Translation from Dutch

PROJECT

Biological and chemical control of vine weevil

(Otiorhynchus sulcatus) (4102)

INTERNAL REPORT

TEST

Control of vine weevil larvae in outdoor containers - 1991

Boskoop 1991 (4102-01)

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SUMMARY

Control of vine weevil larvae in containers - 1991

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Internal Report 4102-01

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Chlorpyrifos* (SusconGreen), at application rates of both 375 kg/ha and 750 kg/ha, chlorpyrifos* (Dursban), imidachlobrid* (Confidor), fonofos* (Dyfonate) and carbofuran (Curater) are effective against the larvae of the vine weevil (Otiorhynchus sulcatus). This trial shows that halving the application rate of chlorpyriphos* (SusconGreen), bringing it to 375 kg/ha, still gives good control of the larvae. Diflubenzuron* (Andalin) and Bacillus thuringiensis* were not effective.

Metarhizium anisopliae (BI01020) produced good results in containers this year, as it did last year. This research has not shown that there is any host-plant sensitivity when the larvae are controlled using this fungus.

Heterorhabditis eelworms, generally speaking, were effective against the larvae. It is noticeable that H. megidis gives better control than H. bacteriophora. Soil-temperature measurements also show that a total of 10 days with a temperature of more than 12 °C was enough to achieve this control. It is even true to say that, of these 10 days, half of them fell within the range of 12 - 13 °C, and that less than one day was warmer than 15 °C. The assessment of the Steinernema eelworms came up against problems when we tried to compare them with the Heterorhabditis eelworms. Owing, amongst other things, to delivery problems, the first application of S. carpocapsae (Koppert) took place a good two weeks later than the other eelworm treatments, and deliveries of S. carpocapsae (Biosys) were halted prematurely, so that only a single, early treatment (a good 20 days earlier

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The products or treatments marked with a * are not approved for the purpose stated in arboriculture.

than the other eelworms) was carried out using this population. This had an enormous influence. S. carpocapsae (Koppert) had almost no period in which the temperature was higher than 12 °C, while S. carpocapsae (Biosys) had an enormously long period with temperatures above 12 °C. In the case of the Biosys eelworms, moreover, the temperature was higher than 15 °C for more than 10 days (for the other eelworms, this figure was less than one day). Seen in this context, the effects of S. carpocapsae (Koppert) cannot automatically be given a negative assessment, nor those of S. carpocapsae (Biosys) a positive one. At the moment, a controlled-environment trial is still in progress, to determine whether there are any differences in the effects of different species and populations of eelworms at lower temperatures. Next year, experiments will be set up both outdoors and in controlled-environment houses in order to discover more information about the correct application time for eelworms and the period that is necessary, at a given temperature, in order to obtain a satisfactory result.

AIM

To determine the effect of insecticides and biological control methods on the larvae of the vine weevil in containers out of doors. The effect of five insecticides is compared with the recommended product carbofuran (Curater liquid). At the same time, the effects of seven populations of insect-parasitic eelworms (Heterorhabditis spp. and Steinernema spp.), the insect-pathogenic fungus Metarhizium anisopliae* and the bacteria Bacillus thuringiensis* were also investigated. In the case of M. anisopliae*, we also looked at the question of whether there is any negative effect, emanating from the host plant, Thuja, on the infection of the larvae by the fungus.

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EXPERIMENTAL SET-UP

Twenty treatments were carried out using eight test plants per parallel, with the exception of treatments O and P. Because there were not enough test plants, in these two cases only four test plants per parallel were used. Treatments A to L inclusive were set up in duplicate, so as to enable early and late harvests to be observed. Owing to circumstances, however, there was no early harvest, and treatments A to L inclusive were harvested at the same time and processed together, so that twice as many test plants were assessed for these treatments. All treatments with the exception of O and P used Thuja occidentalis 'Brabant' as the test plant. O and P used Azalea mollis as the test plant. The treatments were separated from one another by edging plants. In the same way, the entire edges of the container beds consisted of edging plants. The plants were inoculated three times with 15 eggs per plant each time. This was done on 29 July, 12 August and 27 August 1991.

The treatments carried out, together with their application rates, are given in Table 1. On 13 May 1991 the plants were potted up in 1 litre pots using B42 substrate (composition: 60 % peat pellets, 40 % sphagnum-moss peat and 5 % wind-blown sand) and placed in the container beds in positions determined by a system of drawing lots (Basic Information 1). Treatments C, D and K were also mixed into the substrate. The substrate of treatment K was mixed thoroughly with BI01020 (1 g per litre substrate) on 25 April and was then, without further watering, covered and put aside in a warm place until the pottung-up date on 13 May. In the meantime the substrate was shaken and mixed once more, so that no shortage of oxygen could arise. Treatments O and P were started on 27 and 28 May (here too, BI01010 was mixed in and incubated two weeks beforehand). On 25 July and 29 November, soil samples of treatments K and P were taken, and sent to Bayer for determination of spore density in the soil.

