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Effect of benomyl on the potato cyst nematode, Heterodera rostochiensis

H. HOESTRA[1]

Laboratory of Nematology, Agricultural University, Wageningen

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Abstract

Benomyl is a strong fungistat against certain groups of fungi and it does not have much effect on most plant-parasitic nematodes. *Heterodera* spp., however, are affected, and the literature indicated a strong effect on *Heterodera rostochiensis* in the field. Laboratory tests demonstrated that hatching of larvae from *H. rostochiensis* cysts is strongly inhibited by benomyl in vitro. In a pot trial, benomyl effectively suppressed formation of new cysts on the roots of potatoes grown in sand, clay soil or light-sandy loam. There was no such effect in soil rich in organic matter probably because of strong adsorption of benomyl. Control of *H. rostochiensis* by benomyl in soil could be due to suppressed hatch or to other mechanisms, such as inhibition of larval penetration of roots. In view of its long persistence in the soil and of its various side-effects, benomyl does not seem very promising for practical control of *Heterodera rostochiensis*.

Introduction

The fungistatic effects of benomyl – methyl-1-(butylcarbamoyl)-2-benzimidazole-carbamate – have been widely studied. The antifungal activity of benomyl is fairly specific to certain groups of fungi (Bolen and Fuchs, 1970), and benomyl is persistent in soil (Baude et al., 1974).

Effects on other organisms are usually small with amounts used to control fungi (Frahm, 1973; Bunt, 1975). Earthworms are among the noteworthy exceptions; they are quickly eliminated from soil, even with small amounts of benomyl (Stringer and Lyons, 1974). In the aquatic environment, *Daphnia magna* proved to be extremely sensitive to benomyl (Canton, 1975).

Plant-parasitic nematodes are not generally affected severely by benomyl. This is especially so for free-living nematodes such as *Tylenchorhynchus* spp. (Miller and Taylor, 1970; Laughlin and Vargas, 1972). Cyst nematodes, *Heterodera* spp., are more sensitive. Although Price (1971) found no effect on invasion of wheat roots by *Heterodera avenae*, Cook and York (1972) found reduced root invasion, and subsequent larval development was delayed or prevented with *H. avenae* on barley. Miller (1969) studied the effects of benomyl on *Heterodera tabacum*, and found a strong reduction in root invasion. Hide and Corbett (1973, 1974) and Jones (1975) reported strong effects of benomyl on *H. rostochiensis*. In field trials, from the second year onwards, po-

[1] Guest worker of TNO, Netherlands Organization of Applied Scientific Research.

pulations of this nematode were lower in the benomyl-treated plots than in the controls or in plots treated with the nematicide aldicarb or the broad-spectrum soil fumigant methyl bromide. In benomyl-treated plots, nematode multiplication decreased during four successive years of potato cropping after one benomyl treatment of 22.4 kg a.i./ha, demonstrating a remarkable persistence of benomyl.

Materials and methods

Hatching experiments in vitro. These tests were carried out by a technique commonly used in the Netherlands and developed at the Dutch Plant Protection Service, Wageningen, by C. P. Jaspers and J. Kort; the method resembles those used by Sheperd (1959) and Viglierchio (1961). The equipment consists of two Perspex vials (Fig. 1.). The inner vial has a sieve bottom on which the cysts are placed. Hatched larvae concentrate on the bottom of the outer vial, and can be removed for counting. Liquids used in these tests can easily be changed. In standard tests, cytsts are soaked alternately in solutions of hatching factor (potato root diffusate, 3 mg/l) and flavianic acid (2.4.-dinitro-naphthol-7-sulphonic acid, 100 mg/l). Flavianic acid is known to stimulate hatching (Janzen, 1968).

Cysts were first soaked in benomyl solutions. In Experiment 1, the benomyl treatment was repeated once, after the first cycle of treatment with hatching factor and flavianic acid (Table 1). In Experiment 2, benomyl treatments were repeated after each flavianic acid treatment and before the next treatment with hatching factor (Table 2). In both experiments, liquids were changed once every seven days. Experiment 1 was continued for three months; mass concentrations of benomyl a.i. were 10 and 100 mg/l. The duration of Experiment 2 was six months with benomyl solutions of 1 and 10 mg/l. In Experiment 1, there were five replicates and 40 cysts per vial; in Experiment 2, there were 50 cysts and six replicates. The vials were stored at room temperature in covered glass jars in darkness. Hatched larvae were counted at each change of solution.

Fig. 1. Longitudinal cross-section of Jaspers and Kort's hatching tube, actual size.

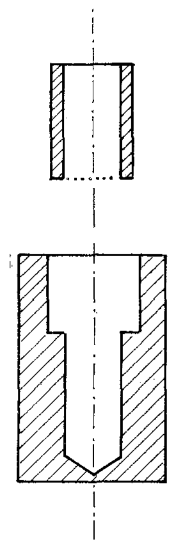

Fig. 1. Overlangse doorsnede van het lokbuisje vgls. Jaspers en Kort, ware grootte.

