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Biological control of Otiorrhynchus sulcatus with Heterorhabditid nematodes in the glasshouse

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Abstract

A *Heterorhabditis* species, found in dead larvae of *Otiorrhynchus sulcatus,* was tested for its efficacy as a biological control agent of this insect in glasshouse experiments. In a preliminary test all weevil larvae were killed in pots with primula, 88% in strawberry and 50% in cyclamen. In a second test with strawberry plants good results were obtained when the nematodes were applied about the hatching time of the weevil eggs. At a dosage of 100 nematodes per cm^2 of soil area, 90-97% of the larvae were killed and 90% of the plants remained undamaged. A dosage of 50 nematodes per cm^2 produced roughly the same level of larval mortality, but left 30% of the plants damaged. Both early and late application of nematodes protected the plants insufficiently, because too many larvae survived. In a third test with strawberry, cyclamen and primula, soil treatment with 50 and 100 nematodes per cm^2 gave comparable results at both application times, i.e. one and three weeks after hatching of the weevil eggs. In strawberry 100% of the larvae were killed and all plants remained in good condition. Also in cyclamen nearly all larvae were killed and the plants remained in good condition, although the root systems had less fine roots in comparison with control plants without insects. In primula 4-12% of the weevil larvae survived, whereas up to 20% of the plants died, indicating that soil structure, soil moisture and condition of the plants have an important impact on the control results. A dosage of 25 nematodes per cm^2 appeared to be too low in all cases. The results of these experiments open new perspectives for control of the black vine weevil in glasshouses.

Additional keywords: black vine weevil, strawberry, cyclamen, primula.

Introduction

The black vine weevil, *Otiorrhynchus sulcatus*, is a serious pest of cultivated plants. Economic injury to glasshouse plants was recorded as early as 1834 (Bouché). Since that time injury to a great variety of plants has been reported from all over the world. In the Netherlands the insect is of economic importance especially in potted plants in glasshouses, in strawberry fields and in tree nurseries. Chemical control with aldrin and dieldrin is effective, but the use op these persistent chlorinated hydrocarbons will probably be prohibited in the near future. Substitutes have been tested in the field and so far carbofuran has given promising results (Evenhuis, 1978). In the same field trials the nematode *Neoaplectana carpocapsae* was tested, but it failed to give satisfactory control.

In other experiments Evenhuis found a few dead red larvae of *O. sulcatus* in soil near strawberry plants. These cadavers were parasitized by nematodes of the genus *Heterorhabditis* Poinar (1976), probably representing a new species. The nematode has been cultured and tested for its suitability as a biological control agent against *O. sulcatus* in three glasshouse experiments with potted strawberry plants, primula and cyclamen.

Materials and methods

In a preliminary control experiment the efficacy of the nematode was tested on strawberry, primula and cyclamen (experiment 1). In a second trial the effect of different dosage levels and times of application on strawberry was studied (experiment 2). A third trial consisted of a combination of the first two studies (experiment 3). All experiments were conducted with potted plants in glasshouses at average air temperatures of 20, 18 and 18 °C in experiment 1, 2 and 3, respectively. Flowers and buds were removed to keep the plants in the vegetative stage as long as possible.

Each plant was infested with 20 eggs of *O. sulcatus*. The eggs came from weevils collected in the field, kept in plastic boxes with moist potting soil and fed with rhododendron leaves.

The nematodes, initially increased on larvae of *O. sulcatus*, were mass-reared on larvae of *Galleria mellonella* by the method of Dutky et al. (1964), slightly modified. Infective nematodes were stored in tap water with continuous aeration at room temperature. The nematodes were applied onto the soil in 20 or 30 ml of tap water per pot. Since this *Heterorhabditis* species appeared to be highly sensitive to daylight (author's unpublished data; cf. Gaugler & Boush, 1978), they were applied on cloudy days after 4.00 p.m. in experiment 2 and 3.

