Neth. J. Pl. Path. 81(1975) 94–101

Evaluation of benomyl and thiophanate-methyl for the control of Verticillium wilt of strawberry in the Netherlands

H. A. Th. VAN DER SCHEER[1,3], H. J. WONDERGEM[1] and B. J. LUIMES[2]

[1] Research Station for Fruit Growing, Wilhelminadorp (Goes)
[2] Horticultural Advisory Service, Tiel

Accepted 9 January 1975

Abstract

In pot experiments the fungicides benomyl and thiophanate-methyl controlled *Verticillium* wilt of strawberry when applied as a soil drench after planting. Both compounds were ineffective as foliar sprays and as root dips prior to planting.

Soil drenches applied to commercially grown runner plants in the waiting field (August) and to the same plants in the greenhouse (December or January) increased the yield.

On infested ground, a soil drench with thiophanate-methyl promoted the occurrence of crown rot caused by *Phytophthora cactorum*.

Introduction

Verticillium wilt is a wide-spread disease in Dutch strawberry plantations and causes losses amounting to 5 to 10 per cent loss of the crop. Control is urgently needed, especially for strawberries under glass, because in this culture with high production costs a small drop in yield can put the grower in the red.

Cultural measures are applied to prevent *Verticillium* wilt infection of the runner plants grown in the open . In glasshouses chloropicrin and chloropicrin-methyl bromide mixtures are well known as fumigants. In the Netherlands, the use of these treatments is restricted because of the noxious effect when incorrectly applied, and therefore effective substitutes are needed. Since benomyl has given promising results in reducing the severity of wilt in the strawberry (Lockhart et al., 1969; Jordan, 1972) this fungicide and thiophanate-methyl were tested for disease control, phytotoxicity, and influence on yield.

Materials and methods

Fungicides. The fungicides Benlate (benomyl, 50 % wettable powder) and Topsin M (thiophanate-methyl, 70 % wettable powder) were applied as soil drenches, root dips, or foliar sprays in several trials.

Host, pathogen, and inoculation. Strawberry plants of the varieties Glasa and Gorella

[3] Guest worker at the Institute of Phytopathological Research (IPO), Wageningen.

were inoculated by immersing the roots of sets of 25 plants in 1 l of a *Verticillium dahliae* culture suspension for 1/2 h before planting. The suspension was prepared by homogenizing 2 (sometimes 3) 14-day-old fungus cultures on Czapek Dox agar in water. Root systems of control plants were immersed in a suspension of macerated Czapek Dox agar medium.

Experimental circumstances. Pot experiments were carried out in a greenhouse. The strawberries were planted in 3-litre buckets hanging in a water bath to keep the soil temperature at 24 °C.

Field trials were carried out on plants grown in the open and on commercially grown plants under glass. The latter were produced on multiplication fields, transplanted to waiting fields at the end of July, and again to greenhouses at the beginning of December (10 plants per m^2). Routine insecticidal sprays and sprays for fruit-rot control with Euparen (dichlofluanid, 50 % wettable powder) were applied if necessary.

Measurement, assessment, and isolation. For the commercially grown plants, yields were expressed as yield per m^2. Plant vigour was assessed by a visual estimate of plant size on a scale from 1 (small) to 10 (large). Isolations from leaf stalks on Czapek Dox agar medium were made to confirm the presence of the pathogen.

Experiment A. In 1970/1971 and 1971/1972 the Horticultural Advisory Service at Tiel performed 3 trials per season on commercially grown plants. Soil drenches consisting of 0.5 l of suspensions with various concentrations of benomyl or thiophanate-methyl per plant were applied to mother plants (May), runner plants on the waiting fields (August), and the same plants in the greenhouses (December in 1970, January in 1972).