Greenhouse pot trial. A pot trial tested the effect of benomyl on *H. rostochiensis* in soil Since soil type was known to influence the effect of benomyl, four soil types were used:
1. Silty river clay; pH 7.3, organic matter 3.6 %, clay 42 %.
2. Light sandy loam; pH 4.7, organic matter 2.1 %.
3. Potting mixture; pH 4.9, organic matter 59 %.
4. River sand.

There were two treatments, 1 and 10 mg benomyl a.i. per pot of 800 ml, and six replicates.

Solutions of benomyl in water were carefully mixed with the soils on 26 April 1974, and potato cv. Pimpernel was planted on the same day. Fifty cysts of *Heterodera rostochiensis* were added to each pot on 1 May 1974. In Mid-July, plants with soil were carefully taken from the pots without disturbance of the soil. Cysts visible on the outside were counted, and plants were then put back into the pots. In November the trial was finished. Soil was allowed to dry. Numbers of cysts per pot and numbers of eggs and larvae per cyst were estimated by Fenwick's methods (Southey, 1970).

Results and discussion

In Hatching Experiment 1 (Table 1), benomyl clearly inhibited hatching. Practically no larvae were found after treatment with hatching factor and flavianic acid immediately after benomyl. Hatching resumed some time after the second (i.e. the latter) benomyl treatment. This demonstrates that at least part of the cyst content was not killed by benomyl. Hatching recovered less and slower with 100 mg/l than with 10 mg/l.

Even with 1 mg/l hatching was clearly affected in Experiment 2 (Table 2) where benomyl treatments were repeated between each treatment with flavianic acid and

Table 1. Counts of larvae in Hatching Experiment 1. Total of five replicates of 40 cysts each after each soaking treatment of one week. B = benomyl, H = hatching factor, F = flavianic acid. Control, water only.

Week	Treatment	Counts of larvae of *Heterodera rostochiensis* with concentration of benomyl a.i.:		
		0 (control)	10 mg/l	100 mg/l
1	B	0	0	0
2	H	2727	0	0
3	F	163	4	1
4	B	44	0	0
5	H	335	0	0
6	F	134	3	0
7	H	5466	569	8
8	F	541	1414	148
9	H	6004	5460	1210
10	F	1205	663	629
11	H	6053	6990	5180
12	F	1676	251	885
13	H	9117	569	2118
Total		33465	15923	10179

Tabel 1. Effect van benomyl in lokproef no. 1. Larvetellingen (totaal van vijf herhalingen met ‹ elk) na de behandelingen van telkens een week. B = benomyl, H = lokstof, F = flaviaanzuur.

Table 2. Counts of larvae in Hatching Experiment 2. Total of six replicates of 50 cysts each after each soaking treatment of one week. B = benomyl, H = hatching factor, F = flavianic acid. Control, water only.

Week	Treatment	Counts of larvae of *Heterodera rostochiensis* with concentration of benomyl a.i.:		
		0 (control)	1 mg/l	10 mg/l
1	B	49	2	3
2	H	18360	3530	23
3	F	2365	1290	2295
4	B	277	359	679
5	H	11470	5690	1042
6	F	2432	4328	4241
7	B	293	353	573
8	H	10700	10020	1801
9	F	578	1312	1706
Total (first 9 weeks)		46524	26884	12363
10	B	200	113	322
11	H	3383	4778	1202
12	F	850	189	1366
13	B	85	19	309
14	H	666	911	390
15	F	229	124	721
16	B	50	12	251
17	H	230	469	149
18	F	269	280	843
Total (second 9 weeks)		5962	6895	5553
19	B	18	9	211
20	H	113	279	82
21	F	139	201	702
22	B	31	50	124
23	H	267	510	132
24	F	81	147	969
25	B	12	80	216
26	H	194	613	72
27	F	55	903	587
Total (third 9 weeks)		910	2792	3095
Total		53396	36571	21011

Tabel 2. Effect van benomyl in lokproef no. 2. Larvetellingen (totaal van zes herhalingen met 50 cysten elk) uitgevoerd bij het wekelijks verwisselen van de oplossingen. B = benomyl, H = lokstof, F = flaviaanzuur.

hatching factor treatment. At this low concentration, the retarding effect was pronounced. In the first nine weeks, hatching was only 40% of the control. In the third period of nine weeks, hatching was well above the control level. At the end of the experiment, total hatch reached 68% of the control. At 10 mg/l, the effect was much stronger, also on the total hatch. But in the last nine weeks, hatching was still above the level for the control series. This suggests again that hatching of at least part of the larvae in the cysts was strongly retarded rather than permanently prevented. The retardation is further illustrated by the observation that for benomyl-treated cysts counts were mostly higher after flavianic acid than after hatching factor, whereas the reverse was true in the control series. The partial recovery of hatching in both benomyl series may have been favoured by the development of bacteria capable of de-activating benomyl in this rather long experiment. Still the greatly reduced total hatch indicates that part of the larvae and eggs were killed. The prolonged soaking of the cysts may have increased mortality in all treatments including control.

