

## The function of an MBC-releasing deposit of benomyl and thiophanates in plant roots and soil

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### Abstract

A comparative study has been made on the effects of short-term treatment versus long-term treatment with benomyl and three thiophanates with regard to distribution of fungitoxicant (MBC, or EBC) within plants and their protection against fungal diseases. In both treatments, plants were exposed to the fungicides for two days and then transplanted to garden soil; long-term treated plants received an additional supply of fungicide as a daily drench for one week.

Results of short-term treatment of cucumber seedlings and tomato plants provided additional proof for the hypothesis, that MBC derivatives, substituted at N-1 in the benzimidazole nucleus, are retained to some extent on or in the roots, and gradually converted to MBC which then moves into the aerial parts of the plant. Correlation of infection with concentration of fungitoxicant within leaves showed 0.35 µg/g fresh weight to be the limiting concentration for symptom expression of cucumber powdery mildew. Only with benomyl was MBC to be released long enough into the aerial parts of the plant to protect leaves, unfolding after termination of the treatment, for some weeks.

With long-term treatment, a concentration of MBC (or EBC) sufficient for protection was found with all fungicides in all above-ground parts of the plant until the end of the experiment. Thus, a reservoir of fungicide in the planting medium guarantees a continuous supply of fungicide only then may new growth be efficaciously protected.

### Introduction

Benomyl, thiophanate (NF 35), thiophanate-methyl (NF 44) and the related compound 2-(3-methoxycarbonyl-thioureido)-aniline (no common name; NF 48) are all converted in vitro to the same fungitoxic compound, methyl benzimidazol-2-yl carbamate (MBC) (or the closely related ethyl ester (EBC)) (Clemons and Sisler, 1969; Selling et al., 1970), at a rate depending on pH and temperature (cf. Fuchs et al., 1972). The transformation rates  $T$  in aqueous suspension were found (Fuchs et al., 1972) to decrease in the order:  $T_{\text{benomyl}} > T_{\text{NF 48}} > T_{\text{thiophanate-methyl}} > T_{\text{thiophanate}}$ . Concentrations  $C$  of fungitoxicant (MBC, or EBC) in above-ground parts of root-treated plants, however, did not reflect the above sequence:  $C_{\text{NF 48}} > C_{\text{benomyl}} > C_{\text{thiophanate-methyl}} > C_{\text{thiophanate}}$ . Neither did the effectiveness  $E$  of these fungicides in protecting plants against fungal attack:  $E_{\text{benomyl}} > E_{\text{thiophanate-methyl}} > E_{\text{NF 48}} > E_{\text{thiophanate}}$ . No appreciable protection resulted from application of heated suspensions of these compounds, which contained almost exclusively MBC (or EBC), although their fungitoxic activity in vitro was increased rather than decreased. These

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phenomena could be ascribed to differences in distribution of MBC (or EBC) within the plant, after root application of the various fungicides: whereas the fungitoxicant from benomyl and thiophanate-methyl was more or less homogeneously distributed over the aerial parts of the plant, with NF 48 and the heated suspensions of the fungicides the fungitoxicant accumulated almost quantitatively in the leaves that were present at the time of treatment. To explain the behaviour *in vivo* of these fungicides the hypothesis has been proposed that only MBC-derivatives substituted at N-1 in the benzimidazole nucleus (benomyl, intermediate conversion product of thiophanate-methyl) are retained in or on the roots and gradually converted to MBC, which then moves into the aerial parts of the plant.

Additional experiments in which the effects of short-term fungicidal root treatment on the distribution of fungitoxicant in above-ground plant parts were compared with those of long-term fungicidal treatment lent further support to this hypothesis. Some details of these experiments have been published before in abstracted form (Davidse and Fuchs, 1971); they are more fully described below.

