The Effects of Single and Multiple Application of Benomyl on Non-Target Soil Bacteria

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INTRODUCTION

There are a number of studies dealing with the anti-fungal effects of benomyl (BOLLEN et al., 1970; EDGINGTON et al., 1971; ERWIN, 1973) but it was not until recently that its effects on soil bacteria were considered. The first bacteria studies suggested that benomyl may have little effect (STAREN et al., 1964; WENSLEY et al., 1970), however, HOFER (1971) found that benomyl reduced rates of nitrification in the soil. These results led us to investigate the effects of benomyl on naturally occurring soil bacteria, and to re-examine the influence of this fungicide on soil nitrification, using the perfusion apparatus.

MATERIALS AND METHODS

A description of the field site and the methods used to obtain and treat soil samples is presented in FOSTER (1975).

To isolate bacteria, soil samples were suspended in autoclaved tap water and through serial dilution, reduced to a power of 10^{-6}. Nutrient agar with a low carbon: nitrogen ratio and without additives was used in the isolations. Many bacteria were found to grow freely on this medium without undue interference from fungal species. Incubation was at 25°C for five days. It is appreciated that this method does not yield an accurate representation of the true soil bacteria populations, however, identical procedures were employed throughout the experiments and do allow comparisons between experimental and control plots.

In the field, two types of experiments were run. In the first, benomyl was applied once to the experimental plots and samples were taken each day for the first 10 days and then on days 12, 14, 16, 18, 20, 24, 28, and 32. In the second set of

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experiments, benomyl was added every 7 days for 11 and 12 weeks (two experiments) and samples were taken on the first 7 days and then on every third and seventh day of each week. In all cases benomyl was added in a soil drench, suspended in 10 l of water.

In the laboratory, a positive pressure soil perfusion apparatus (LEES et al., 1946; KAUFMAN, 1965) was used. Twelve units were used, and to start each experiment, 50 g of soil was dried and placed in the sample tube of each unit. The reservoirs contained 500 ml of N/50 ammonium sulphate which was perfused through the sample. The reservoirs also contained benomyl in the quantities: 0 ppm = 3 controls, 0.1 ppm = 3 experimentals, 1.0 ppm = 3 experimentals, 10.0 ppm = 3 experimentals. The perfusate was sampled on days: 1, 2, 3, 5, 7, 10, 14, 18, 22, 26, and 30. The nitrate nitrogen was measured volumetrically by the method described by CHASE (1948).

RESULTS

SINGLE APPLICATION FIELD EXPERIMENTS

In this series, experiments 1 and 2 (Fig. 1) involved the application of recrystallized benomyl at the concentration of 0.5 g/m^2 dispersed in water. In both there was no significant difference (α = 0.05) between control and experimental plots in the 24-hour sample but there was a significant difference between the readings at 48 hours. However, though these significant differences continued for several days, the total numbers of bacteria in the experimental plot returned to those in the control plot within 14 to 16 days. These results suggest that benomyl reduced isolations of viable forms of bacteria from the soil, but that the effect was somewhat shorter-lived than that upon fungal populations (FOSTER, 1975). In experiment 3 (Fig. 1) Benlate, the commercial form of the fungicide was applied so as to produce the same concentration of active material used in experiments 1 and 2. The reduction of bacterial isolations from the experimental plot was, however, considerably greater than in the two previous experiments. This was probably associated with the uniformity of the suspension prepared from the commercial product compared with that prepared from recrystallized benomyl. Significant reductions (α = 0.05) of isolations from the experimental plot, compared

