The *in vivo* Antifungal Effects of Benomyl on Non-Target Soil Fungi

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INTRODUCTION

The effectiveness of the fungicide, benomyl in controlling many plant pathogens is well documented, for example, DELP et al., (1968), reported that benomyl inhibited a wide range of pathogens but had little effect against the Phycomycetes; GILPATRICK (1969) documented the effects of benzimidazoles against powdery mildew of apple and cherry and WENSLEY et al., (1970) demonstrated the inhibitory effects of benomyl against Fusarium wilt of muskmelon. The in vitro fungitoxic spectrum of benomyl has also been examined by BOLLEN et al., (1970) and EDGINGTON et al., (1971) who reported high sensitivity by many Deuteromycetes and Ascomycetes, varying sensitivity by Basidiomycetes and little sensitivity by Phycomycetes. This work correlates well with effective disease control in the field by the fungicide as catalogued by BOLLEN et al., (1970). However, little attention has been paid so far to the effects of benomyl on non-target microorganisms such as those occurring naturally in the soil ecosystem.

Benomyl may be applied as a soil drench (WENSLEY et al., 1970, JORDAN, 1971) to control soil-borne pathogens and systemically protect the plant against susceptible pathogenic fungi and mites. If such fungicidal soil treatments become widespread agricultural practices then more information is required on the in vivo effects of benomyl on soil microorganisms, particularly the fungi and bacteria. With this in mind, a number of preliminary experiments were undertaken to investigate the effects of benomyl on populations of soil fungi and bacteria in the field. Total population fluctuations were determined along with the population density of the five principal genera: Mucor, Rhizopus, Penicillium, Trichoderma and Fusarium.

MATERIALS AND METHODS

Soil cores to a depth of 6 in. were removed from adjacent one square metre 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. Experimental plots were treated with aqueous suspensions of benomyl or Benlate and the control plots with equivalent water blanks (Table 1). The soil was sampled over a 32 day period following application of the fungicide.

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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 figures per gram of the dry weight soil.

TABLE 1

Times and Dosages of the six Fungicide Treatments

EXPERIMENT NO.	STARTING DATE	FUNGICIDAL TREATMENT	CONCENTRATION OF ACTIVE FUNGICIDAL SPRAY IN P.P.M.
1	May 1971	0.5g. Benomyl/M^3	50
2	June 1971	0.5g. Benomyl/M^3	50
3	October 1971	1.0g. Benlate/M^3	50
4	June 1972	1.0g. Benlate/M^3	50
5	July 1972	0.5g. Benlate/M^3	25
6	August 1972	2.0g. Benlate/M^3	100

In experiment four, 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 32 day 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, experimentals and controls, valid comparisons can be made between the various experimental plots and an evaluation can be made of the non-target effects of the fungicide for the prevailing environmental conditions.

RESULTS

The results from the single application experiments have been summarized in Figures 1-6. In each experiment there was a rapid decline in the total number of fungi isolated after application of benomyl. This depletion was discernable after only 24 hours in all except experiment 2 (Fig. 1b), in which the experimental plot isolations were not significantly different ($P=0.05$) until the second soil sample was taken after 48 hours.

Experiments 1-4 each received the same amount (0.5g.) of active material. In experiments one and two this was in the form of recrystallized benomyl, while experiments three and four em-