### Materials and methods

One week old cucumber seedlings, cv. 'Lange Gele Tros', were placed with their roots in 400  $\mu$ M fresh or heated (1 h, 100°C) suspensions of the following fungicides: benomyl, thiophanate, thiophanate-methyl and NF 48. All the fungicides were applied as 50% wettable powders (for further details see Fuchs et al., 1972). Controls were immersed with their roots in water. The twelve seedlings used for each treatment remained in the suspensions for 48 h. Each group of twelve seedlings was then divided into two lots of six, and each lot transplanted into a pot (13.5 cm diam.) containing about 600 g garden soil. One pot from each treatment received an additional daily application of fungicide (50 ml of the 400  $\mu$ M suspensions) as a soil drench, for one week (long-term treatment), while the other pot received only water (short-term treatment). Eleven days after transplanting one plant from each pot was sampled and the remaining five were inoculated with powdery mildew (*Sphaerotheca fuliginea*). At intervals, whole inoculated plants or plant parts from each treatment were removed. Immediately after harvesting, whole plants were subdivided into several parts (hypocotyls plus cotyledons, and internodes plus adjacent leaves), and all plant samples weighed and subsequently frozen at -21°C. All frozen plant sections were ground with sand in a mortar, and extracted in 5 ml of 96% ethanol per g fresh weight. Aliquots of 50  $\mu$ l of each extract representing 10 mg fresh weight, were spotted onto silicagel plates and these developed in ethyl acetate and bioassayed using *Penicillium expansum* as the test organism (Homans and Fuchs, 1970). The inhibition zones, that were visible two days after spraying and caused solely by MBC, were outlined on tracing-paper (Schoellershammer Hochtransparent, Nr. 205 glatt; Düren, Germany), cut out and weighed in triplicate. A series of known amounts of MBC produced a standard curve<sup>2</sup> (Fig. 1), from which unknown amounts of fungitoxicant in inhibition zones could be calculated. With *P. expansum* amounts as low as 2.5 ng could be as-

<sup>2</sup> The change in slope of the dosage-response curve at about 20 ng is the result of the presence, at the origin, of some MBC in an ionic form, in addition to 'normal' MBC, when quantities of MBC are spotted, which exceed 20 ng.

Fig. 1. Sizes of inhibition zones on thin-layer chromatograms (Merck DC-Alufolie Kieselgel 60 F 254; test organism *Penicillium expansum*) in relation to amounts of MBC spotted; solvent ethyl acetate.