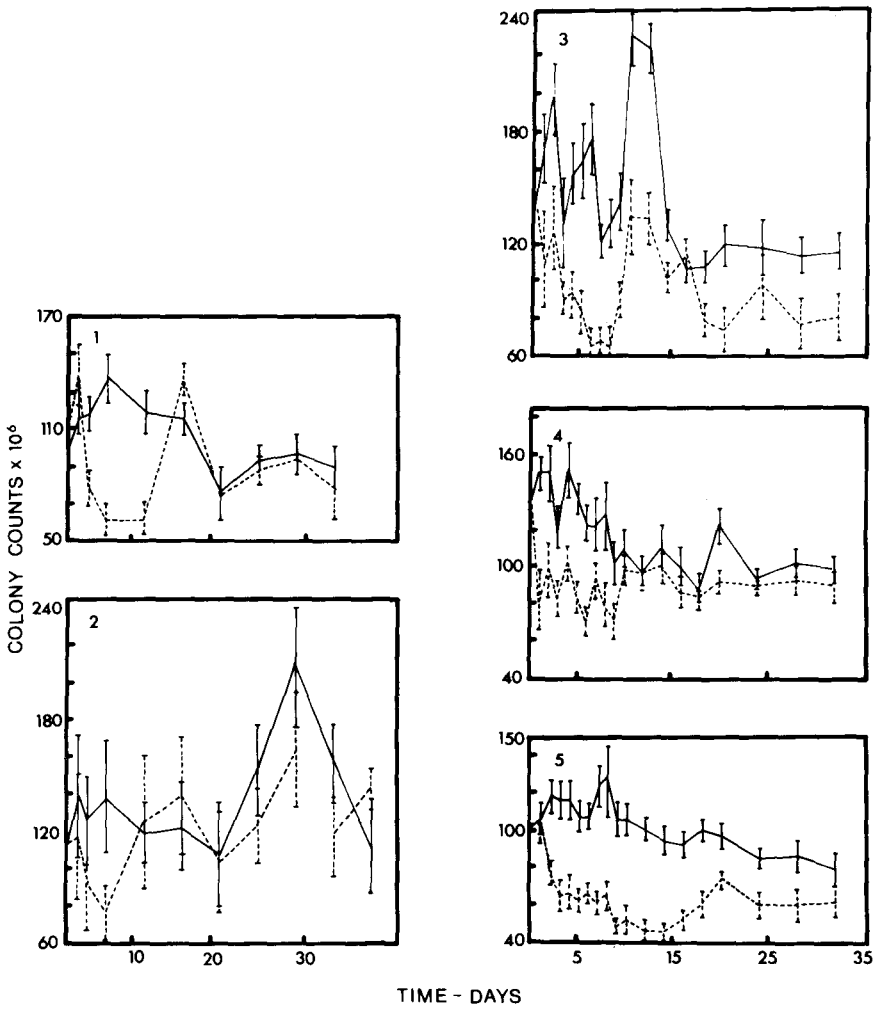

Figure 1. Colony counts x 10^6 plotted with respect to time (in days). Means $\pm$ 95% confidence limits) are plotted for both controls (solid lines) and experimental plots (dotted lines).

with those from the control plot, were evident after 24 hours. Moreover, this reduction remained significant for 16 days despite considerable bacterial growth in both experimental and control plots between days 8 and 14. Experiment 4 (Fig. 1) also received 1.0 g/m^2 of Benlate. The reduction of isolations from the experimental plot were not so marked as in experiment 3, however, all but two samples over the 32-day experimental period displayed significant differences between control and experimental isolates. In experiment 5, 1.0 g/m^2 of active fungicide was applied to the experimental plot. There was a significant ($\alpha = 0.05$) depletion of isolations from the experimental plot after 48 hours in this experiment, followed by a continued and relatively enormous difference between the experimental and control plots which continued to register through the completion of the experiment. It is apparent from this experiment that an increased concentration of fungicide considerably reduced the total number of bacteria isolated compared with isolations at the lower concentration of the fungicide.

MULTIPLE APPLICATION FIELD EXPERIMENTS

Two experiments were run in which 0.5 g/m^2 of benomyl (added as Benlate) was applied every seven days. In both, the experimental populations fell to a level of 50% of the control populations within 48 hours, and remained at that level for the duration of the experiment (10 weeks in the first experiment and 11 weeks in the second). The initial response was identical to that observed in the single application work, but the effect persisted because of the constant reinforcement by the continued addition of benomyl (Fig. 2).

