Multiple Applications of Benomyl and Effects on Non-Target Soil Fungi

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INTRODUCTION

The fungicide benomyl, has been shown to control a variety of plant pathogens. In vitro work by BOLLEN et al., (1970) and EDGINGTON et al., (1971) indicated that many Deuteromycetes and Ascomycetes were very sensitive, that Basidiomycetes showed varied sensitivity and that Phycomycetes were not sensitive. This pattern is supported by the work of DELP et al., (1968) who reported that while benomyl inhibited many plant pathogens, it had little effect on Phycomycetes; also by GILPATRICK (1969) who found that benzimidazoles were effective against powdery mildew of apple and cherry and by WENSLEY et al., (1970) who found that benomyl inhibited Fusarium wilt of muskmelon. The results of these studies are also supported by field studies on non-target organisms (FOSTER, 1975) which indicated that Trichoderma, Fusarium and Penicillium were affected, but Mucor and Rhizopus were not.

The work of FOSTER (1975) was prompted by the dearth of information on the effects of benomyl on non-target soil organisms and by the fact that the chemical is widely used as a soil drench (SCHROEDER et al., 1968; WENSLEY et al., 1970; SCHREIBER et al., 1971; SMALLEY, 1971; JORDAN, 1971; HARE, 1973; and ILYAS et al., 1976). Briefly stated, this work suggested that at levels below 0.5 g of benomyl in a fungicidal spray applied to one square meter of soil, total colony counts decreased within 48 hours of treatment and returned to control levels within 32 days. At levels > 1.0 g of benomyl per m^2, colony counts remained low throughout the 32-day period studied, and the Penicillium, Trichoderma and Fusarium were strongly suppressed while the Phycomycete genera were not.

The work presented here follows directly from FOSTER (1975) and is intended to investigate the effects of multiple

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applications, such as may be advocated during epidemic conditions. The object of the study was to determine whether or not multiple applications had a cumulative effect on colony counts, and to determine whether succeptibility of certain genera is altered by continuous exposure.

MATERIALS AND METHODS

Soil cores to a depth of 6 inches were removed from adjacent one square meter experimental and control field plots, situated in a grain field in Maple, Ontario. The soil was of a clay-loam type, pH 7.3 and sown with winter wheat. Two sets of experiments were run, the first lasting eleven weeks and the second twelve weeks. Benlate (1 g/m^2) was added to the treated plots in aqueous suspension once every seven days, and the control plots were treated with equivalent water blanks. Samples of soil were removed daily for one week after initial treatment and thereafter upon the third and seventh day of each week. At each sampling, three soil cores were removed randomly from each of the experimental and control plots and dilution spread plates were prepared from each core. Malt extract agar (Oxoid), containing streptomycin and rose bengal was used as the isolating medium for fungi. Plates were counted after five days of incubation at 25°C and the colony counts were converted to numbers per gram of the dry weight soil.

In experiment one, the abundance of the predominant fungal genera as well as total fungal isolation counts, were studied. The total number of species within each of the genera: Mucor, Rhizopus, Penicillium, Trichoderma and Fusarium, were counted on each of the field isolation days during this experiment.

It is appreciated that the dilution-plate method does not provide a true representation of the total soil fungal populations, in that it is selective for the faster-growing, prodigious spore-formers. However, as the same isolation procedures were employed throughout, for both the treated plots and the controls, valid comparisons can be made between the various treated plots and an evaluation can be made of the non-target effects of the fungicide for the prevailing environmental conditions.

RESULTS

In experiment I (Fig. 1), pre-application samples from treated and control plots indicated a marginally higher population count in the treated plot, however, within 24 hours of application this count fell to a level significantly below that of the control. Local soil environmental conditions were such that the control population increased rapidly over the next 24 hours and remained high while isolations from the treated plot continued to fall. Populations in both the control and treated plots stabilized somewhat after seven days, and during the remainder of the experiment the number of isolations from the treated plot fell to approximately 30% of those from the control plot. Throughout the experiment, the treated plot populations displayed no signs of returning to the levels of the control populations.