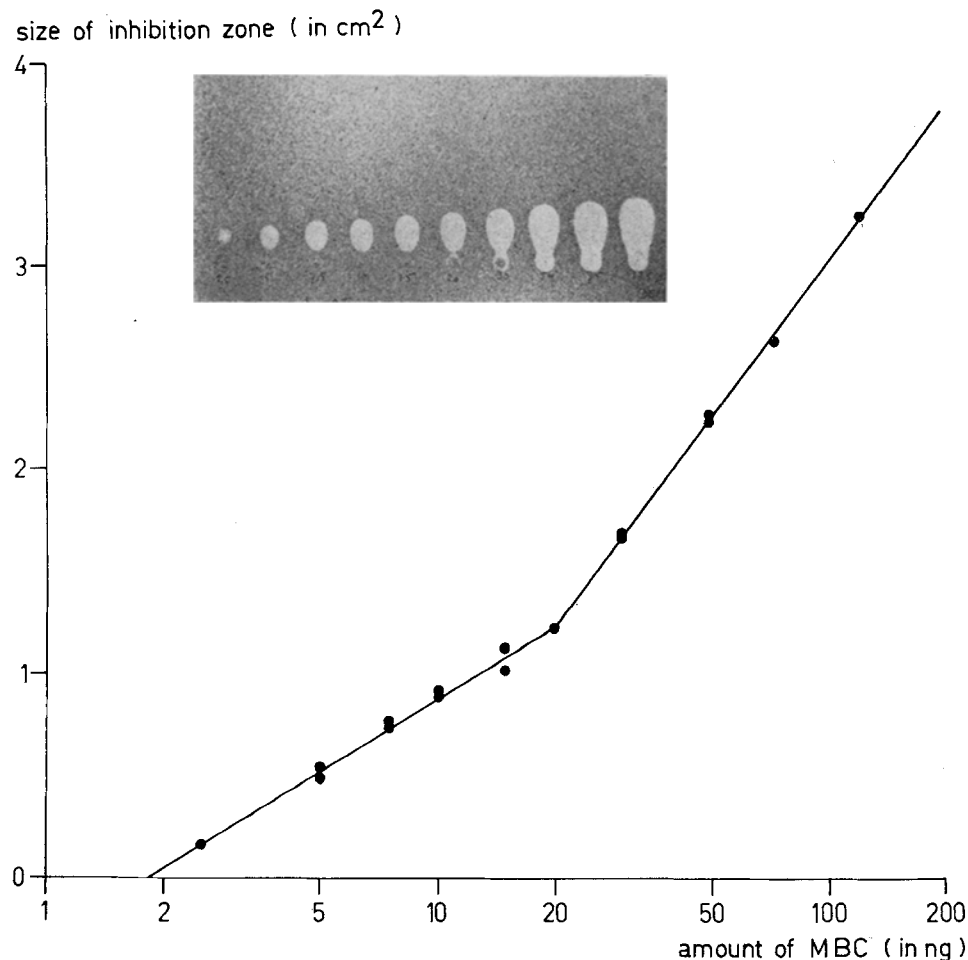


Fig. 1. Grootte van de remmingszones op dunnelaag chromatogrammen (Merck DC-Alufolie Kieselgel 60 F 254; toetsorganisme *Penicillium expansum*) in relatie tot hoeveelheid MBC; loopvloeistof ethyl-acetaat.

sayed accurately; therefore, in the above mentioned extracts concentrations as low as  $0.25 \mu\text{g/g}$  fresh weight could be measured quantitatively.

Seven to eight weeks old tomato plants, cvs 'Moneymaker' and 'Bonner Beste', were treated like the cucumber seedlings. However, each plant was potted separately; those subjected to a long-term treatment each received an additional daily amount of fungicide (50 ml of  $400 \mu\text{M}$  suspensions) for one week. All plants were uninoculated. At intervals, the stem of one plant from each treatment was cut off obliquely at approximately 5 cm above soil level, and the bleeding sap collected overnight, by placing a small tapered paper wick on the cut stem and allowing the bleeding sap to drip into a small test tube. The bleeding sap was examined for the presence of fungi-

toxicant as described above. Unfortunately, amounts of bleeding sap and concentrations of fungitoxicant were quite variable from plant to plant, and there was no distinct correlation between these parameters in any type of treatment applied. Therefore, only semi-quantitative data will be given.

### Results

Nine days after inoculation with powdery mildew conidia, symptoms were seen in the youngest (second) leaves of cucumber plants treated for two days (short-term treatment) with fresh suspensions of both thiophanate and NF 48 and with heated sus-

Fig. 2. Second leaves of cucumber seedlings showing (left) degree of infection with powdery mildew (*Sphaerotheca fuliginea*) and (right) concentration of fungitoxicant in  $\mu\text{g/g}$  fresh weight in the same leaves; EBC – the fungitoxic principle of thiophanate (NF 35) – expressed as MBC. Leaves sampled 9 days after inoculation. In fresh NF 48 treated plants (top row; right) the second leaves had not yet unfolded completely at the time of sampling; therefore, instead, a first leaf – not showing any symptoms of powdery mildew – has been used in taking the photograph and for the determination of the concentration of fungitoxicant. A: short-term treatment; B: long-term treatment; NF 44 = thiophanate-methyl; for further details see text.

