632.953 : 632.954 : 631.461

Soil Biol. Biochem. Vol. 6, pp. 131 to 133. Pergamon Press 1974. Printed in Great Britain.

SHORT COMMUNICATION

Effect of the fungicide benomyl on some metabolic processes, and on numbers of bacteria and actinomycetes in the soil

H. G. VAN FAASSEN
Institute for Soil Fertility, Haren (Gr.), The Netherlands

(*Accepted* 20 *October* 1973)

INTRODUCTION

In view of the increasing use of the systemic fungicide benomyl the possible side-effects of this compound on the soil microflora and on metabolic processes in the soil need to be studied.

MATERIALS AND METHODS

In laboratory experiments we treated humus-rich sandy soils (Table 1) with Benlate (spray formulation containing 50% benomyl) suspensions or with water (controls). Moisture content about 50 per cent of the water holding capacity. The samples were incubated at 15 or 20°C. Soil I (from Naaldwijk) had been used for glasshouse cultures and had neither been treated with benomyl nor had been fumigated or steamed. Soil II (from Venlo) came from an experimental field, where in plots IIB the soil around gherkin plants had been disinfected twice with 2 g Benlate/plant (2·5 plants/m^2). Samples were taken about 2 months after the last treatment.

Bacteria and actinomycetes in the soils were counted by the dilution plate method. Agar media used were based on soil extract, glycerol–asparaginate, casein–glucose, chitin or casein–starch–nitrate; the pH was adjusted to about 7. Cycloheximide (*ca.* 100 mg/l) was used as an antifungal antibiotic, added after sterilization and cooling; sometimes Benlate (*ca.* 100 mg/l) was also added to investigate its effect on bacteria and actinomycetes *in vitro*. In the samples IIA and IIB the following functional groups of microbes were estimated by dilutions of soil suspensions into liquid media (Pochon and Tardieux, 1962): total microflora, amylolytic, cellulolytic, nitrifying microbes and algae; ammonification of urea and *Azotobacter* were investigated in soil.

Dehydrogenase activity of soil I was measured (Casida *et al.*, 1964, slightly modified).

Table 1. Some properties of the sandy soils used in the experiments

	Organic matter (%)	Mineral particles $<16\,\mu m$ (%)	pH (1 N KCl)
Soil I (untreated)	8·9	7·4	7·0
Soil IIA (untreated)	9·8	12·4	7·4
Soil IIB (treated)	9·7	10·0	7·3

The CO_2 evolution from soil I, kept in columns through which CO_2-free air (0·5 l/h) was passed, was measured during 1 month at 29°C. CO_2 was absorbed in N NaOH and twice a week determined by differential titration with hydrochloric acid with phenolphtalein and methyl orange, respectively as indicators.

Nitrogen mineralization was determined quantitatively as follows. Total inorganic nitrogen was found by the method of Cotte and Kahane (1946); ammonium nitrogen was determined by distillation following addition of MgO, and nitrate + nitrite nitrogen was found by difference. A second analysis after destroying nitrite with sulphamic acid was used to see, how much nitrite had been present. In pure cultures of nitrifiers in liquid media the oxidation of ammonium and nitrite was checked qualitatively by colour reactions on nitrate and nitrite separately. Media used were for *Nitrosomonas*: $(NH_4)_2SO_4$ 1·0 g, K_2HPO_4 0·5 g, NaCl 2·0 g, $MgSO_4 7H_2O$ 0·2 g, $FeSO_4 7H_2O$ 0·05 g, $CaCO_3$ 6·0 g, and water 1000 ml; for *Nitrobacter*: $NaNO_2$ 1·0 g, K_2HPO_4 0·5 g, $MgSO_4 7H_2O$ 0·5 g, $FeSO_4 7H_2O$ 0·05 g, and water 1000 ml, pH = 7·6.

RESULTS AND DISCUSSION

Numbers of bacteria and actinomycetes

The numbers of bacteria plus actinomycetes of Soil I were counted after 6 and 14 days of incubation respectively (Table 2), and higher numbers of microbes were found with increasing benomyl concentration which is in accordance with the data of Hofer *et al.* (1971). Such findings suggest that this can be explained by the fact that microbes utilize compounds added with the benomyl formulation or that benomyl has a vitamin-like action. Unfortunately, the composition of the "inert" part of the formulation is not supplied by industry for use in tests. Weeks and Hedrick (1971) have suggested that in soils where Benlate is applied some detoxification will occur by bacterial utilization of the active ingredient as a source of carbon. The increase in numbers may also result from a change in the population with bacterial numbers increased and fungal reduced.