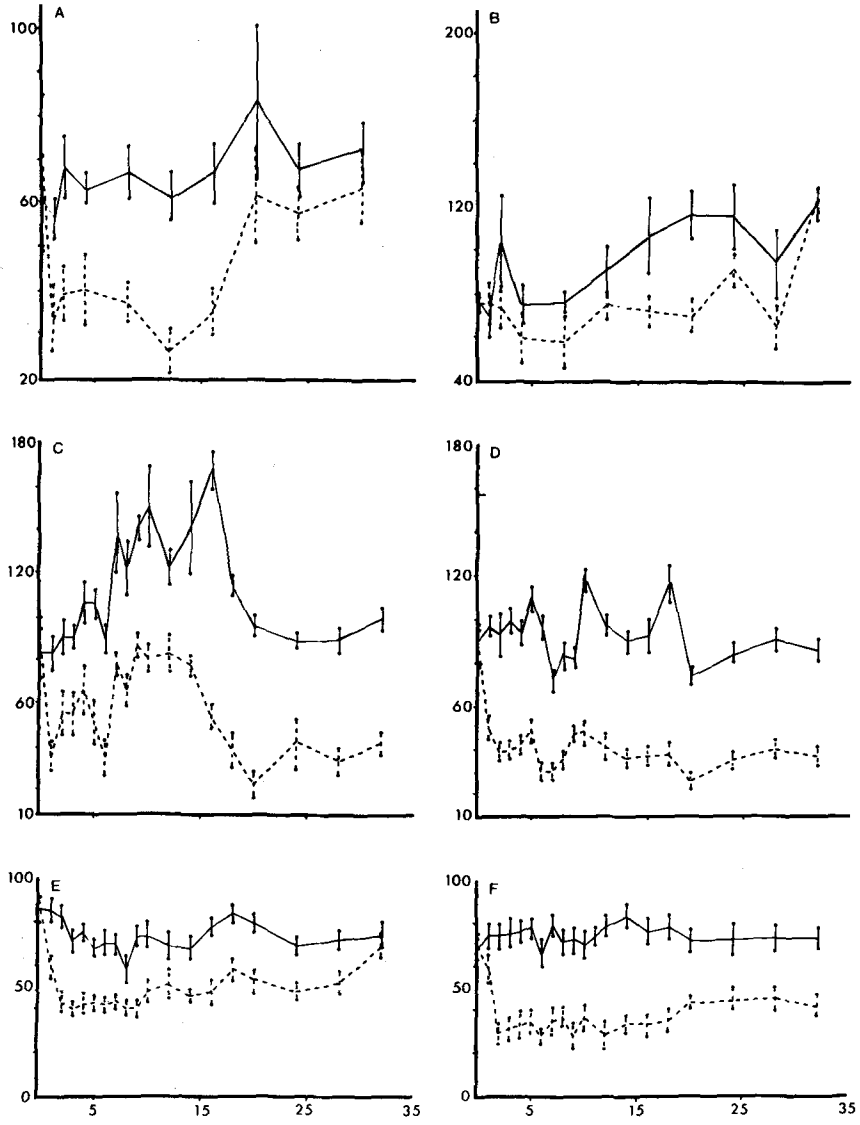
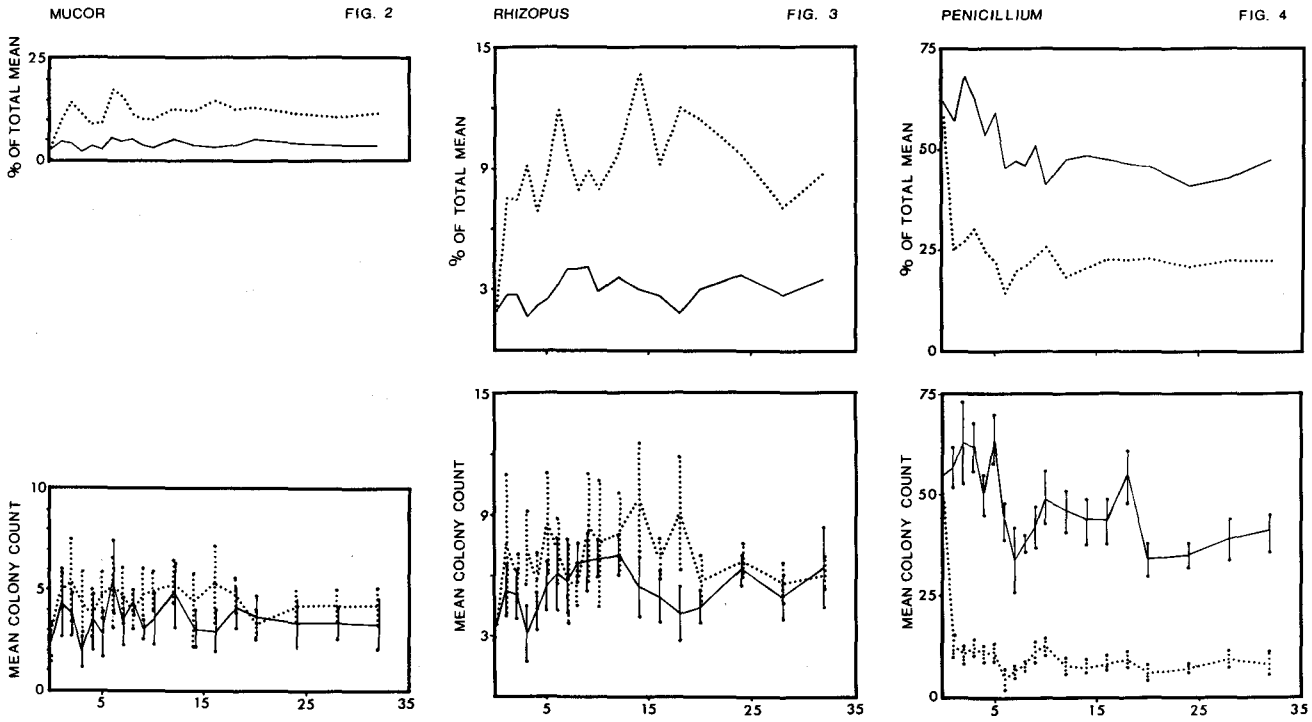