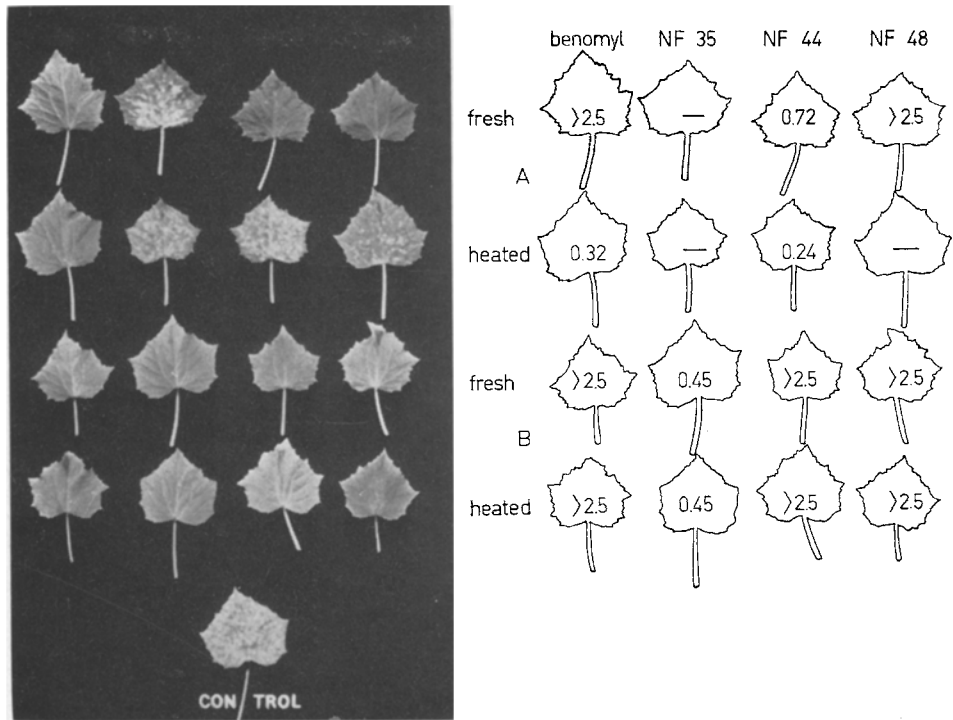


Fig. 2. Bladeren van op verschillende wijze behandelde komkommerzaailingen (cv. 'Lange Gele Tros') (links) al of niet geïnfecteerd door komkommermeeldauw (*Sphaerotheca fuliginea*) en (rechts) met de concentratie van MBC (resp. EBC) in  $\mu\text{g/g}$  vers gewicht in dezelfde bladeren aangegeven. Bladeren 9 dagen na inoculatie geoogst. A: kortdurende behandeling; B: voortgezette behandeling; NF 35 = thiophanaat; NF 44 = thiophanaat-methyl.

pensions of thiophanate, thiophanate-methyl and NF 48. On comparable heated benomyl-treated plants infection was barely visible. Degree of infection was correlated with concentration of MBC (or EBC) within the leaves (Fig. 2), 0.35  $\mu\text{g/g}$  fresh weight being the limiting concentration for symptom expression. Later, plants from the following short-term treatments became infected successively, first heated benomyl (distinct symptoms 17 days p.i.) and then fresh thiophanate-methyl-treated plants (22 days p.i.); at that time neither fresh benomyl-treated plants from the short-term treatment nor any of the plants from the long-term treatments were showing signs of mildew.

MBC (or EBC) distribution within all plants, both healthy and inoculated, showed concentration gradients that appeared to depend directly upon the treatment and the fungicide applied. From Table 1 it is evident, that up to 23 days after inoculation, in all long-term treatments with both fresh and heated fungicides enough MBC (or EBC) was present even in the youngest leaves to ensure complete protection against powdery mildew infection. However, after short-term treatment only in fresh benomyl-treated plants were concentrations (0.4  $\mu\text{g/g}$  fresh weight) of MBC up to the youngest (third) leaves large enough to keep the plants free from powdery mildew. With the other

Table 1. Concentrations of fungitoxicant in  $\mu\text{g/g}$  fresh weight in cotyledons and first, second, and third leaves (plus adjacent internodes) of powdery mildew inoculated cucumber seedlings (cv. 'Lange Gele Tros') in relation to treatment (short-term versus long-term; for details see text and legend). Age of plants at time of harvesting – 23 days after inoculation – was 43 days; all leaves with concentration  $> 0.35 \mu\text{g/g}$  fresh weight were free from powdery mildew.

Fungicide applied	Short-term treatment <sup>1</sup>				Long-term treatment <sup>2</sup>			
	cot.	1st	2nd	3rd <sup>3</sup>	cot.	1st	2nd	3rd <sup>3</sup>
benomyl fresh	1.05	$>2.5$	$>2.5$	0.40	$>2.5$	$>2.5$	$>2.5$	$>2.5$
benomyl heated	0.27	$>2.5$	0.25	– <sup>4</sup>	$>2.5$	$>2.5$	$>2.5$	$>2.5$
thiophanate fresh	0.86	–	–	–	2.12	$>2.5$	1.14	0.43
thiophanate heated	–	–	–	–	1.60	$>2.5$	1.38	0.45
thiophanate-methyl fresh	1.14	$>2.5$	0.96	–	$>2.5$	$>2.5$	$>2.5$	$>2.5$
thiophanate-methyl heated	1.03	$>2.5$	0.33	–	$>2.5$	$>2.5$	$>2.5$	$>2.5$
NF 48 fresh	0.71	$>2.5$	–	–	$>2.5$	$>2.5$	$>2.5$	$>2.5$
NF 48 heated	0.26	1.25	–	–	1.66	$>2.5$	$>2.5$	$>2.5$

<sup>1</sup> One week old seedlings immersed with their roots in 400  $\mu\text{M}$  aqueous suspensions of fungicide for 2 days.

<sup>2</sup> Idem, plus, after transplanting into soil, a daily drench of 50 ml of 400  $\mu\text{M}$  aqueous suspensions of the same fungicide to potted seedlings for one week.