PERFUSION EXPERIMENTS

The results from the field experiments involving soil drenches with Benlate indicated that the fungicide was not without effect upon soil bacteria. Significant declines in the bacterial populations of the experimental plots compared with those of the control plots were observed but these quantitative experiments gave no indication of which bacterial groups were affected. Consequently, it was decided that one of the essential physiological groups of soil bacteria, the nitrifiers, should be investigated in relation to the soil applications of Benlate. Two thirty-day experiments were run with concentrations of Benlate at 10.0, 1.0, and 0.1 ppm in the soil. Three perfusion units were used at each concentration and the remain-

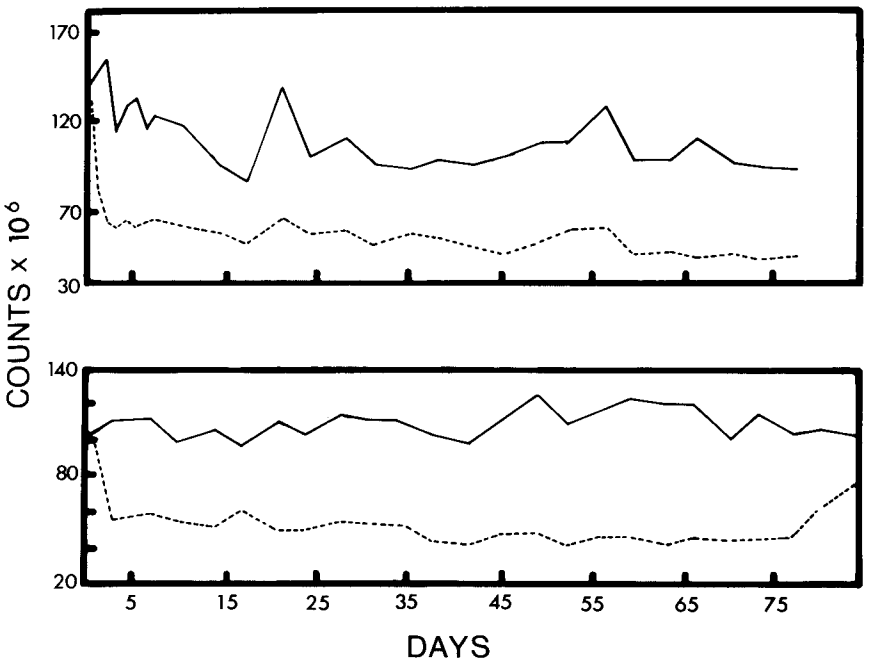

Figure 2. Colony counts x 10^6 plotted with respect to days for two long-term experiments. Benlate was applied every seven days in both experiments. Controls are represented by solid lines and experimentals by dotted lines.

ing three units acted as controls.

In the first perfusion experiment (Fig. 3) the control was found to rapidly convert the ammonium nitrogen to nitrate nitrogen. There was a short initial lag period followed by an increase in the conversion rate which showed no signs of leveling off before a concentration of 100 ug/ml was reached at approximately fifteen days after commencing the experiment. Compared with the control, the soils in which the Benlate was incorporated displayed varying reactions. At a concentration of 0.1 ppm Benlate, the initial rate of nitrification was somewhat more rapid than that of the control soil. The lag period was less pronounced and until the 10th day, the nitrification rate remained higher than that of the control. The rate, however, dropped and by the 14th day of the experiment was below that of the control. At the end of the sampling period