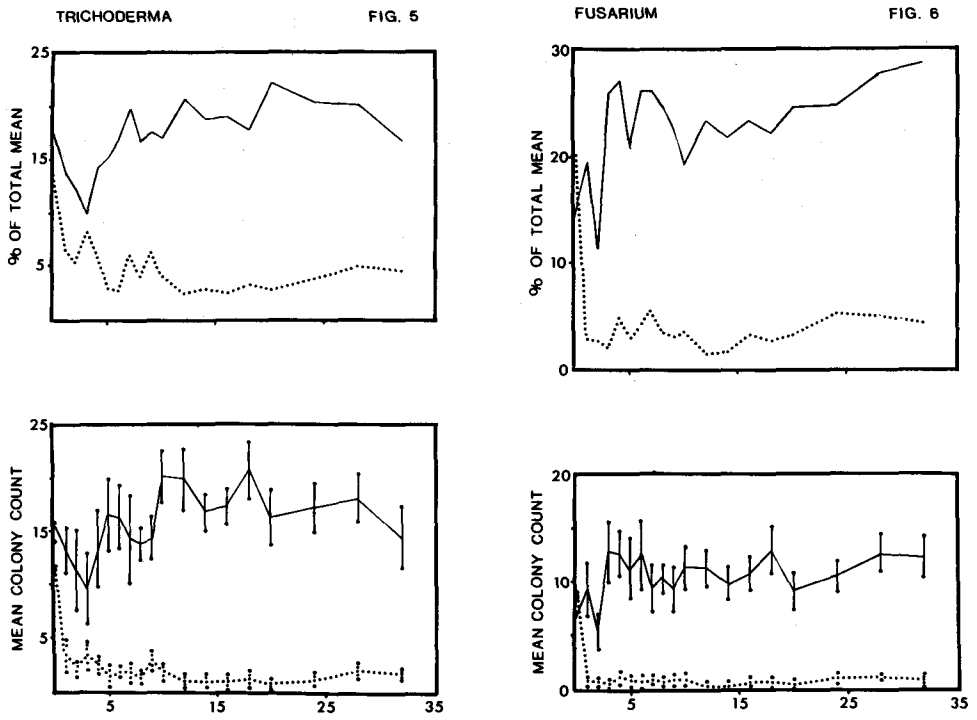