<sup>3</sup> Cotyledons and 1st, 2nd, and 3rd leaf, respectively.

<sup>4</sup> –: fungitoxicant below detection level (0.20  $\mu\text{g/g}$  fresh weight).

*Tabel 1. Concentraties van MBC (resp. EBC) in  $\mu\text{g/g}$  vers gewicht in cotylen en 1e, 2e en 3e bladeren (met bijbehorende internodiën) van met komkommermeeldauw geïnoculeerde komkommerzaailingen (cv. 'Lange Gele Tros') in relatie tot de behandelingswijze (kortdurend tegenover voortgezet; zie Samenvatting). De leeftijd der planten op het moment van monsternamen – 23 dagen na inoculatie – was 43 dagen; alle bladeren met concentraties  $>0,35 \mu\text{g/g}$  vers gewicht waren vrij van meeldauw.*

fungicides, the time of first appearance of powdery mildew symptoms as well as the degree of infection completely reflected the concentration gradients of MBC (or EBC) in the leaves. Another feature of mildewed plants, when treated with fresh suspensions of thiophanate, thiophanate-methyl and NF 48 (short-term treatment) was the frequent presence of a clear, disease-free margin around the edge of infected leaves (Fig. 3). In these cases 'overall' concentrations of MBC (or EBC) were apparently insufficient to protect the leaves from becoming infected, but in the leaf margins the fungitoxicant still accumulated above the limiting concentration for symptom expression. A similar lack of protection in the center of cucumber leaves has been observed by Schroeder and Provvidenti (1968) when low dosages of benomyl were applied.

Table 2 summarizes results of the experiments with tomato plants. It appears, that the presence of fungitoxicant in the bleeding sap again greatly depended upon the treatment and the fungicide applied: after short-term treatment MBC was detected in fresh NF 48-treated plants up to only 7 days, but in fresh benomyl-treated plants up to 49 days after discontinuation of the treatment. However, after long-term treatment MBC was present at least up to 42 days after termination of the treatment (fresh thiophanate-methyl-treated plants), but usually until the end of the experimental period (85 days after discontinuation of the treatment). After thiophanate treatment, concentrations of EBC in bleeding sap decreased rapidly and became virtually undetectable with the bioassay employed within 2-3 weeks after discontinuation of the drenching.

Table 2. Presence of fungitoxicant in bleeding sap of tomato plants (cv. 'Bonner Beste') in relation to treatment (short-term versus long-term; for details see text and legend of Table 1). Age of plants at time of start of treatment (13-3) 53 days.

	Short-term treatment <sup>1</sup>				Long-term treatment <sup>2</sup>					
dates of sampling days from start of treatment	22-3	5-4	19-4	3-5	22-3	5-4	19-4	3-5	25-5	15-6
	9	23	37	51	9	23	37	51	73	94
fungicide applied										
benomyl fresh	+	+	±	trace	+	+	+	+	+	+
benomyl heated	+	+			+	+	+	+	+	±
thiophanate fresh	±	trace			±	±				
thiophanate heated	±				±	trace	trace			
thiophanate-methyl fresh	+	+	trace		+	+	+	+		
thiophante-methyl heated	+	+	±		+	+	+	+	+	±
NF 48 fresh	+				+	+	+	+	+	
NF 48 heated	+	+			+	+	+	+	+	+

<sup>1</sup> Plants immersed with their roots in 400 µM aqueous suspensions of fungicide for 2 days.

<sup>2</sup> Idem, plus, after transplanting into soil, a daily drench of 50 ml of 400 µM aqueous suspensions of the same fungicide to potted plants for one week.

*Table 2. Aanwezigheid van MBC (resp. EBC) in het bloedingssap van tomatplanten (cv. 'Bonner Beste') in relatie tot de behandelingswijze (kortdurend tegenover voortgezet; zie Samenvatting). De leeftijd der planten was bij de aanvang der behandeling (13 maart) 53 dagen.*

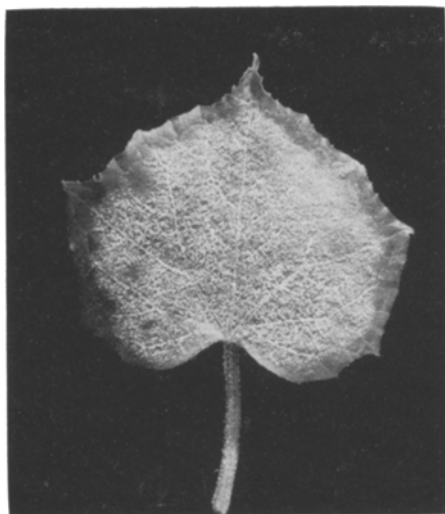


Fig. 3. Leaf of fresh thiophanate-methyl (NF 44) treated cucumber seedling showing disease-free margin.