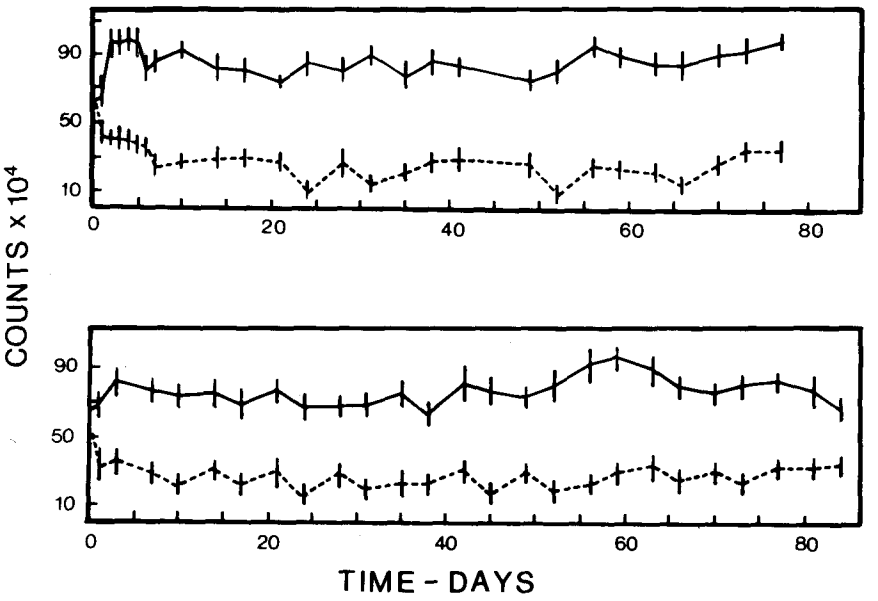

Figure 1. Colony counts (times 10^4/g of soil) with respect to time (days) for experiments I (top) and II (bottom). Solid lines are control plots and dotted lines are treated plots. Data are presented as means $\pm$ 95% confidence intervals.

In order to test the accuracy and consistency of experiment I, a second multiple application experiment was undertaken. This was experiment II (Fig. 1), and it began approximately one month after experiment I. The results obtained were very similar to those in experiment I, with isolations from the treated plot dropping rapidly to a level of approximately one-half that of the control plot within the first 24 hours. Levels of isolations from both control and treated plots remained relatively stable throughout the experiment exhibiting no overlap of confidence limits at any time. The experiment was terminated after 12 weeks (one week longer than experiment I), with isolations from the treated plot remaining significantly ($\alpha = 0.05$) below those from the control plot.

In experiment I, five genera were treated separately. For each genus the mean colony count was calculated along with the percentage of the total colony mean. This method indicated both relative and absolute changes in the composition of the fungal population.

Throughout the experiment there was no inhibitory effect from multiple applications of benomyl upon isolations of the genus Mucor (Fig. 2A). The total number of isolations from control and treated plots were not significantly different throughout the experiment excepting two samples removed after 31 and 52 days respectively. Such deviations are expected when sampling for soil fungi and they were not considered to be caused by the benomyl applications. However, when considering the percentage of the total fungal population represented by Mucor species, there is a significant rise. Within 48 hours of application, there is a great difference between the experimental and control plots with regard to the percentage of the total population comprised by Mucor species. At no point during the experiment are the treatment plot figures less than 100% higher than those of the control, with a maximum difference of over 400% between the fifteenth and seventh day of the experiment.

Essentially the same picture was obtained with Rhizopus species (Fig. 2B). Over the eleven-week experiment, control and treatment plot isolation counts differed significantly only once and this was probably not associated with fungicide applications. Rhizopus species also greatly increased their percentage representation of the total mean isolations. This genus comprised less than 10% of the total fungi isolated from