I did not investigate whether the observed retardation was due to an effect on growth processes such as cell division, or to inhibition of hatching of the second stage larvae from the eggs and cysts. At 10 mg/l, the effect was stronger after the first six weeks in Experiment 1 than in Experiment 2. Differences in the condition of the cysts or other experimental conditions, such as temperature, were probably responsible for this difference, as illustrated by the lower hatch in the control also in the first experiment.

The pot trial (Table 3) demonstrated that the benomyl effect closely depended on soil type. Mobility of benomyl is generally low in soil (Rhodes and Long, 1974). In studies on the uptake of benomyl by plants, it was demonstrated that benomyl was

Table 3. Effect of soil treatment on *Heterodera rostochiensis*. Six weeks after inoculation plants were carefully removed temporarily from their pots. Cysts visible on the outside of the root balls were counted.

	Benomyl, mg a.i. per pot	Cysts per pot, six weeks after inoculation	Cysts per pot at the end of the trial	Eggs and larvae per cyst
Potting mixture	0	114	1525	367
	1	109	1687	316
	10	109	1408	368
Light sandy loam	0	104	1015	360
	1	71	703	365
	10	0	65	88
Clay soil	0	147	1356	354
	1	90	564	297
	10	0	115	216
Sand	0	76	1718	294
	1	0	52	146
	10	0	57	184

Tabel 3. Effect van benomyl-behandeling van de grond op Heterodera rostochiensis. 'Cysts per pot, six weeks after inoculation' heeft betrekking op een 'potkluittoetsing', waarbij, zes weken na de inoculatie, de planten voorzichtig tijdelijk uit de pot gelicht worden voor de telling van de aan de buitenzijde van de potkluit zichtbare cysten.

bound to soil in proportion to the organic matter content (Schreiber et al., 1971). In our pot trial, the strong adsorption of benomyl in soils rich in organic matter was clearly demonstrated by the abscence of any effect on *H. rostochiensis* in potting soil. In sand, with practically no organic matter, benomyl prevented the formation of new cysts, even the lesser amount (1 mg per pot). The other two soils were intermediate. The lesser amount of benomyl had a noticeable effect, especially in clay soil but permitted the formation of a fair number of cysts. The higher amount (10 mg per pot) drastically reduced new cyst formation. Numbers of eggs and larvae per cyst were reduced whenever total numbers of cysts were reduced. This phenomenon was also observed by Hide and Corbett (1974) in their field trials. They used about as much benomyl as the higher amount in our pot trail.

Although hatching in vitro was strongly reduced by benomyl, it is doubtful whether the strong effect of benomyl in soil can be explained entirely by an inhibition of hatching. The retardation observed in the tests in vitro would probably have permitted the formation of some cysts in all benomyl treatments. Reduced invasion of roots as observed for other *Heterodera* spp. by Cook and York (1972) and Miller (1969) may well have played a role, as well as other effects on the nematode. Further research is needed to clarify these questions. Cook and York (1972) found that benomyl as a soil treatment was effective in reducing formation of new cysts of *Heterodera avenae*, but higher amounts were required than in our work on *H. rostochiensis*. In vitro, they found no effect on hatching, even at 250 mg/l benomyl. These data suggest that *H. rostochiensis* is much more sensitive to benomyl than other nematode species, including *H. avenae*.

In view of its long persistence in the soil and of its various side-effects, benomyl does not seem very promising for practical control of *Heterodera rostochiensis*.

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Samenvatting

Het effect van benomyl op het aardappelcystenaaltje, Heterodera rostochiensis

Benomyl is bekend als een middel met sterk fungistatische eigenschappen ten aanzien van bepaalde groepen schimmels. Het heeft in het algemeen weinig effect op planteparasitaire aaltjes. Cystevormende aaltjes echter, en in het bijzonder *Heterodera rostochiensis*, zijn volgens aanwijzingen uit de literatuur gevoeliger voor benomyl.

Onder laboratoriumomstandigheden bleek het uitkomen van de larven van *H. rostochiensis* uit de cysten sterk door benomyl te worden geremd (Tabel 1 en 2). In een potproef werd door een grondbehandeling met benomyl de vorming van nieuwe cysten op aardappelwortels onderdrukt, afhankelijk van de grondsoort (Tabel 3). Naarmate de grond meer organische stof of klei bevat, is het effect minder groot. Bij gebruik van rivierzand trad reeds bij een dosering van ongeveer 1 ppm benomyl een volledige onderdrukking van cystevorming op. Maar bij potgrond (59 % organische stof) was er, ook bij een tien maal hogere dosering, geen effect. Verder onderzoek zal moeten uitwijzen of het effect in grond berust op de remming van het uitkomen van larven uit de cysten, of (ook) op de beïnvloeding van andere aspecten van het parasitair gedrag van dit aaltje, zoals de penetratie in de wortels.

Gezien de grote persistentie van benomyl in de grond, en de nevenwerkingen, lijkt het middel minder geschikt voor toepassing in de praktijk.

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Address

Afdeling Nematologie LH, Binnenhaven 10, Wageningen, the Netherlands.