Benomyl treatment of the soil samples (up to 100 parts/10^6 of benomyl) did not influence the numbers of actinomycetes that were counted after 6, 21, 50 and 80 days.

The soil samples IIA and IIB did not show significantly different numbers of bacteria and actinomycetes on agar plates. This was also found when counts were made 14 days after treating both samples in the laboratory with 20 parts/10^6 of benomyl (giving a concentration comparable with that applied in practice).

Table 2. Influence of benomyl on numbers of bacteria and actinomycetes in soil I

Benomyl in the soil (parts/10^6)	Agar plate media (No./g dry soil × 10^{-5}) Without benomyl 6 Days incubation	Without benomyl 14 Days incubation	With benomyl (approx. 50 mg/l) 6 Days incubation	With benomyl (approx. 50 mg/l) 14 Days incubation
Glycerol–asparaginate				
0	39 (±6)*	55 (±12)	35 (±37)	49 (±13)
50	186 (±60)	198 (±38)	184 (±10)	161 (±17)
Casein–glucose				
0	59 (±14)	N.D.	20 (±4)	N.D.
50	157 (±24)	N.D.	139 (±21)	N.D.
Soil extract				
0	1·2 (±1·3)	6·2 (±2·0)	0·2	0·5
20	2·5 (±0·9)	9·8 (±2·0)	0·6	4·2 (±2·3)
50	2·2 (±0·7)	18·4 (±0·7)	0·6	12·4 (±3·4)
100	3·4 (±1·5)	24·6 (±2·5)	0·5	17·8 (±5·6)

* Confidence limits of 95 per cent reliability.
N.D. not determined.

Samples IIA and IIB moreover, showed no significant differences in most probable numbers or activities of the functional groups of the microflora. Fifteen days after treatment of IIA and IIB with 20 parts/10^6 of benomyl in the laboratory the most probable numbers of the total microflora were 200 and 1000 times lower, respectively, than before treatment; the numbers of nitrite-oxidizing microbes were also considerably lower. Amylolytic, cellulolytic and ammonia oxidizing microbes were not significantly influenced by the treatment. Neither ammonification of urea nor the development of *Azotobacter* and of algae in the benomyl treated soil were different from that in the control soil. Other workers (e.g. Hugé, 1970) have shown that differences found by this method may be very large and can seldom be ascribed with certainty to the use of pesticides. Furthermore, Ponchet and Tramier (1971) with this method found no disturbances of the soil microflora by high doses or repeated application of benomyl.

Differences in the results of the experiments with soils I and II may be due to the different benomyl concentrations applied or to different soil properties. Hofer *et al.* (1971) found large differences between two soils treated with equal benomyl concentrations. Their results may be explained from differences of the soils in humus content that is expected strongly to influence the availability of Benlate in soil.

CO_2 evolution from the soil

Total CO_2 evolved from soil I was only slightly infl
enced by benomyl even at high concentrations (Table
It may be assumed that the breakdown of soil organic ma
ter, cellulose or chitin in this soil is not disturbed by norm
benomyl application (at concentrations about 20 parts/10
Even with 200 parts/10^6 of benomyl in the soil and 0·2
cellulose or chitin added, maximum CO_2 evolutic
occurred only a few days later than the controls; though a
increased lag phase was found for cellulose breakdow
Hofer *et al.* (1971) found some increase in CO_2 evolutic
without added substrate and a small decrease with adde
starch upon benomyl treatment during 3 days incubatio
which agrees rather well with our findings for these le
rapidly degradable substrates.

