowe, R. C.; Beute, M. K.; Wells, J. C.; Wynne, J. C. *Plant Dis. Rep.* 1974, **58**, 348.
6. Rush, M. C.; Averre, C. W.; Jenkins, S. F.; Khan, A. *Phytopathology* 1970, **60**, 1311.
7. Weaver, D. J. *Plant Dis. Rep.* 1971, **55**, 1094.
8. Birchfield, W.; Parr, J. F.; Smith, S. *Plant Dis. Rep.* 1969, **53**, 923.
9. Bradbury, F. R.; Campbell, A.; Suckling, C. W.; Jameson, H. R.; Peacock, F. C. *Ann. Appl. Biol.* 1957, **45**, 241.
10. Colby, F. *Weed Sci.* 1967, **15**, 163.
11. Kelley, W. D.; Rodriguez-Kabana, R. *Can. J. Microbiol.* 1975, **21**, 565.
12. Thornton, H. G. *Ann. Appl. Biol.* 1922, **9**, 241.
13. Skujins, J. J. Enzymes in Soil; in *Soil Biochemistry* Dekker, New York, 1967, p. 371.
14. Nelson, N. *J. Biol. Chem.* 1944, **153**, 375.
15. Rodriguez-Kabana, R.; Truelove, B. *Enzymologia* 1970, **39**, 217.
16. Adams, P. B.; Warren, H. L.; Tate, C. J. *Plant Dis. Rep.* 1971, **55**, 1077.
17. Parochetti, J. V.; Warren, G. F. *Weed Sci.* 1970, **18**, 5555.
18. Marx, D. H.; Bryan, C.; Cordell, E. *For. Sci.* 1976, **22**, 91.