Table 1 - Treatments and application rates

Active substance #	Trade name	Rate	% a.s.	Number	Tnts applied
A. Untreated	-	-	-	-	
B. Carbofuran	Curater liquid	37.5 l/ha	20	2x	
C. Chloropyrifos*	SusconGreen	375 kg/ha	10	1x	
D. Chloropyrifos*	SusconGreen	750 kg/ha	10	1x	
E. Imidachlobrid*	Confidor	37.5 l/ha	20	2x	
F. Heterorhabditis megidis	Nemasys H	15,000/l	-	2x	26 Sept 24 Oct
G. Heterorhabditis megidis	Groene Vlieg (HSH)	15,000/l	-	2x	10 Oct 24 Oct
H. Steinernema carpocapsae	Koppert	15,000/l	-	2x	15 Oct 24 Oct
J. Heterorhabditis megidis	Westerman (HF85)	15,000/l	-	2x	26 Sept 24 Oct
K. Metarhizium anisopliae*	BI01020	1 g/l	-	1x	
L. Fonofos*	Dyfonate liquid	37.5 l/ha	25	2x	
M. Heterorhabditis bacteriophora	Bio-erre	15,000/l	-	2x	1 Oct 24 Oct
N. Chloropyrifos*	Dursban liquid	19.0 l/ha	48	2x	
O. Untreated (Azalea)	-	-	-	-	
P. M. anisopliae* (Azalea)	BI01020	1 g/l	-	1x	
R. Heterorhabditis bacteriophora	Otinem	15,000/l	-	2x	26 Sept 24 Oct
S. B. thuringiensis*	Brinkman	16.0 l/ha	2	1x	26 Sept
T. B. thuringiensis*	Brinkman	16.0 l/ha	2	2x	26 Sept 24 Oct
V. Diflubenzuron*	Andalin SC - 10	50 kg/ha	10	1x	3 Sept
X. Steinernema carpocapsae	Biosys	15,000/l	-	1x	6 Sept

O and P = Azalea as test plant; all other treatments had Thuja as their test plant.

% a.s. = percentage of active substance.

Number = Number of repeat applications.

plants assessed 25-29 Nov

Nemasys H 15000/l in 1l (13cm²) pot
⇒ 1.15×10⁹/m²
×2 applications

(10⁹/m² ⇒ 13300 nems/13cm pot)

On 22 July 1991, treatments B, E, L and N were carried out for the first time. These treatments were repeated on 3 September 1991. Owing to circumstances, treatments V and X could be carried out only once, on 3 and 6 September respectively. Treatments F, J, R, S and T were carried out for the first time on 26 September 1991. Treatments G, H and M could not be applied on that date, because delivery was delayed. G and M were therefore carried out for the first time on 1 October 1991 and H on 15 October. On 24 October 1991, treatments F, G, H, J, M, R and T were applied for the second time. For the liquid products, the eelworms and B. thuringiensis, 25 ml of spray liquid per plant was applied, using a dispenser.

OBSERVATIONS

The plants were harvested between 25 and 29 November. The substrate of each test plant was searched to determine the presence of vine weevil larvae. The number of larvae found per test plant was noted. At the same time, the root systems of the test plants were assessed for biting damage. This was done by means of an assessment score (on a scale from 0 to 5, in which 0 signified an undamaged root collar and 5 signified biting damage all the way round the root collar. The observations are given in Basic Information 2. At the same time, the temperature of the potting compost was measured from the time of inoculation with eelworms to the end of the trial. By means of a data logger and a thermocouple, the temperature was measured every two hours. Basic Information 3 gives these measurements.

RESULTS AND DISCUSSION

Table 2 gives a summary of the results. The number of larvae is an average of 4 parallels and is shown as the number of larvae per plant. The same applies to the assessment score for the root system. The results have been statistically processed using ANOVA (See Basic Information 4). The result of this processing is given in the table. In order to analyse the number of larvae, it was necessary to convert the figures. In this case we chose the square root of the figures.

no. of 8 plants = total 32 plants

Table 2 - Average number of larvae per plant and average assessment score for root-system damage per plant