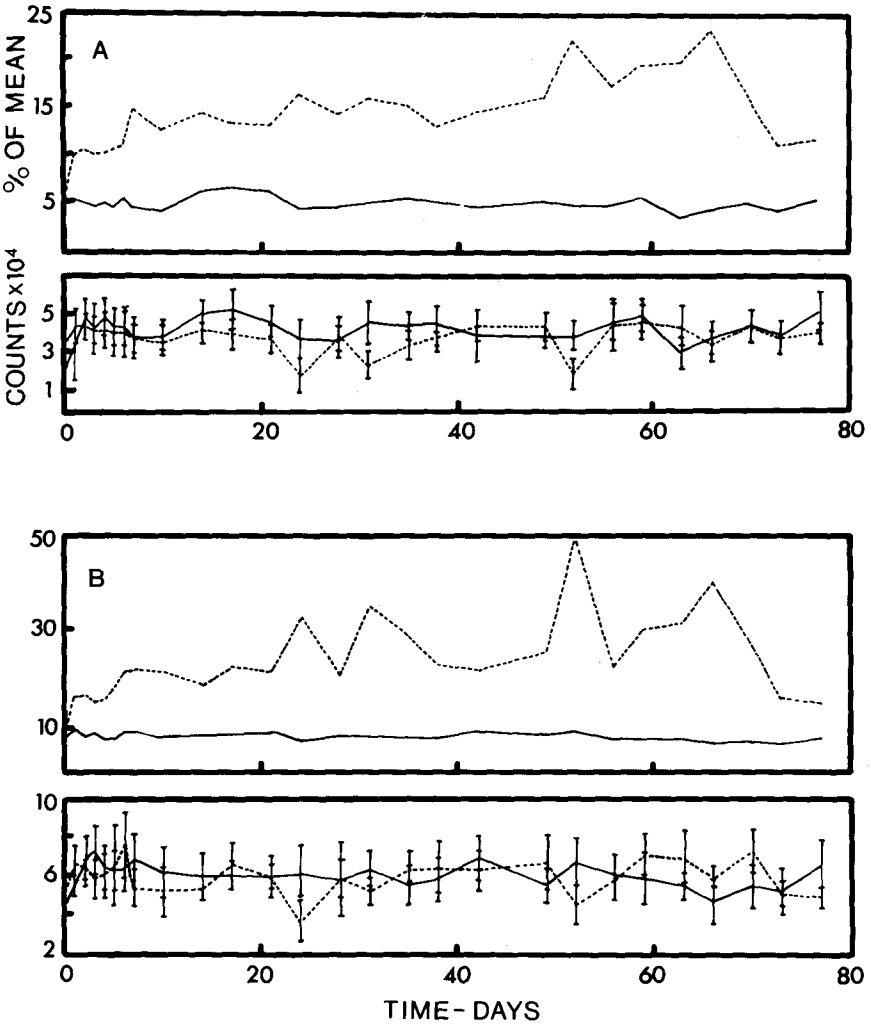

Figure 2. Individual data for Mucor (part A) and Rhizopus (part B). The top figure in each set represents individual colony counts expressed as a percentage of the total, the bottom figure in each set represents individual colony counts (times 10^4/g of soil). Dotted lines are treated plots and solid lines control plots. Means $\pm$ 95% confidence intervals are shown.

the control plot throughout the experiment, whereas in the treated plot, Rhizopus generally comprised more than 20% of the isolations and peaked with a high of 50% of the total fungal population on the 52nd day of the experiment.

Figure 3 summarizes the effect of weekly applications of benomyl on the Deuteromycete genera; Penicillium, Trichoderma and Fusarium. These three genera displayed very different responses when compared to the Zygomycetes. Penicillium (Fig. 3C) species were extremely sensitive to the fungicide and there was an immediate and rapid decline in isolations of the genus from the treated plot after application of benomyl. Isolations of Penicillium from the control plot were never less than 30% higher than those from the treated plot and peaked on several occasions at levels of more than 400% higher. This corresponded with a sharp decline in the percentage of the total mean comprising Penicillium species. This percentage fell to approximately one-third of the control plot percentage.

A similar response pattern was observed in the isolation of Trichoderma (Fig. 3D). Again there was a rapid drop in the number of isolations of this genus from the treated plot and the Trichoderma population continued at an extremely low level throughout the experiment, dropping to zero count on two occasions. The level of isolations from the control plot remained relatively constant, maintaining Trichoderma at levels of at least 700% over those of the treated plot. There was also a corresponding drop in the percentage of the total fungal mean comprised by Trichoderma.

The third Deuteromycete genus, Fusarium, displayed the most sensitive reaction to benomyl, as it did in the single application experiments (FOSTER, 1975). In the treated plot Fusarium dropped immediately by a factor of 10 compared to the control plot and this difference was maintained throughout the experimental period (Fig. 3E).