Experiment B. The effects of foliar sprays with fungicide suspensions at a dosage of 1500 l/ha were tested on 'Gorella' plants grown in the open in 1972/1973 and 1973/1974. In the first trial 3 randomized blocks of 10 treatments were subdivided into plots, each with 6 plants. The second trial comprised 12 randomized blocks of 10 treatments subdivided into plots, each with 3 plants.

Experiment C. In a pot experiment, methods of application of benomyl to cold-storage runner plants of the variety Gorella were tested in 1973. A root dip was performed by immersing the root systems in a suspension of benomyl for 1/2 h. The plants were then allowed to dry for 2 days, after which they were inoculated and potted. A soil drench was carried out by pouring 100 ml of a benomyl suspension into each bucket 7 days after inoculation. Foliar sprays with benomyl were applied once a week during 6 weeks, starting 7 days after inoculation. During each spray application the soil surface was covered with plastic sheeting to prevent contact with the fungicide. The buckets were filled with peat-sand mixtures on 8 July. Each treatment comprized 5 buckets, each holding 2 plants.

Experiment D. In another pot experiment, the effect of a soil drench with benomyl (1 or 7 days after planting) in 2 types of soil (silty clay-loam and peat-sand) was tested on 'Gorella' plants. The experiment comprized 12 treatments, each with 6 buckets holding two plants, and was started on 11 October 1973.

Experiment E. On outdoor-grown 'Glasa' plants soil drenches (0.5 l per plant) with suspensions of dexon, thiophanate-methyl, and etridiazol were carried out on 11 August 1971. The plants were grown on soil known to be infested with *Phytophthora cactorum*. Due to severe losses, the plants were not transferred to a greenhouse. *P. cactorum* attack was visually evaluated on 19 October 1971. Isolations from some plants were carried out to verify the presence of the fungus. The trial comprized 4 treatments in 4 replicates, each with 20 plants.

Results

1. *Effect of soil drenches on yield of fruit.* In 1970/1971 the fungicide treatments on the waiting field, and in the greenhouse enhanced the yield by about 25%. The results indicated no effect on the yield by the fungicide application to the plants on the multiplication field.

In the next season the trials were repeated on a larger scale. The treatments gave the same effects on yield as those in the preceding season, but the results were statistically significant only between some of them (Table 1).

At the moment of transferring to the greenhouse, the treated plants showed more extensive root systems than untreated ones.

Table 1. Effects of soil drenches with benomyl (b) and thiophanate-methyl (t) on the yields of the strawberry varieties Glasa (farms 1 and 2) and Gorella (farm 3) in 1971/1972.

Soil drench with 0.5 litre fungicide suspension per plant			Yield (g) per m^2		
multiplication field	waiting field	greenhouse	farm 1	farm 2	farm 3
0.042% t	untreated	untreated	2159a[1]	1631a	3517a
0.042% t	0.042% t	untreated	2439ab	1697ab	
0.042% t	untreated	0.042% t	2646b		3360a
0.042% t	0.042% t	0.042% t	2432ab	1835b	3885a
untreated	0.042% t	0.042% t			3736a
0.042% t	0.02 % b	untreated			3696a
0.042% t	0.02 % b	0.02 % b	2556b	1860b	3406a

[1] a and b indicate groupings of treatments not differing significantly at the 5% level.

Tabel 1. Invloed van het aangieten van aardbeiplanten met benomyl (b) en thiofanaat-methyl (t) op de opbrengst van de rassen Glasa (bedrijf 1 en 2) en Gorella (bedrijf 3).

2. *Effect of foliar sprays on size of plants grown in the open.* In two trials in consecutive seasons the strawberry plants were not protected against *Verticillium* wilt by foliar sprays with a suspension of 0.05% benomyl or 0.07% thiophanate-methyl, applied after artificial inoculation (Table 2). *Verticillium dahliae* was also present in uninoculated plants, probably due to the fact that the trials were performed on a site which had a previous history of strawberry wilt.

Table 2. Control of *Verticillium* wilt of artificially inoculated plants of the strawberry variety Gorella by fungicide sprays (spray dosage 1500 l/ha) in trials in 1972/1973 and 1973/1974.