*Fig. 3. Blad van met thiophanaat-methyl (NF 44) behandelde komkommerzaailing met meeldauwvrije rand.*

## Discussion

Short-term treatment of cucumber seedlings with fresh and heated aqueous suspensions of benomyl and three closely related thiophanates for 48 h resulted in distribution patterns of fungitoxicant in above-ground plant parts (Table 1; short-term treatment) comparable with those of bean plants (Fuchs et al., 1972). In leaves unfolded after termination of the treatments, only with fresh benomyl and thiophanate-methyl were sufficient amounts of fungitoxicant present to protect these leaves against powdery mildew infection; with fresh benomyl this protection continued until the end of the experiment, 34 days after termination of the treatment. Periodic examination of the bleeding sap of tomato plants, treated for 2 days with the same fungicides, again showed fresh benomyl to be superior, fungitoxicant being present longer (49 days) than with any of the other compounds (Table 2, short-term treatment). The data of both cucumber seedlings and tomato plants thus support our hypothesis that N-1 substituted MBC derivatives (benomyl, intermediate conversion product of thiophanate-methyl) are retained to some extent in or on the roots, and gradually converted to the fungitoxicant, which then moves into the shoots (Fuchs et al., 1972).

The amount of fungicide adsorbed to – or taken up by – the roots during the treatment period probably depends on the concentration of the fungicide applied and the size of the root system as well as its adsorptive, or absorptive, capacity. Part of the adsorbed fungicide might be released again into the planting medium after transplanting the seedlings. When plants are transplanted into soil, this release might be of lesser significance than when they are transferred back to a liquid planting medium after the fungicide treatment, as in the experiments of Siegel and Zabbia (1972). After a four days root treatment of dwarf pea plants with  $^{14}\text{C}$ -benomyl dispersed in Knop's solution, in their experiments, the roots were washed in running water and the plants transferred to fresh nutrient solution. The label in the roots proved to be translocated primarily to the foliage present at the time of treatment, 10 times less label being found in the foliage grown after discontinuation of the  $^{14}\text{C}$ -benomyl

treatment. Since the label present in the lower leaves did not appear to be remobilized and translocated to the upper foliage, Siegel and Zabbia (1972) inferred that the small amount of radioactivity in the latter leaves could have been translocated from the roots. Although there were differences in the method of application of benomyl and in the nature of the planting medium to which the seedlings were transferred after the fungicide treatment, their results thus confirm our observations.

In other respects their data also corroborate our results: no  $^{14}\text{C}$ -benomyl was recovered from the plants at any time,  $^{14}\text{C}$ -MBC being the main metabolite present. As distinct from these findings and those of others (Peterson and Edgington, 1970, 1971; Pionnat, 1971; Siegel and Zabbia, 1971, 1972) chromatographic analysis of extracts of benomyl-treated creeping bentgrass (Meyer et al., 1971; Nicholson et al., 1971) and strawberry plants (Nicholson et al., 1972) revealed translocation of both benomyl and MBC up to the growing point and throughout all tissues. In a comparative study on the systemic activity of MBC, benomyl and thiophanate-methyl in bean plants, Leroux and Gredt (1972) made similar observations. On root application of benomyl, they detected not only MBC, but also the parent compound, although the latter was confined to the roots. Thiophanate-methyl root-treated bean plants, on the other hand, showed the presence of both MBC and the parent compound also in stems and primary leaves. In our experiments, only thiophanate-methyl and NF 48 were detected very rarely in above-ground plant parts (cf. Fuchs et al., 1972, Fig. 5).