FIG. 1. (A-F) Responses of six isolated total fungal populations over 32 day periods, to a single application of fungicide.



FIGS. 2-4. Responses of individual genera of fungi to a single application of fungicide. Figures represent mean colony count and the percentage of the total mean colony count.



FIGS. 5-6. Responses of individual genera of fungi to a single application of fungicide. Figures represent mean colony count and the percentage of the total mean colony count.

ployed the commercial form of the fungicide, Benlate. The compounding of the fungicide with a spreader in the commercial form facilitates its manipulation in suspension. It seems likely that the more pronounced effect of the fungicide in experiments three and four (Figs. 1c and 1d) in reducing the numbers of isolated fungi was due to the more efficient application of the commercial fungicide. In experiments 5 and 6, the amount of Benlate applied was 0.25g and 1.0g of active material respectively, which were one half and double the amount of fungicide applied in the first four experiments. However, the pattern of reduction in numbers of isolations remained basically the same (Figs. 1e and 1f).

The effect of a single application of benomyl at concentrations used in soil drenches, upon soil fungi in vivo, was surprisingly long-lasting. When using benomyl, which was difficult to suspend finely during application, there was a significant difference in numbers of fungi isolated, between the control and experimental plots for at least 24 days, (Figs. 1a and 1b). The equivalent amount of Benlate produced significant differences between control and experimental plots for at least 32 days, at which time the experiment was terminated (Fig. 1c and 1d). At a concentration of 0.25g/plot of active material, significant differences between experimental and control results were still apparent after 28 days although the isolation counts overlapped on the final day of sampling. This suggests that the soil fungal populations may have been recovering from the effect of the fungicide (Fig. 1e). At 1.0g active material per plot the experimental and control plot counts remained significantly different throughout the period of the 32 day experiment, (Fig. 1f).

The effect of a single application Benlate on the five principle genera isolated from these field plots is summarized in Figures 2-6. The figures are presented as mean colony counts for experimental and control plots and from these data the percentage of the total mean of experiment 4 (Fig. 1d) of each of the five genera was calculated and expressed graphically. The two Phycomycetes, Mucor and Rhizopus were not significantly affected by a single application of benomyl, although the numbers isolated from experimental plots were generally higher than those isolated from control plots (Figs. 2 and 3 - MEAN COLONY COUNT). The lack of fungistatic or fungicidal effect against these Phycomycetes is further emphasized by their increased percentage of the total mean population. This increase is approximately 100% in Mucor species and approximately 200% in Rhizopus (Figs. 2 and 3 - % OF TOTAL MEAN).

The isolations of the three genera, Penicillium, Trichoderma and Fusarium from the experimental plots were dramatically affected by the application of Benlate. The numbers of Penicillia and Trichoderma were reduced by approximately 70% and those of Fusarium by approximately 90% (Figs. 4, 5 and 6 - MEAN COLONY COUNT). Moreover, these three genera represented a smaller percentage of the total fungal population than in the control plots (Figs. 4, 5 and 6 - % OF TOTAL MEAN). Therefore, not only had the number of isolations of these three genera fallen in absolute terms but their

abundance relative to other isolations had also declined. This is probably due to a greater selective effect of Benlate upon these more sensitive genera.

DISCUSSION

It would appear that the use of benomyl as a soil drench is not without effects upon non-target fungi within the soil. The total numbers of fungi isolated from experimental plots dropped dramatically below those from the control plots indicating at least a reduction of sporulation in many species. The two Phycomycete genera studied appeared to be unaffected by the fungicide in vivo which confirms the in vitro experiments of EDGINGTON et al., (1970) and BOLLEN et al., (1971) in which little sensitivity to benomyl by the Phycomycetes was found. However, isolations of the three genera, Penicillium, Trichoderma and Fusarium were greatly reduced, confirming the sensitivity of these genera to benomyl as found by the above workers. The reduction in activity of these genera may also have been responsible in part for the apparent slight increase in activity by the Phycomycete genera, due to a reduction of the competitive effects of the three sensitive genera.

The results indicate that a single application of benomyl at a soil drench concentration of 50ppm. in aqueous suspension considerably disrupts the soil fungal populations, having effects upon the beneficial saprophytic fungi as well as the potentially dangerous plant pathogens. Furthermore, continuing work in this area suggests that a series of applications of benomyl, such as may be advocated during epidemic conditions, had an even more pronounced effect upon non-target fungi (unpublished data, M. Foster). Effects upon sensitive genera have proven to be significant ($P<0.05$) and have lasted for at least one month after application in most experiments. The range of concentrations employed appeared to have no significant effect upon the numbers and types of fungi isolated. However, as the experimental applications were equivalent to recommended treatment levels, such relatively long-term effects could have important implications concerning the ecology of soil fungi. It is hoped that further work elucidating more precisely the roles of fungi in the soil will aid the understanding of the complex effects of benomyl upon these fungi.

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