Fungicide treatment	Inoculation[1]	1972/1973		1973/1974	
		plant size on 25 July 1973 (1–10 scale)	number of *Vert.*-infected plants (max. 18)	plant size on 18 May 1974 (1–10 scale)	number of *Vert.*-infected plants (max. 36)
0.07% thiophanate-methyl 1×[2]	+	4.0 ab[3]	16	4.9 ab	17
0.07% thiophanate-methyl 1×	−	6.7 bcd	8	6.5 d	10
0.07% thiophanate-methyl 3×	+	4.0 ab	15	5.7 bcd	23
0.07% thiophanate-methyl 3×	−	6.5 bcd	4	6.7 d	18
0.05% benomyl 1×	+	2.7 a	15		
0.05% benomyl 1×	−	6.5 bcd	7		
0.05% benomyl 3×	+	4.5 abc	14		
0.05% benomyl 3×	−	8.0 d	5		
untreated	+	3.3 a	13	4.6 a	28
untreated	−	7.0 bcd	6	6.8 d	10

[1] + = performed, − = not performed.
[2] 1× = 1 fungicide application, 1 week after planting.
3× = 3 fungicide applications, 1 week after planting, $4^1/_2$ weeks after planting, and at restart of growth in the next year.
[3] a–d indicate groupings of treatments not differing significantly at the 5% level.

Tabel 2. Bestrijding van Verticillium verwelkingsziekte bij kunstmatig besmette planten van het aardbeiras Gorella door middel van fungicidebespuitingen (hoeveelheid spuitvloeistof 1500 l/ha) in een proef in 1972/1973 en in 1973/1974.

Table 3. Effect of benomyl treatments applied to artificially inoculated strawberry plants of the variety Gorella on plant size and infection of the petioles in 1973.

Method of application	Inoculation[1]	Plant size (1–10 scale)		Percentage of *Vert.*-infected petioles
		15 Aug. 1973	3 Sept. 1973	
soil drench	+	7.2	6.8 b[2]	4
leaf sprays	+	6.0	4.4 a	–
soil drench + leaf sprays	+	7.8	7.0 b	15
root dip	+	5.8	4.0 a	–
soil drench + leaf sprays	−	8.0	8.3 b	–
untreated	+	5.6	4.3 a	23
untreated	−	7.4	8.1 b	0

[1] + = performed, − = not performed.
[2] a and b indicate groupings of treatments not differing at the 5% level.

Tabel 3. Invloed van benomyl behandelingen op plantgrootte en bladsteelbesmetting van kunstmatig besmette aardbeiplanten van het ras Gorella in 1973.

3. *Comparison of various methods of fungicide application.* The potted plants were not protected against *Verticillium* wilt by the applied root dip or by foliar sprays with a

suspension of 0.005% benomyl (Table 3). The soil drench (100 ml of 0.1% benomyl suspension) prevented visible wilt symptoms, although *Verticillium dahliae* was present in the petioles.

Table 4. Effect of soil drenches with benomyl on strawberry plants of the variety Gorella grown in different types of soil.