When the plants died the soil was immediately examined for the presence of weevil larvae. Replicates with surviving plants and their soil were examined after the control plants (plants that had been infested with insects but not treated with nematodes) had died or when no further changes in the condition of these plants were expected.

Experiment 1. Strawberry plants were taken from the field and potted in fresh potting soil. Full-grown primula in potting soil and cyclamen in loamy soil came from commercial nurseries. These plants were not repotted.

Twenty insect eggs and freshly hatched larvae were applied to 22 plants of each species. Eleven pots were treated with 100 nematodes per cm^2 of soil area. They were applied 16 days after insect introduction on strawberry and primula and after 20 days on cyclamen. The other plants served as the control. The experiment was terminated 7 weeks after nematode application on strawberry and 11 weeks after application on primula and cyclamen.

Experiment 2. Strawberry plants of about equal size taken from the field were planted in sterile potting soil after the roots had been washed free of soil. Two weeks later 20 weevil eggs were introduced into each of 160 pots. The pots were then inoculated with 0, 25, 50 or 100 nematodes per cm^2 soil area at 0, 1, 2 and 5 weeks

intervals after infestation with the insect eggs. Each treatment was replicated 10 times. A group of 10 control plants was neither inoculated with insect eggs nor with nematodes. The experiment was terminated 13 weeks later. The foliage was treated with dienochlor to control red spider mites in the 5th week. The soil surface was covered during treatment.

Experiment 3. Well developed strawberry, cyclamen and primula plants were replanted in potting soil after washing the roots. They were given 6 weeks to become established before infestation with 20 insect eggs per plant. Nematodes were introduced at the levels used in experiment 2 at 3 and 5 weeks after infestation with insect eggs. Each treatment was replicated 10 times.

The air temperature was increased to 22 °C for the first two weeks following infestation with insect eggs to accelerate hatching. Aphids were chemically controlled in strawberry with pirimicarb in the 4th week and strawberry mites with dicofol in the 6th week after application of the eggs. Cyclamen was treated with thiram to control *Botrytis* in the first week and with pirimicarb against aphids in the 6th week. The soil was always covered during spraying.

All plants were placed on a moisture retaining cotton watering cloth. The experiment was terminated 11, 12 and 14 weeks after nematode application on cyclamen, strawberry and primula, respectively.

Results

Experiment 1. The first strawberry plants in the controls died 4 weeks after infestation with *O. sulcatus* and, with the exception of one replicate, all others after 9 weeks. Conversely only one of the nematode treated plants was in a poor condition after nine weeks. Most untreated primula plants died after 13 weeks, whereas all nematode treated plants were still in a good condition. The cyclamen were examined after 13 weeks when about half of both treated and untreated plants had died.

The numbers of surviving insect larvae and of dead plants are given in Table 1. There was no correlation between the number of insect larvae recovered and time of plant death.

Experiment 2. Strawberry plants not infested with *O. sulcatus* remained healthy. All plants inoculated with the insect alone, however, died 7 to 8 weeks after inoculation. A number of nematode treated plants also died. The presence of wilt symptoms in the plants to be inoculated with nematodes 5 weeks after infestation with insect eggs indicated that nematode introduction was too late. Therefore treatment with the lower dosages of nematodes was omitted and only the highest dosage was applied to determine wether damage could be reduced. This was not the case.

The number of non-parasitized insect larvae and the number of surviving plants are given in Table 2. The number of dead nematode infested weevil larvae found in the soil of both dead and living plants are presented in Table 3. The ultimate condition of the surviving plants did not differ from insect free plants. A few plants exhibited some wilting during the test period, but recovered with time.

Table 1. Numbers of living *O. sulcatus* larvae, recovered from soil treated (+) or not treated (−) with Heterorhabditid nematodes, after death of the plants or at the end of the test. Each plant had been infested with 20 insect eggs.