DISCUSSION

The objectives of this study were to determine whether or not multiple applications of benomyl could cause cumulative decreases in the numbers of non-target fungi, and to determine whether or not succeptibility of certain genera was altered by continuous exposure. With respect to the first question, it

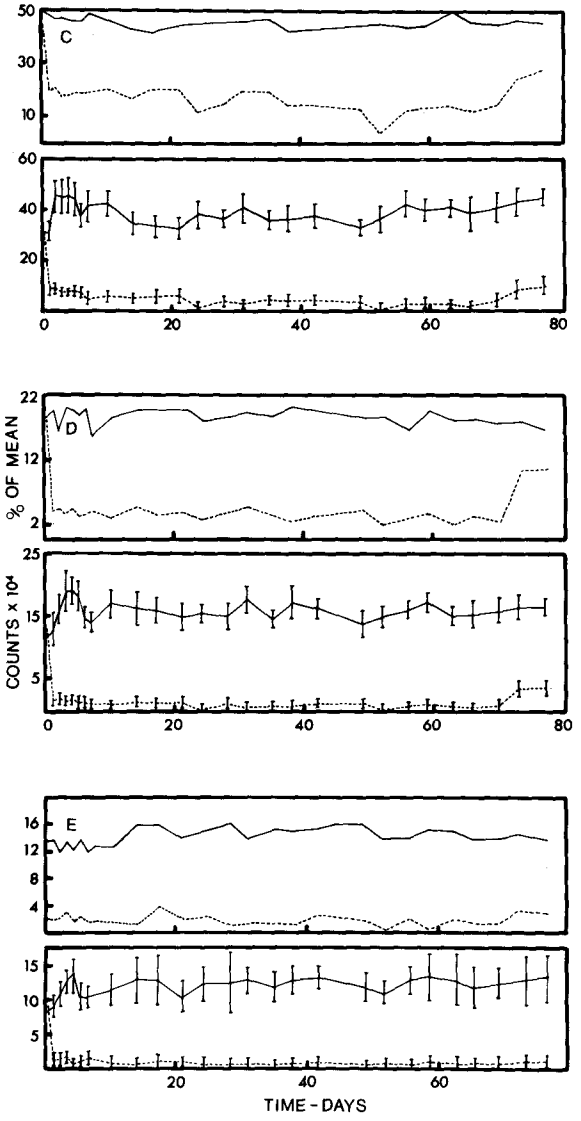

Figure 3. Individual data for Penicillium (part C), Trichoderma (part D) and Fusarium (part E). The top figure in each set represents individual colony counts expressed as a percentage of the total, the bottom figure in each set represents individual colony counts (times 10^4/g of soil). Dotted lines are treated plots and solid lines control plots. Means $\pm$ 95% confidence intervals are shown.

appears that compared to single application experiments (FOSTER, 1975) multiple applications of benomyl do not result in progressive decreases in total colony counts. Multiple applications do, however, have the effect of preventing a return to control levels.

With respect to the second objective, it appears that different genera were affected differently. Although the amount of benomyl applied was ten times that of the single application experiments (10 applications as opposed to one), there was still no significant inhibitory effect upon the two Zygomycete genera. Levels of both Mucor and Rhizopus remained relatively constant and without significant population differences throughout the experiment. This confirms laboratory studies on the in vitro specificity of benomyl by BOLLEN et al., (1970) and EDGINGTON et al., (1971) and the performance of the fungicide in controlling Zygomycete pathogens in the field (DELP, 1968). On the other hand the Deuteromycete genera that were studied, displayed high levels of sensitivity and populations of these genera were sharply reduced after fungicide applications. This again confirms observations made by the above workers, who found many of the representatives of the form-class Deuteromycetes sensitive to benomyl in laboratory studies. As in the single application experiments (FOSTER, 1975), the differential sensitivity towards benomyl exhibited by these fungal groups was responsible for a quantitative alteration in the composition of the total fungal community in the treated plot with the three Deuteromycete genera dropping in relative abundance and the two Zygomycetes increasing their relative percentage composition of the total fungal community.

One interesting point which is suggestive but requires experimental scrutiny, is that both Penicillium and Trichoderma show some recovery near the end of the experiment (Figs. 3C, 3D). This might be due to acquired resistance resulting from continuous exposure to benomyl (BOLLEN et al., 1971, RUPPEL, 1975, and POLACH et al., 1975) or to changes in species composition, with relatively tolerant forms replacing those sensitive to the fungicide.

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