Nitrogen mineralization

After 12 weeks' incubation significantly more (NO_2 -
NO_3)–N ($P < 0{\cdot}05$) was found in the benomyl-treated so
(regardless of the amount of benomyl added) than in the ur
treated soil (Table 4). Nitrite–nitrogen after 12 weeks wa
less than 2·5 mg/kg and no ammonium–nitrogen was foun
at any time during the experiment; so the ammonificatio
was immediately followed by nitrification. In contrast wit
this, Hofer *et al.* (1971) found a more or less strong inhib

Table 3. Relative values of CO_2 evolution of benomyl-treated soil I after 1 month of incubation (control soil without benomyl = 100 per cent)

Substrate added to the soil	Benomyl concentration in the soil (parts/10^6) 50	200	Significance of the differences found between untreated and benomyl-treated soil (50 as well as 200 parts/10^6)
No substrate	91	104	$P > 0{\cdot}3$
Cellulose (0·2%)	88	82	$P < 0{\cdot}01$
Chitin (0·2%)	N.D.	86	$P < 0{\cdot}01$

N.D. not determined.

Table 4. Amounts of nitrate plus nitrate–nitrogen formed in benomyl-treated soil I during 3-, 6- and 12-week incubation at 29°C

Benomyl-concentration in the soil (parts/10^6)	*Nitrite plus nitrate–nitrogen formed* (mg/kg) 3 weeks	6 weeks	12 weeks
0	6	19	30
10	8	18	36
25	8	18	36
100	10	23	38

tion of nitrification after 4 weeks incubation with 15 and 150 parts/10^6 of benomyl in a humiferous sandy soil. A contribution to nitrate from "mineralization" of benomyl is not evident from Table 4, as the higher amounts of nitrate found in the treated samples were independent of the benomyl concentration in the soil. The higher concentration of $(NO_2 + NO_3)$-N found in the treated samples may result from an enhanced ammonification of soil organic nitrogen.

However, addition of benomyl at a level of 200 mg/l to liquid media with a mixed culture of *Nitrosomonas* and *Nitrobacter* (isolated from soil) caused in our experiments a delayed oxidation of ammonium to nitrite and inhibited the (further) oxidation of nitrite to nitrate (at 15°C and at 25°C). With 20 mg/l of benomyl in the medium only the oxidation of nitrite was delayed. Thus the oxidation of nitrite to nitrate *in vitro* was found to be more sensitive to benomyl than the oxidation of ammonium to nitrite.

Dehydrogenase activity

The dehydrogenase activity of soil I was not significantly affected by 20 or 200 parts/10^6 of benomyl in the soil; this was the case without further addition of substrate and with simultaneous addition of 200 parts/10^6 of glucose.

CONCLUSIONS

(a) Repeated applications of benomyl or the use of a high dose of benomyl—bearing in mind the uncertainty about its persistence in soil—can affect the total numbers of bacteria and actinomycetes and may cause a shift in the bacterial flora of the soil.

(b) Addition of benomyl to liquid or agar media inhibits the outgrowth in these media, of part of the soil bacteria, especially that of nitrite oxidizers. In soil this inhibition may be less because of adsorption of benomyl to soil particles.

(c) Serious disturbances of the conversion of carbon compounds in the soil by benomyl application apparently need not to be feared, but at present no generalization can be made as to the effect of benomyl on nitrification in the soil.

REFERENCES

Casida L. E., Klein D. A. and Santoro T. (1964) Soil dehydrogenase activity. *Soil Sci.* **98**, 371–376.

Cotte J. and Kahane E. (1946) New reduction method for the determination of nitrates. *Bull. Soc. Chim. Fr.* **1946**, 542–544.

Hofer I., Beck Th. and Wallnofer P. (1971) Der Einfluss des Fungizids Benomyl auf die Boden-Mikroflora. *Z. PflKrankh. PflPath. PflSchutz* **7**, 398–405.

Hugé P. L. (1970) Contribution à l'étude de l'influence du methbenzthiazuron sur les microorganismes du sol. *Meded. Fac. LandbWet. Gent* **35**(2), 811–827.

Pochon J. and Tardieux P. (1962) *Techniques d'analyse en microbiologie du sol.* Editions de la Tourelle, Saint-Mandé (Seine).

Ponchet J. and Tramier R. (1971) Effets du bénomyl sur la croissance de l'oeillet et la microflore des sols traités. *Annls Phytopath.* **3**(3), 401–406.

Weeks R. E. and Hedrick H. G. (1971) Screening microorganisms from two soils for utilization of the systemic fungicide Benlate. *Bact. Proc.* 1971, Abstract A78.