Treatment	Time of application	Soil type	Inoculation[1]	Plant size (1–10 scale) 12 Dec. 1973	16 Jan. 1974	7 March 1974	Plant weight (g) 11–15 March 1974
benomyl	D 1[2]	silty clay-loam	+	6.3 ab[3]	7.0 b	6.5 a	21.6 ab
benomyl	D 7	silty clay-loam	+	6.7 ab	7.4 bc	6.7 a	19.7 ab
untreated		silty clay-loam	+	5.2 a	5.7 a	5.7 a	14.0 a
benomyl	D 1	silty clay-loam	−	6.8 ab	7.3 bc	6.7 a	23.7 ab
benomyl	D 7	silty clay-loam	−	6.3 ab	7.0 b	6.2 a	20.0 ab
untreated		silty clay-loam	−	5.8 ab	5.9 a	6.1 a	19.4 ab
benomyl	D 1	peat-sand	+	7.6 b	8.3 cd	7.7 b	34.6 cd
benomyl	D 7	peat-sand	+	7.0 ab	7.7 bcd	7.9 b	34.8 cd
untreated		peat-sand	+	5.0 a	6.1 a	5.9 a	19.3 ab
benomyl	D 1	peat-sand	−	7.7 b	8.5 d	8.1 b	40.2 d
benomyl	D 7	peat-sand	−	7.7 b	8.2 cd	8.3 b	39.3 cd
untreated		peat-sand	−	7.0 ab	7.3 bc	7.5 b	29.2 bc

[1] + = performed, − = not performed.
[2] Soil drench, 100 ml per bucket 0.1% benomyl 1 day after planting (D1) or 7 days after planting (D7).
[3] a–d indicate groupings of treatments not differing significantly at the 5% level.

Tabel 4. Invloed van het aangieten met benomyl van planten van het aardbeiras Gorella, die in verschillende grondsoorten groeiden.

Table 5. Effect of soil drenches with fungicide suspensions on plants of the variety Glasa on *Phytophthora cactorum* attack.

Treatment		Percentage dead and diseased plants, 70 days after treatment
0.042%	thiophanate-methyl	21 b[1]
0.21 %	dexon	5 a
0.07 %	etridiazol	10 ab
0.042%	thiophanate-methyl	23 b
0.21 %	dexon	4 a
	untreated	10 ab

[1] a and b indicate groupings of treatments not differing significantly at the 5% level.

Tabel 5. Invloed van het aangieten van planten van het ras Glasa met fungicidenoplossingen op de aantasting door Phytophthora cactorum.

4. *Effect of a soil drench applied to different types of soil on the size and weight of potted plants.* Table 4 shows the effects on the size and weight of the plants. At the second evaluation, made 97 days after inoculation, differences in plant size between treatments were more evident than 62 and 147 days after inoculation. The benomyl-

treated, uninoculated plants were larger than untreated, uninoculated plants, both grown on the clay-loam soil, indicating (indirect) growth stimulation by benomyl. No interaction was found between soil type, fungicide treatment, and inoculation. Plant growth was better in peat-sand than in silty clay-loam, and benomyl has a greater positive effect on plant size in the latter soil type.

5. *Effect of soil drenches with fungicide suspensions on Phytophthora cactorum attack to outdoor-grown plants.* The trial in which some treatments were done in duplicate to be split up at a later stage, was ended prematurely due to the increase of *Phytophthora* crown rot by the soil drench with thiophanate-methyl (Table 5). The dexon-treated plants showed less crown rot, while plants treated with etridiazol did not differ from the untreated control.

Discussion

Verticillium wilt of chrysanthemum leaves was controlled by foliar application of benomyl in a very low dosage, whereas untreated leaves on the same plant showed wilt symptoms (Busch and Hall, 1971). Jordan (1973) detected no pathogen in the leaves of strawberry plants given a leaf dip in a suspension 0.1 % benomyl, until the 28th day after inoculation, after which the pathogen level remained very low until the end of the experiment, 70 days after inoculation. Our results show that leaf sprays with suspensions of 0.05 % benomyl or 0.07 % thiophanate-methyl had very little effect on strawberry plant size 10 months after inoculation (the interval between planting and harvest). This is in agreement with the results of Lockhart et al. (1969). Both compounds are fungistatic in their action on *Verticillium dahliae* (Jordan, 1973). It seems possible that with foliar application the concentration of these compounds on the root surface dropped below or never reached the fungistatic levels required to prevent wilt symptoms in the plants.

In pot experiments soil drenches of 0.1 g benomyl per plant controlled wilt symptoms for as long as 5 months (up to the end of the experiments), but the fungus was present in the petioles of the inoculated plants. Jordan (1972) obtained similar results in a field trial with uninoculated plants on infested soil.