Plant No.	Strawberry		Primula		Cyclamen	
	+	−	+	−	+	−
1	3	(11)[1]	0	(10)	(15)	(18)
2	0	(11)	0	(16)	(13)	(10)
3	0	(0)	0	(12)	(1)	(12)
4	2	(8)	0	(16)	(7)	(12)
5	0	(13)	0	(11)	(9)	(9)
6	2	(7)	0	(8)	0*	(10)
7	0	(9)	0	(13)	1*	(13)
8	0	(12)	0	(13)	0	4
9	0	(13)	0	(11)	4	2
10	5*	(5)	0	(11)	0	3
11	0	(8)	0*	0	0	3
12	0	0	0*	8	1	6

[1] ( ) = dead plant. * = Examined half-way.

Tabel 1. Aantallen levende larven van O. sulcatus, teruggevonden in grond behandeld (+) en niet behandeld (−) met Heterorhabditis, na afsterven van de planten of aan het einde van de proef. Iedere plant was met 20 kevereieren geïnfecteerd.

Table 2. Numbers of *O. sulcatus* larvae surviving different dosages of nematodes applied at various times on soil of potted strawberries.

Plant No.	Time of application[1]										
	0				1			2			5
n.n.[2]	0	25	50	100	25	50	100	25	50	100	100
1	(9)[3]	(12)	(9)	(6)	(0)	(0)	(2)	(5)	(4)	(8)	(13)
2	(10)	(8)	(7)	(0)	(1)	(6)	0	(7)	(5)	0	(4)
3	(12)	(2)	(3)	(4)	(10)	(0)	0	0	(0)	0	(5)
4	(5)	(5)	(7)	(5)	(5)	1	0	0	0	0	(5)
5	(7)	(6)	(0)	(0)	(10)	0	0	0	0	0	(5)
6	(7)	0	(0)	0	0	0	0	0	0	0	(6)
7	(6)	0	0	0	0	0	0	0	0	0	(4)
8	(6)	0	3	0	0	0	0	0	0	0	(8)
9	(8)	0	5	0	0	0	0	0	0	0	0
10	(13)	0	0	0	0	0	0	0	0	0	0

[1] Weeks after infestation with insect eggs.
[2] Number of nematodes per cm² of soil area.
[3] ( ) = dead plants.

Tabel 2. Aantallen levende larven van O. sulcatus na een bodembehandeling met verschillende aaltjesdoseringen op verschillende tijdstippen bij opgepotte aardbeiplanten.

Table 3. Numbers of dead parasitized larvae of *O. sulcatus* recovered from soil of potted strawberry plants treated with different dosages of nematodes at various times.

Plant No.	Time of application[1]										
	0				1			2			5
n.n.[2]	0	25	50	100	25	50	100	25	50	100	100
1					5(10)[3]	5(7)					
2			1(8)	1(9)	6(7)		2	1(6)		1	
3			2(9)	2(10)		6(10)	3	1	1(11)	2	
4			2(8)			2				2	3(3)
5				5(12)						1	
6			4(12)		3						
7					2						2(4)
8				1							1(4)
9											
10											

[1] Weeks after infestation with insect eggs.
[2] Number of nematodes per cm^2 of soil area.
[3] ( ) = dead plants.

Tabel 3. Aantallen dode geparasiteerde larven van O. sulcatus teruggevonden in grond van opgepotte aardbeiplanten, die op diverse tijdstippen was behandeld met verschillende hoeveelheden aaltjes.

Experiment 3. During the first ten weeks after infestation with insect eggs no differences between treatments were observed in strawberry. Whereas nematode-free plants began to wilt after 10 weeks with death occurring two weeks later, all nematode treated plants survived. Data on the condition of the plants and on recovered weevil larvae are given in Table 4.

In primula no differences between treatments were observed until the tenth week, when more than half of the nematode-free plants wilted. After 14 weeks they were all dead. During this period of 14 weeks some of the nematode treated plants also died. A comparison of the aerial plant parts was difficult because the surviving plants aged near the end of the test period. Therefore, only the root systems were assessed; the results are given in Table 5, along with the number of recovered weevil larvae.