Treatment#	Larvae	Damage	Stage
A. Untreated	2.2 ab	1.7 ab	4.0
B. Carbofuran	0.1 fh	0.1 lm	3.2
C. Chlorpyrifos (SuscGr.)*	0.3 def	0.3 jklm	4.7
D. Chlorpyrifos (SuscGr.)*	0.0 h	0.0 m	-
E. Imidachlobrid*	0.5 ce	0.2 lm	3.0
F. H. megidis (Nemasys)	0.1 fh	1.1 cdg	2.3
G. H. megidis (Gr.Vlieg, HSH)	0.3 de	0.8 fgh	3.3
H. S. carpocapsae (Koppert)	2.1 ab	1.3 cde	3.9
J. H. megidis (Westerman, HF85)	0.0 gh	0.7 fghk	1.0
K. M. anisopliae (BI01020)*	0.3 ef	0.3 jlm	2.7
L. Fonofos*	0.3 efg	0.1 lm	4.1
M. H. bacteriophora (Bio-erre)	0.7 ce	0.7 eghl	3.2
N. Chlorpyrifos liquid (Dursban)*	0.6 c	0.3 jklm	3.7
O. Untreated (Azalea)	5.5 A	-	1.8
P. M. anisopliae (BI01020)*	0.8 B	-	1.8
R. H. bacteriophora (Otinem)	0.8 c	1.6 bc	3.6
S. B. thuringiensis*	1.8 b	0.9 dhj	4.2
T. B. thuringiensis*	2.7 a	2.3 a	4.1
V. Diflubenzuron*	2.0 ab	1.3 bdf	3.7
X. S. carpocapsae (Biosys)	0.7 cd	0.4 hm	3.3

O and P = Azalea as test plant; all other treatments have Thuja as their test plant.

Larvae = average number of larvae per plant.

Damage = damage to root collar (scale: 0 to 5).

The figures in the table followed by the same letter are not significantly different, with a 95 % confidence limit.

Treatment P was tested only against treatment O.

Stage = average stage of the larvae (1 to 5)

Table 3 shows the number of days for which the pot temperature was higher than 12 °C, from the time the eelworms were introduced to the time of harvesting. These results are a summary of the extensive measurements which were carried out (every 2 hours). These measurements are given in Basic Information 3. The selection of 12 °C as a minimum working temperature for the eelworms is based on the results of the controlled-environment test in 1991 (see Internal Report 49/91 (4102-3)). The results were compared with the percentage control of the number of larvae, with reference to the untreated plants. Untreated was taken as 100 % survival. The significance of these percentages is based on the figures in Table 2.

Table 3 - Number of days with a pot temperature of more than 12 °C, and percentage control of the larvae, compared with the untreated plants

Treatment	Days	% Control
A. Untreated	not applic.	0 a
B. Carbofuran	not applic.	95 d
F. H. megidis (Nemasys)	11.5	96 d
G. H. megidis (Gr. Vlieg, HSH)	9.2	85 c
H. S. carpocapsae (Koppert)	1.2	5 a
J. H. megidis (Westerman, HF85)	11.5	99 d
M. H. bacteriophora (Bio-erre)	9.2	70 bc
R. H. bacteriophora (Otinem)	11.5	63 b
X. S. carpocapsae (Biosys)	27.6	69 bc

The figures in the table followed by a letter indicate the significance of the number of larvae as shown in Table 2, and not the significance of the percentage control. An identical letter in this column means that the treatments concerned are not significantly different as regards the number of larvae, with a confidence limit of 95 %.
Untreated = 0 % control.

Tables 2 and 3 would appear to indicate a difference in larvae control levels between H. megidis and H. bacteriophora. With the aid of orthogonal coefficients, we tested this difference to determine whether it was significant. From the analysis it emerged that this difference is significant, i.e. H. megidis gave better control of the larvae than H. bacteriophora. S. carpocapsae cannot be tested in the same way, owing to the large difference in the number of days with a temperature higher than 12 °C (see Table 3). Details of these statistical analyses are given in Basic Information 4.

The results given in Tables 2 and 3 indicate the following:
1) Of the chemical products, both chlorpyrifos* (SusconGreen) in both low and high concentrations (C and D) and fonofos* (Dyfonate) (L) were just as effective as carbofuran (B). Chlorpyrifos liquid* (Dursban) (N) and imidachlobrid* (Confidor) (E) also gave a good level of larvae control, though not quite as good as carbofuran (B).
2) Diflubenzuron* (Andalin) (V) and Bacillus thuringiensis* (S and T) did not have any effect. In fact, when B. thuringiensis* was applied twice, this actually had a favourable effect on the survival of the larvae, and resulted in extra damage compared with the single application.
3) Metarhizium anisopliae* (BI01020) (K and P) had a good effect on the weevil larvae. No evidence could be found of the host plant's having any effect on control. Both treatments, K and P, gave approximately 85 % control compared with the untreated plants.
4) Eelworm populations Nemasys (F) and Westerman (J) were just as effective as carbofuran against the weevil larvae. H. bacteriophora (Otinem) (R), H. bacteriophora (Bio-erre) (M) and H. megidis (Gr.Vlieg) (G) were also reasonably effective, but not as good as carbofuran. Of these last three, H. megidis (Gr.Vlieg) (G) was the best. S. carpocapsae (Koppert) had no effect at all.
5) The number of days with a soil temperature higher than 12 °C was so different, particularly for treatments H and X, that comparisons with the other eelworm populations are irrelevant (see Table 3). The low number of