Mother plants treated with benomyl may produce more runner plants than the controls (Lockhart et al., 1969). In our trials plants treated with benomyl or thiophanate-methyl in the waiting field showed a larger root system than controls at the time of transfer to the greenhouse. Another example of the (indirect) effect of benomyl on plant growth was the larger size of treated, uninoculated plants as compared with the controls in pot experiments.

In the culture under glass, benomyl- or thiophanate-methyl-treated plants had fewer dead leaves after regrowth in spring. Therefore, the cultural measure of removing dead leaves from greenhouse plants after regrowth required much less time for the treated plants. In addition, less heart rot (infection of petioles by *Botrytis cinerea* in the heart of plants) occurred in the fungicide treated plants. Control of heart rot in outdoor-grown strawberry plants of the variety Senga Sengana by a benomyl treatment is reported by Wijshoff (1973).

Use of benomyl for control of *Verticillium* wilt may be dangerous with respect to the development of tolerant or resistant strains of *Botrytis cinerea*, as reported by

Jarvis and Hargreaves (1973). We found that thiophanate-methyl favoured crown rot of strawberry caused by *Phytophthora cactorum*.

Presumably, thiophanate-methyl acted as a suppressor of antagonists of *Phytophthora cactorum*, because this fungicide behaves like benomyl (Bollen, 1972), which does not inhibit growth of Oomycetes (Bollen and Fuchs, 1970).

An other dangerous aspect of the replacement of unwanted chloropicrin and chloropicrin-methyl bromide mixtures by (high dosages of) benomyl or thiophanate-methyl is the appearance of persistent residues in the soil with unpredictable consequences.

Benomyl and thiophanate-methyl may raise the yield of strawberry plants, as was seen in trials in 1970/1971. In the next year the same results were only significant in some treatments. In these trials only a few symptoms of infection by *Verticillium* spp. were present, and therefore the effect of benomyl and thiophanate-methyl must be ascribed to other factors as well. Due to the (indirect) stimulating influence on plant growth and the possible positive effect on yield, both fungicides are used as leaf sprays in commercial strawberry growing, regardless of the presence of *Verticillium* spp. or the risk of favouring other diseases such as crown rot or heart rot.

Samenvatting

Toepassingsmogelijkheden van benomyl en thiofanaat-methyl voor de bestrijding van Verticillium verwelkingsziekte bij aardbei in Nederland

Een onderzoek werd ingesteld naar de effecten van verschillende manieren en tijdstippen van fungicidetoediening op groei en opbrengst van aardbeiplanten, aangetast door *Verticillium dahliae*.

In potproeven kon de schimmel niet worden bestreden door de wortels te dompelen in, of het gewas te bespuiten met fungicidenoplossingen (Tabel 3). Ook in veldproeven was de stand van besmette aardbeiplanten, die na het planten bespoten werden met fungicidenoplossingen, tijdens de oogstperiode slecht (Tabel 2).

Als besmette planten na het oppotten aangegoten werden met 0,1 g benomyl in 100 ml water per pot, groeiden ze goed en vertoonden ze geen ziekteverschijnselen (Tabel 3 en 4). Op zavelgrond was de groei van de aardbeiplanten slechter dan op een mengsel van veen en zand. De groeiverbetering door aangieten met benomyl was dan ook duidelijker merkbaar bij planten in zavelgrond (Tabel 4).

Het aangieten van aardbeiplanten op het wachtbed en in de kas van produktiebedrijven met benomyl of thiofanaat-methyl gaf een hogere opbrengst in 1971. Bij proeven in het volgende seizoen werd niet altijd een hogere opbrengst verkregen (Tabel 1).