As already pointed out by Siegel and Zabbia (1972), failure to recover the parent compound as such from benomyl-treated plants could be due to its rapid breakdown to MBC either in the aqueous suspension, in the plant or during the extraction procedure. Although even at room temperature and almost independently of pH, extensive decomposition of benomyl takes place in aqueous suspensions (Fuchs et al., 1972), our results indicate, that fresh suspensions – at least at the concentrations we used – still contain appreciable amounts of undegraded benomyl. Because the presence of MBC as the sole fungitoxicant in plants was independent of the extraction procedure employed, the complete absence of benomyl in the extracts of above-ground plant parts could, therefore, be due to hydrolytic breakdown of this compound within the plant. In this connection it is worth mentioning, that Matta and Gentile (1971) showed, that plant tissues are able to ‘activate’ thiophanates with production of MBC (or EBC). Indirect proof for the presence in shoots of MBC only can be derived from experiments of Reilly and Klarman (1972). They observed that MBC, as distinct from benomyl and butylamine (which arises from the butylcarbamoyl side chain of benomyl) did not induce phytoalexin production in excised soybean hypocotyls. Since exposure of soybean roots to benomyl did not result in the induction of phytoalexin in the above-ground plant portions, it seemed tempting to suggest that neither benomyl nor butylamine were present there; thus, the fungitoxicant recovered could be assumed to correspond to MBC, as was further substantiated by TLC bioassays.

Depending on the amount of fungicide adsorbed to or absorbed by the roots during short-term treatment, there will be a shorter or longer lasting ‘constant’ supply of the aerial parts with fungitoxicant, and accumulation in leaf tips and margins. This means that as long as MBC (or EBC) moving to the leaves with the transpiration stream (Peterson and Edgington, 1971) is continuously replenished to a concentration exceeding the limiting concentration for symptom expression (0.35



µg/g fresh weight in the case of cucumber powdery mildew) no disease symptoms will become apparent.

As has been repeatedly observed (Mercer, 1971; Smith and Spencer, 1971; Siegel and Zabbia, 1972) no redistribution of fungitoxicant from older to younger leaves seems to take place. The cotyledons, however, might constitute an exception to this rule: in our experiments concentrations of fungitoxicant in cotyledons at the time of extraction (Table 1) were not only lower than those in the first leaves, but quite frequently symptoms were found to appear on the cotyledons at an advanced stage of the disease, suggesting either inactivation or removal of the fungitoxicant. Similar observations with different plants have been made by other authors (cf. Thapliyal and Sinclair, 1971).

Although benomyl and thiophanate-methyl are retained to some extent on or in the roots, the results of our short-term treatments indicate, that under such circumstances the roots of plants become depleted too soon after transplanting to non-treated garden soil to afford long-term protection against fungal attack. In other words, the reservoir function of roots is evidently only of limited practical importance, so that long-term application, for instance as soil drenches, is necessary for continued protection. Pots from long-term treatments containing either six cucumber seedlings or one tomato plant, were drenched with a total quantity of 140 µmole of the respective fungicide within one week, which corresponds to 40.6, 51.8, 47.9, and 31.5 mg of benomyl, thiophanate, thiophanate-methyl, and NF 48, respectively. These additional supplies of fungicide were able to give 100% protection against cucumber powdery mildew for the entire experimental period (23 days after inoculation) even in the case of thiophanate, which is the least effective of all fungicides tested. Mercer (1971) found ED<sub>90</sub>-values for cucumber powdery mildew control 6 weeks after fungicide application, which ranged from 2.5 mg/kg air dry soil for NF 48 and 2.7 mg/kg for thiophanate-methyl to 4.9 mg/kg for thiophanate. Since the above-mentioned quantities were added to about 600 g soil, in our experiments concentrations of fungicides have been well above the latter; hence, our results confirm these of Mercer. Usually, also in tomato plants fungitoxicant was detectable until the end of the experimental period, 85 days after termination of the treatment.

For benomyl, similar observations on the differential effect of short-term treatment, followed by transplanting to a non-fungicide containing planting medium, versus long-term treatment without transplanting have been made by numerous investigators with a large variety of crops. Upon transplanting to fresh medium, concentrations of fungitoxicant within plant roots and stems usually decline rapidly with concomitant accumulation in leaf tips and margins (Peterson and Edgington, 1969, 1970; Biehn and Dimond, 1970; Gray and Sinclair, 1970; Hock et al., 1970; Attabhanyo and Holcomb, 1972) however, sometimes even in the stems fungitoxicant remained detectable for many months (Shanmuganathan and Bopearatchy, 1971).