days with a temperature of more than 12 °C for treatment H (S. carpocapsae (Koppert)), hardly gave this eelworm a chance to be reasonably effective. For treatment X (H. carpocapsae (Biosys)), the reverse is true, i.e. in this case there were far more days with a temperature higher than 12 °C after the application of the eelworms, so that these eelworms had more chance of bringing about a high level of infection of the larvae. The exact measurements (Basic Information 3) also show that for all the treatments with the exception of treatment X there was less than one day of temperatures higher than 15 °C, whereas in the case of treatment X there were more than ten such days.

6) The analysis in which H. megidis was compared with H. bacteriophora shows that when H. megidis is applied, control of the larvae is better than with H. bacteriophora.

7) The general trend, though this was not tested statistically, is that the development stages of the surviving larvae were somewhat less advanced in the case of the biological products H. megidis and M. anisopliae than in the case of the control and the other products, which suggests that the bigger larvae in particular (stages 4 and 5) are more effectively controlled by these products. When the host plant was Azalea, the development of the larvae remained considerably behind, compared with the cases where Thuja was used as the host plant (see Table 2).

PROVISIONAL CONCLUSION

The products chlorpyrifos* (SusconGreen) (at application rates of both 375 kg/ha and 750 kg/ha), chloropyrifos* (Dursban), imidachlobrid* (Confidor), fonofos* (Dyfonate) and carbofuran (Curater) were highly effective against the larvae of the vine weevil (Otiorhynchus sulcatus). For all these products it is true to say that the results correspond to the results obtained in previous years, with the exception of imidachlobrid, which has not been tested before. This test shows that even when the application rate of chlorpyrifos* (SusconGreen) is halved to 375 kg/ha, this still gives a good level of control of the larvae. Diflubenzuron* (Andalin) and Bacillus thuringiensis* did not have any effect.

Metarhizium anisopliae* (BI01020) gave good results this year as regards control in containers, as it did last year. As was shown in previous tests (see Internal Report 39/91 (4007-24)), reducing the weevil-egg inoculum and at the same time increasing the pot volume is very important in order to eliminate any distortion or overshadowing of the result by a high natural mortality rate. This could be an important reason for the variable and often poor results that have often been obtained in the past. This applies to the chemical products as well as to the biological products. This study does not reveal any host-plant sensitivity when this fungus is used to control the larvae. At the present time, laboratory tests are still being carried out to determine whether exudates from the roots of Thujas have any direct or indirect influence on the process of infecting the larvae with this fungus. Research is also being carried out to find out what the minimum working temperature is for M. anisopliae.

Heterorhabditis eelworms were generally effective against the larvae. It is noticeable that better control was achieved with H. megidis than with H. bacteriophora. Soil-temperature measurements (Table 3, Basic Information 3) also show that a total of 10 days with a temperature higher than 12 °C was sufficient to obtain this control. It is even the case that of these 10 days half were between 12 and 13 °C and less than one day was warmer than 15 °C.

In the case of Steinernema eelworms, problems arise if we attempt to make a comparison between them and the Heterorhabditis eelworms. Owing, amongst other things, to problems with delivery, the first application of S. carpocapsae (Koppert) took place a full two weeks later than the other eelworm treatments, and the delivery of S. carpocapsae (Biosys) was halted prematurely, so that only one early treatment was carried out using this population (20 days earlier than the other eelworms). Table 3 shows that this must have had an enormous influence. S. carpocapsae (Koppert) had almost no period in which the temperature was above 12 °C, whereas S. carpocapsae (Biosys) had an enormously long period with temperatures higher

than 12 °C. In fact, for the Biosys eelworms the temperature was higher than 15 °C for more than 10 days (for the other eelworms the figure was less than one day). Seen in this context, the effects of S. carpocapsae (Koppert) cannot automatically be given a negative assessment, nor those of S. carpocapsae (Biosys) a positive one. At the moment, a controlled-environment trial is still in progress, to determine whether there are any differences in the effects of different species and populations of eelworms at lower temperatures. Next year, experiments will be set up both outdoors and in controlled-environment houses in order to discover more information about the correct application time for eelworms and the period that is necessary, at a given temperature, in order to obtain a satisfactory result.