De behandelde planten hadden minder last van stengelrot, veroorzaakt door *Botrytis cinerea*. Het aangieten met thiofanaat-methyl van 'Glasa' aardbeiplanten, die op een met *Phytophthora cactorum* besmet veld stonden, resulteerde in een versterkte rhizoomaantasting door deze schimmel (Tabel 5).

Ondanks het gevaar voor ontstaan van fungicide-resistente *Botrytis cinerea* stammen en het in versterkte mate optreden van stengelbasisrot, alsmede het optreden van persistente residuen in de grond bij toepassing van hoge doseringen benomyl en thiofanaat-methyl, worden beide middelen in de praktijk gebruikt omdat ze op (in)-direkte wijze de plantengroei bevorderen en de produktie verhogen.

Acknowledgment

The authors are indebted to Mrs I. Seeger for correction of the English text and to Mr J. P. A. van Dieren for statistical analyses.

References

Bollen, G. J., 1972. A comparison of the in vitro antifungal spectra of thiophanates and benomyl. Neth. J. Pl. Path. 78: 55–64.

Bollen, G. J. & Fuchs, A., 1970. On the specificity of the in vitro and in vivo antifungal activity of benomyl. Neth. J. Pl. Path. 76: 299–312.

Busch, L. V. & Hall, R., 1971. *Verticillium* wilt of chrysanthemum: suppression of fungal colonization of leaves and prevention of wilt symptom development by foliar applications of benomyl. Can.J.Bot. 49: 1987–1991.

Jarvis, W. R. & Hargreaves, A. J., 1973. Tolerance to benomyl in *Botrytis cinerea* and *Penicillium corymbiferum*. Pl. Path. 22: 139–141.

Jordan, V. W. L., 1972. Evaluation of fungicides for the control of *Verticillium* wilt (*V. dahliae*) of strawberry. Ann. appl. Biol. 70: 163–168.

Jordan, V. W. L., 1973. The modes of action of two benzimidazoles and thiophanate-methyl used for the control of *Verticillium* wilt of strawberry. Ann. appl. Biol. 75: 41–47.

Lockhart, C. L., MacNab, A. A. & Bolwyn, B., 1969. A systemic fungicide for control of *Verticillium* wilt in strawberries. Can. Pl. Dis. Surv. 49: 46–48.

Wijshoff, J., 1973. Bestrijding stengelrot bij aardbei. Fruitteelt 63: 148–149.

Addresses

H. A. Th. van der Scheer and H. J. Wondergem: Proefstation voor de Fruitteelt, Wilhelminadorp (Goes), the Netherlands.

B. J. Luimes: Consulentschap voor de Tuinbouw, Dodewaardlaan 5, Tiel, the Netherlands.

(Continuation from page 93)

Patterns of diseases caused by fungi and bacteria (8) have continuously changed with changes in agricultural practice. In some instances, chemical control has been unavoidable, but biological control is being applied on a practical scale already. Many examples are described in which research on disease resistance, supply of inoculum to plant breeders and varietal testing for disease resistance have allowed introduction of resistant varieties.

Viruses (9) are especially hard to study and to control. IPO is concentrating on various ways of identifying viruses in Dutch crops and on epidemiology. This knowledge is necessary for interference with virus overwintering and spread. Crop sanitation includes the building up of virus-free mother stocks by using heat treatment or meristem tip-culture, for instance. Examples are given of IPO's contribution to the use of virus resistant varieties.

Air pollution (10) is of increasing importance to agricultural and horticultural crops in the Netherlands because of rapid industrialization and urbanization. Research on the influence of air pollution on plants is also of interest to environmental research as a whole because plants often are more sensitive than man or animals, and so may serve as indicators.

The editors are to be commended on the well-balanced way in which the information has been assembled and the book has been composed, indicating that they did not merely edit it.

The layout of the book is very good. The illustrations are informative and of excellent quality.

This issue, although unfortunately published in Dutch, will certainly be of value not only to all those who work on crop protection, but also to everybody interested in ecology as a whole.

J. Dijkstra