The nematode-free cyclamen also began to die in the tenth week and they were all dead after eleven weeks. The corms were completely void of roots. None of the nematode treated plants showed this phenomenon. Aging of the plants hindered comparison of the aerial plant parts. Comparison of the root systems produced no differences between treatments with regard to application time or nematode inoculum levels. Control plants without insects had more fine roots than the nematode treated infested plants. The numbers of recovered weevil larvae are given in Table 6.

Table 4. Condition of strawberry plants and numbers of living and parasitized larvae of O. sulcatus recovered from soil infested with 20 eggs per plant and treated with Heterorhabditid nematodes 3 or 5 weeks after insect introduction.

Plant No.	Nematode application[1] after 3 weeks															
	0				25				50				100			
	P[2]	R	lv l	lv d	P	R	lv l	lv d	P	R	lv l	lv d	P	R	lv l	lv d
1	+[3]	+		6	±	±		1	±	±			±	±		
2	−	−	11		+	±		1	+	+			±	±		
3	−	−	11		+	±		1	+	+			+	±		1
4	−	−	13		+	±			+	+			+	+		
5	−	−	13		+	±			+	+			+	+		
6	−	−	6		+	±			+	+			+	+		
7	−	−	10	3	+	+		4	+	+			+	+		
8	−	−	7	2	+	+		2	+	+			+	+		
9	−	−	4	3	+	+		1	+	+			+	+		
10	−	−	6	5	+	+		2	+	+			+	+		
	no insects						nematode application after 5 weeks									
1	±	±			±	±		2	±	±		2	±	±		1
2	+	+			±	±		1	+	±		1	+	±	1	
3	+	+			+	±		2	+	±			+	±		
4	+	+			+	±		1	+	+			+	±		
5	+	+			+	±			+	+			+	+		
6	+	+			+	+			+	+			+	+		
7	+	+			+	+			+	+			+	+		
8	+	+			+	+		2	+	+			+	+		
9	+	+			+	+		5	+	+			+	+		
10	+	+			+	+			+	+			+	+		

[1] Number of nematodes per cm^2 soil area.
[2] P = aerial plant parts, R = root system, lv = weevil larvae, l = living, d = dead parasitized.
[3] + = normal, ± = reduced, − = dead.

Tabel 4. De toestand van aardbeiplanten en de aantallen levende en geparasiteerde larven van O. sulcatus die zijn teruggevonden in de grond, die met 20 kevereieren was geïnfecteerd en 3 of 5 weken daarna met Heterorhabditis was behandeld.

Discussion

Considering the condition of the plants and the numbers of surviving insect larvae in the nematode treated pots of the preliminary experiment, control results were very good for primula and strawberry. The poor results in cyclamen might have been caused by unfavourable conditions for nematode movement in the loamy soil. An adverse effect of daylight can not be excluded either, since the extreme sensitivity of

Table 5. Condition of the root system of primula and numbers of living and parasitized larvae of *O. sulcatus* recovered from soil infested with 20 insect eggs per plant and treated with Heterorhabditid nematodes 3 or 5 weeks after insect introduction.

Plant No.	Nematode application[1] after 3 weeks											
	0			25			50			100		
	R[2]	lv		R	lv		R	lv		R	lv	
		l	d		l	d		l	d		l	d
1	–[3]	17		–	13		–	6	4	–	5	
2	–	16		–	9	3	–	1		+		3
3	–	15		±	1	2	±	3	4	+		1
4	–	15		±		2	±		1	+		
5	–	11		+	1	1	+	2		+		
6	–	11		+		2	+	1		+		
7	–	10		+		1	+			+		
8	–	6		+			+			+		
9	–	3		+			+			+		
10	–	6	4	+			+			+		
	no insects			nematode application after 5 weeks								
1	+			–	11		±	1	1	–	7	
2	+			–	7	2	±		2	–		4
3	+			–	2	3	±		2	+		1
4	+			±	1	4	+	2		+		1
5	+			±		2	+	1		+		
6	+			+		3	+		3	+		
7	+			+		2	+		2	+		
8	+			+			+		1	+		
9	+			+			+			+		
10	+			+			+			+		

[1] Number of nematodes per cm^2 soil area.
[2] R = root system, lv = weevil larvae, l = living, d = dead parasitized.
[3] + = normal, ± = reduced, – = dead.