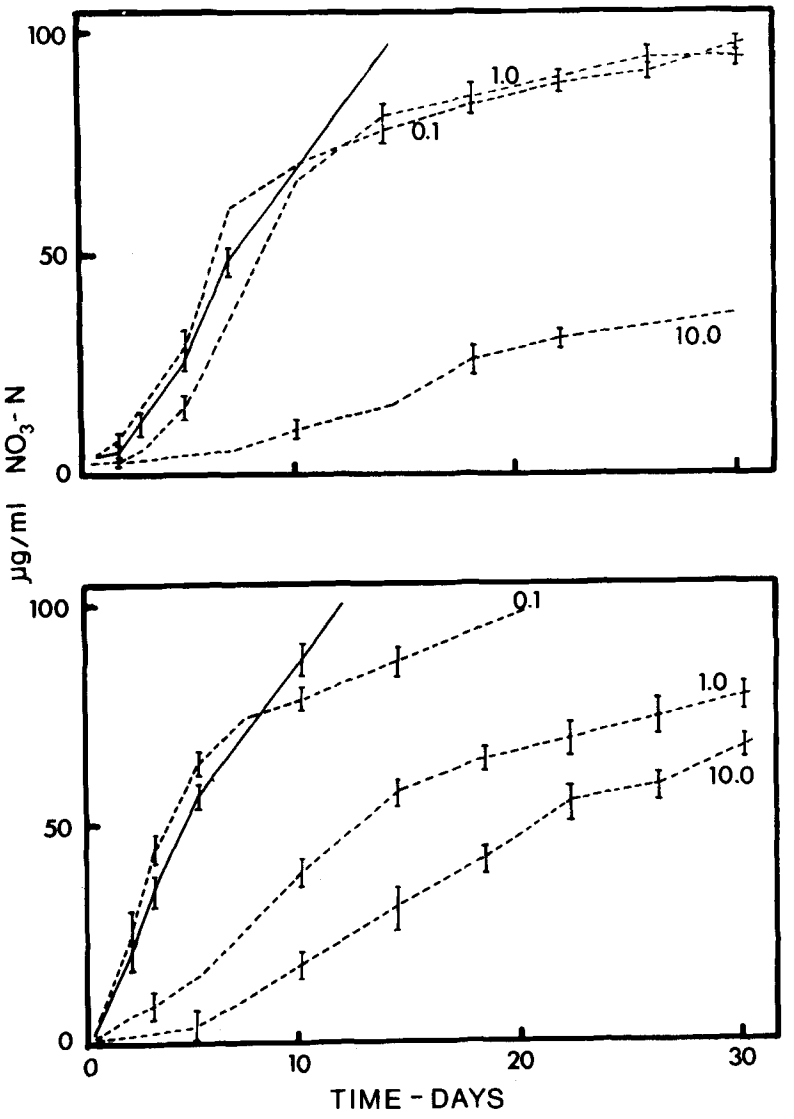

Figure 3. Results of two nitrification experiments expressed in terms of ug/ml of NO_3 - N (means $\pm$ 95% confidence intervals) plotted with respect to time measured in days.

the nitrate-nitrogen levels were showing signs of leveling off. Benlate, at a concentration of 1.0 ppm in soil caused a slightly longer lag period in the nitrification curve but after three days the rate of nitrification increased and paralleled that of the control until the 10th day of the experiment. At this point the rate began to diminish and the 30-day level of nitrate-nitrogen was very close to that of the 0.1 ppm Benlate treatment. The highest concentration of Benlate at 10.0 ppm in soil produced a protracted lag period in the rate of nitrification and severely lowered the concentration of nitrate produced. At the termination of the experiment, the nitrate-nitrogen level at this fungicide concentration, was less than 50% of that of the other Benlate treatments.

In the second experiment (Fig. 3), 0.1 ppm Benlate again stimulated nitrification. The nitrate-nitrogen concentration remained higher than that of the control for seven days and then fell sharply below the control level. The 1.0 and 10.0 ppm concentrations of Benlate both produced significant declines in the rates of nitrification compared with that of the control although the reduction of rate in the 10 ppm concentration was much closer to that at 1.0 ppm than in the previous experiment.

DISCUSSION

The single and multiple application experiments indicate that there is some inhibitory effect of benomyl upon soil bacteria. At the active concentration of 0.5 g/m^2 the fungicide reduced the isolations of bacteria from the experimental plot within 48 hours after fungicide application. However, significant differences in the number of bacteria isolated between control and experimental plots did not continue throughout the duration of the experiments and the number of bacteria from the experimental plots tended to return to the levels of those from the control plots within the experimental time period. When an active concentration of 1.0 g/m^2 of fungicide was used, the differences in numbers of isolations from control and experimental plots were found to be significantly greater than those obtained at the lower concentration of fungicide. Moreover, the number of bacteria isolated from the experimental plot had not returned to the level of the control plot at the end of the experimental period. It appears, therefore, that the increased concentration of benomyl had an increased

inhibitory effect upon the isolation of bacteria from the soil. While it must be recognized that because this study only considers total soil bacteria, it is impossible to draw conclusions about the effects benomyl might have on soil ecology, but the data do suggest that more detailed work is warranted.

One such approach is exemplified by the perfusion experiments which indicate that Benlate affects the rate of nitrification when incorporated into the soil at levels of 0.1, 1.0, and 10.0 ppm. At 0.1 ppm there appears to be some intitial stimulation of the nitrification in the soil but this rate is depressed below that of controls after approximately seven days. At higher concentrations Benlate retards rates of nitrification over the first 30 days although it does not inhibit this process completely. This supports the work of HOFER (1971) which suggests that benomyl affects the nitrifying bacteria in soil in a manner not yet established.

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