Observations on considerable reduction of disease incidence in carnations after a short 'dip treatment' before planting (Tramier and Antonini, 1971) are in agreement with these reports. However, a much longer lasting protection resulted from soil treatments without the plants being transferred after treatment (Tramier and Antonini, 1971). Because of its extreme persistence in soils (and in plant tissues) (Hock et al., 1970; Jacobsen and Williams, 1970; Netzer and Dishon, 1970; Schreiber et al., 1971; Riesselman and Weihing, 1972; Sabet et al., 1972) benomyl can often completely

protect plants for many months, sometimes for up to 3 years after its application (Biehn and Dimond, 1971; Biehn, 1973). This residual activity of benomyl might imply that a single application to the soil might be effective over several successive crops (Jacobsen and Williams, 1970).

The nature of the planting medium undoubtedly plays an important role. From comparative studies on the influence of different soil types (sand, silt loam, and various potting mixtures) on benomyl uptake (Hock et al., 1970; Hock and Schreiber, 1971a, b; Pellissier et al., 1971; Schreiber et al., 1971) it can be concluded that the planting medium affects total amount and rate of uptake of the fungitoxicant considerably. Highest levels of accumulation are found to be correlated with lowest proportion of organic matter<sup>3</sup> and highest pH (Schreiber et al., 1971). Heat sterilization of soil has also proved to have some effect on uptake of benomyl (Hock and Schreiber, 1971b; Schreiber et al., 1971). Further, root uptake of fungicides might be appreciably affected by addition of surfactants (Biehn and Dimond, 1971; Pitblado and Edgington, 1971; Biehn, 1973; see, however, Hock and Schreiber, 1971a), which might increase mobility of benomyl in soils. On the other hand, movement will also vary due to, for instance, buffering capacity of the soil, proportion of organic matter, cation exchange capacity, soil moisture, etc. (Pitblado and Edgington, 1972).

It is evident, that the formation and maintenance of a deposit of benomyl (or thiophanates) in plant roots and soil is highly complex. Long-term chemotherapeutic effects of MBC will be dependent on the type of planting medium, with its variable physico-chemical characteristics, on the mode of application and on the concentration of fungicide used, which finally determine distribution and concentration of fungitoxicant within the plant. That soil application of such persistent fungicides like benomyl and thiophanates might, however, have its serious drawbacks, for instance through disturbance of soil ecosystems, should be recognized.

## Samenvatting

### *De functie van een MBC leverend reservoir van benomyl en thiophanaten in plantewortels en in grond*

Een vergelijkend onderzoek werd ingesteld naar de effecten van kortdurende behandeling tegenover voortgezette behandeling met benomyl en drie thiophanaten wat betreft de verdeling van de eigenlijke fungitoxische verbinding (MBC, respectievelijk EBC) in planten en hun bescherming tegen schimmelziekten. Bij beide behandelingen werden de planten gedurende twee dagen aan de fungiciden blootgesteld en daarna in tuingrond geplant; in het geval van de voortgezette behandeling werd dagelijks gedurende één week een hoeveelheid fungicide in het gietwater toegevoegd.

De resultaten van de kortdurende behandeling van komkommerzaailingen en tomatplanten ondersteunden de hypothese, dat MBC-derivaten, die op de N-1 plaats in de benzimidazoolring gesubstitueerd zijn, tot op zekere hoogte in de wortels worden 'vastgelegd', waar ze geleidelijk in MBC worden omgezet en vandaar als zodanig

<sup>3</sup> Because of the relatively high organic matter content (about 60%) of the garden soil we used in our experiments, conditions in the long-term treatments were, in fact, far from optimal for maximal fungicide uptake.

naar de bovengrondse delen worden getransporteerd. Correlering van infectie met concentratie van het MBC in de bladeren liet zien, dat 0,35  $\mu\text{g/g}$  vers gewicht de grensconcentratie is, waarboven geen symptomen van komkommermeeldauw meer optreden. Alleen in het geval van benomylbehandeling werd MBC voldoende lang naar de bovengrondse delen vervoerd om ook die bladeren die zich na beëindiging der behandeling ontplooiden, gedurende enkele weken te beschermen tegen meeldauw.

Bij voortgezette behandeling werd de genoemde concentratie met alle fungiciden in alle bovengrondse plantedelen tot het einde van de onderzoeksperiode gehandhaafd. Klaarblijkelijk garandeert een zekere voorraad van het fungicide in de grond een continue toevoer van het fungicide aan de plant; alleen onder die omstandigheden kunnen nieuwgevormde plantedelen doeltreffend worden beschermd.

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