Tabel 5. De toestand van de wortelstelsels van primula's en de aantallen levende en geparasiteerde larven van O. sulcatus die zijn teruggevonden in de grond, die met 20 kevereieren per plant was geïnfecteerd en 3 of 5 weken daarna met Heterorhabditis was behandeld.

these nematodes was not considered in this experiment. Moreover, the nematodes were applied to the cyclamen four days later than to the other plants. The relative early death of the strawberries can be explained by plant age. The plants had been taken from the field only two weeks prior to insect inoculation, whereas the other plants had well developed root systems.

In experiment 2 half of the plants died when nematodes were applied immediately

Table 6. Numbers of living and parasitized larvae of *O. sulcatus* recovered from soil of cyclamen infested with 20 insect eggs per plant and treated with Heterorhabditid nematodes 3 or 5 weeks after insect introduction.

Plant No.	Time of nematode application[1]						
	3 weeks				5 weeks		
	0	25	50	100	25	50	100
1	11	1(1)[2]	1		(6)	16	
2	16	9			1		
3	10	1			1		
4	9	1			(3)		
5	15						
6	14						
7	6(2)						
8	12(2)						
9	11(1)						
10	6(1)						

[1] Number of nematodes per cm^2 of soil area.
[2] () = parasitized larvae.

Tabel 6. Aantallen levende en geparasiteerde larven van O. sulcatus, die zijn teruggevonden in grond van cyclamen, die was geïnfecteerd met 20 kevereieren per plant en 3 of 5 weken daarna met Heterorhabditis was behandeld.

upon depositing insect eggs, although more weevil larvae were killed by higher nematode dosages (Table 2). Application of nematodes one or two weeks after insect infestation gave better results. The highest dosage protected the plants well and killed most of the weevil larvae. Nematode dosage seems to be less important when nematodes are applied two weeks after the introduction of the insect eggs. Nematode application 5 weeks after insect introduction gave poor results.

The number of dead parasitized weevil larvae and the time required for death (Table 3) indicate a slow efficacy of the nematodes in soil. Parasitized weevil larvae will die within 48 h through the action of the symbiotic bacteria carried by the nematodes. Development and reproduction of the hermaphroditic nematodes continues inside the dead insect. A new generation of infective juveniles develops in approximately 15 days at 18-20 °C. During this period the insect cadavers remain more or less preserved. In this experiment red parasitized larvae were found 3 tot 13 weeks ater nematode application. The dead larvae, therefore, had been parasitized between 1 and 12 weeks after nematode application. This may have been caused by a slow rate of nematode movement in soil, related to porous structure. Normally the nematode can move 10 cm in 48 h (author's unpublished data). Maybe newly hatched weevil larvae are too small to attract the nematode (see also Schmidt and All, 1978). Nematode movement would then be at random and only those larvae that are accidentally encountered would be killed. However, these young weevil larvae will hardly provide enough food for the nematodes to complete their life

cycle. The nematodes will die without producing a new generation of infective juveniles. If the nematodes are able to survive extended periods in soil, attraction may increase with insect development. A new generation of nematodes will develop in these larvae parasitized at a later stage and they will be able to parasitize the insect larvae that have escaped the initial nematode treatment.

The low levels of control and the high number of both living and parasitized larvae following early nematode application in experiment 2 can be explained by the fact that (1) it takes two weeks for the eggs to hatch, (2) the nematodes are attracted to the young larvae in very low numbers and many die by starvation and (3) a new generation of infective nematodes only develops after the insect larvae have developed, so that the second generation of nematodes comes too late for adequate control. Delayed application of nematodes means: (1) presence of more developed insects, producing stronger attraction, (2) higher levels of mortality and (3) stimulated reproduction of nematodes.

The dosage of 100 nematodes per cm^2 protected the plants best when applied 1 and 2 weeks after the insect eggs, although the results of the lower dosage suggest that this might be as effective when applied after 3 weeks. This was confirmed by the results of experiment 3, in which 50 and 100 nematodes per cm^2 applied 3 weeks after infestation with eggs gave similar results in strawberry and cyclamen. The plants were well protected and all but a single weevil larva were killed. The one cyclamen with 16 living weevil larvae probably had not been treated with nematodes. Nematode application 5 weeks after infestation with eggs gave good control results, which was not the case in experiment 2. Possible causes for this dissimilarity are differences in soil structure, condition of the root systems or temperature fluctuations. In experiment 3 the results with primula were comparatively moderate. The number of surviving and of parasitized larvae indicate inadequate nematode movement, perhaps caused by unfavourable soil structure due to repotting of the excessively rooted plants, resulting in unfavourable moisture conditions.

In most cases parasitized larvae were found in the nematode-free control pots in experiment 3. The pots were placed at random on a moisture retaining cotton watering cloth. This setup allowed nematode movement from the treated pots via the holes in the bottom and over the watering cloth to the untreated pots. This means that the nematode can be very active and traverse long distances in a suitable medium. The fact that only one of the untreated primula pots contained parasitized larvae, affirms the assumption of inadequate nematode movement in these pots due to unfavourable soil structure.

It can be concluded that the application of *Heterorhabditis* can give efficient control of *O. sulcatus* larvae and that introduction time is extremely important. Aspects still to be studied are the influence of insect number, plant age, soil type, temperature and split application on control efficacy.

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Samenvatting

Biologische bestrijding van Otiorrhynchus sulcatus met Heterorhabditide nematoden in kassen

Een inheemse nematode van het geslacht *Heterorhabditis* werd door middel van potproeven in kassen getoetst op zijn werkzaamheid als biologisch bestrijdingsmiddel tegen larven van de gegroefde lapsnuitkever, *Otiorrhynchus sulcatus*. In een oriënterende proef werden in potten met primula's alle keverlarven gedood, bij aardbeiplanten werden 88% gedood en bij cyclamen 50%. In een tweede proef met alleen aardbeiplanten werden goede resultaten verkregen als de aaltjes werden toegediend in de periode dat de kevereieren uitkwamen. Bij een dosering van 100 aaltjes per cm^2 grondoppervlak werd 90-97% van de keverlarven gedood en bleef 90% van de planten onbeschadigd. Bij een dosering van 50 aaltjes per cm^2 was de doding van de keverlarven weliswaar vrijwel hetzelfde, maar de schade aan de planten was te groot; 30% van de planten ging dood. Ook bij een eerder of later bestrijdingstijdstip was de schade aan de planten aanzienlijk en was de doding van de keverlarven onvoldoende. In een derde proef werd in zowel aardbei als in cyclamen vrijwel 100% van de keverlarven gedood bij een dosering van zowel 50 als 100 aaltjes per cm^2 en op beide bestrijdingstijdstippen, te weten toen de keverlarven circa 1 en 3 weken oud waren. Alle aardbeiplanten en cyclamen bleven in goede staat, hoewel bij cyclamen het aantal fijne wortels minder was dan bij de controle planten zonder keverlarven. De resultaten met primula's waren in deze proef iets minder goed. Het percentage overlevende keverlarven variëerde van 4 tot 12, terwijl tot 20% van de planten dood ging. Een dosering van 25 aaltjes per cm^2 was in alle gevallen te laag. De resultaten van deze proeven bieden gunstige perspectieven voor toepassing van dit aaltje bij de bestrijding van de gegroefde lapsnuitkever in kassen.

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