

PROJECT

Field efficacy testing of the entomopathogenic nematode *Steinernema kraussei* against the vine weevil (*Otiorhynchus sulcatus*)

REPORT (PPO) - 1399

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SUMMARY

Report number: 1399

Title: Field efficacy testing of the entomopathogenic nematode *Steinernema kraussei* against the vine weevil (*Otiorhynchus sulcatus*)

Author: ir. R.W.H.M. van Tol

Purpose of this research is to show the efficacy of the nematode *Steinernema kraussei* strain N0093 at several application concentrations for control of larvae of the vine weevil in the field.

The results show that the winter application of *Steinernema kraussei*, strain N0093 is not effective for control of the vine weevil larvae. The autumn application, however, is giving good control of the larvae. The control for the higher application rate (0.5 million/m², 75% control) is significantly better than the lower rate (0.125 million/m², 66% control) (one-way analysis; $H_0 = P_B < P_A$). It is possible that a higher rate than 0.5 million/m² will give better control results but this has not been tested in this trial.

Application of Larvanem at the rate of 0.5 million/m² applied in autumn and winter is giving no control. The recommended application rate of 1.0 million/m² has not been tested in this trial.

In previous trials we have seen that application of *Heterorhabditis megidis* at lower rates than 1.0 million/m² is giving unpredictable control results and is therefore not advised for practice. Considering that Larvanem would have been effective at the practical rate of 1.0 million/m² the Steinernema strain N0093 is more effective at lower application rates than Larvanem. However, both strains should first be tested at 0.5 and 1.0 million/m² to proof that the Steinernema strain is more effective than Larvanem at the lower application rates.

Root-collar damage is low in all treatments but the differences in damage are in agreement with the control results of the larvae.

Co	NT	ΈN	ΤS
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1. SUBJECT	3
2. MATERIALS AND METHODS	3
2.1 TRIAL SET UP	3
2.1.1 Trial schedule	3
2.1.2 Crops	3
2.1.3 Treatments/ insecticides	3
2.1.4 Sprayings and inoculations	3
2.1.5 Statistics	4
3. OBSERVATIONS	4
3.1 PEST INDEX	4
3.2 PHYTOTOXICITY	4
3.3 CROP GROWTH AND DEVELOPMENT	5
4. RESULTS AND DISCUSSION	5
4.1 PEST INDEX	5
4.2 PHYTOTOXICITY	6
4.3 CROP GROWTH AND DEVELOPMENT	6
5. CONCLUSIONS	7
6. APPENDIX	7
Appendix 1. Data	7
Appendix 2. Statistic analysis	, 10

1. SUBJECT

Purpose of this research is to show the efficacy of the nematode *Steinernema kraussei*, strain N0093 for control of larvae of the vine weevil in the field

2. MATERIALS AND METHODS

2.1 TRIAL SET UP

2.1.1 Trial schedule

Each treatment consists of 20 trial plants (4 blocks of 5 plants). The test treatments are coded with the capital letters A, B, C and D. The standard treatments are coded S1 and S2. The control (untreated) treatment is coded O.

2.1.2 Crops

The tested crop is Thuja occidentalis 'Smaragd'.

2.1.3 Treatments/ insecticides

code	product	active ingredient	application	dose x 10 ⁶	eggs [#]
0	untreated	-	-	-	2x20
S1	Larvanem	H. megidis	3 October '00	0.5	2x20
S2	Larvanem	H. megidis	26 March '01	0.5	2x20
A	N0093	S. kraussei	3 October '00	0.125	2x20
в	N0093	S. kraussei	3 October '00	0.5	2x20
С	N0093	S. kraussei	26 March '01	0.125	2x20
D	N0093	S. kraussei	26 March '01	0.5	2x20

double inoculation (two times 20 eggs) with vine weevil eggs.

2.1.4 Sprayings and inoculations

The plants of all treatments were twice inoculated with 20 eggs of the vine weevil each time on respectively 26 July and 10 August 2000. Percentage living nematodes in the products were determined several hours before application. Concentration nematodes applied were based on the percentage living nematodes found. Nematodes were applied with a watering can. Three-liter water with nematodes was applied per m².

2.1.5 Statistics

Data were statistically analyzed with ANOVA after square root transformation of the original data. The data are shown in **appendix 1** and the statistic analysis is shown in **appendix 2**.

3. OBSERVATIONS

3.1 PEST INDEX

Plants were harvested on 7 to 10 May 2001. From each plant the number of larvae were counted as well as the developmental stages of the larvae. For the developmental stages we used an index based on the length of the larval body and the transparency of the larvae (between brackets).

larval development index:

stage 1: 0-1 mm (transparent)

stage 2: 2-3 mm (transparent)

stage 3: 4-6 mm (transparent)

- stage 4: 7-10 mm (transparent)
- stage 5: 7-10 mm (milky white)

For the pest index we also used the degree of damage to the root-collar (see index below). root-collar damage index:

- 0 = no damage
- 1 = <25% girdling of the root collar
- 2 = 25 50% girdling of the root collar
- 3 = 50 75% girdling of the root collar
- 4 = 75 100% girdling of the root collar
- 5 = 100% girdling of the root collar

3.2 PHYTOTOXICITY

For phytotoxicity of the green plant parts we looked at the discoloration and/or necrosis of these parts (see index below). For phytotoxicity the control treatment O (no larvae, no chemical) is compared with the other treatments.

phytotox. index green plant parts:

- 0 = no necrosis/discoloration
- 1 = little necrosis (<10%)/discoloration
- 2 = moderate necrosis (10-25%)/discoloration
- 3 = large necrosis (>25%)/discoloration
- 4 = plant dead

3.3 CROP GROWTH AND DEVELOPMENT

Not performed

4. RESULTS AND DISCUSSION

4.1 PEST INDEX

Table 2. Mean number of larvae per plant (n) and percentage reduction compared tothe control treatment O.

code	e treatment	n	total%#	L1% [#]	L2% [#]	L3% [#]	L4% [#]	L5% [#]
0	untreated	3.8 [@]	-	-	-	-	-	-
S 1	Larvanem	3.6	6	-	40	12 *	-	0
S2	Larvanem	4.0	0	-	8	0	-	0
А	N0093	1.3	66 *	-	75 *	35	-	79 *
В	N0093	1.0	75 *	-	50 *	55 *	-	92 *
С	N0093	3.5	8	-	42	25 *	-	0
D	N0093	4.7	0	-	50	25	-	0

Percentage reduction based on number of larvae. L1 and L4 too low number of larvae (4%) for analysis

@ The population in the control (O) consisted for 4% of L1-larvae, 16% of L2-larvae, 26% of L3-larvae, 4% of L4-larvae and 50% of L5-larvae.

* Values followed by an asterisk are significantly different from the control treatment O

Table 3. Root-collar damage expressed as percentage damage compared to the control treatment O (n = mean number of larvae per plant).

code	e treatment	n	%damage [#]
0	untreated	3.8	100
S1	Larvanem	3.6	46 *
S2	Larvanem	4.0	14 *
А	N0093	1.3	14 *
В	N0093	1.0	0 *
С	N0093	3.5	57 *
D	N0093	4.7	71

percentage root-collar damage based on index numbers (see Chapter 3.1)

* Values followed by an asterisk are significantly different from the control treatment O

The percentage mortality of the nematodes in the products prior to application revealed the following results:

October 2000 application

• Larvanem: 44% mortality

• N0093: 57% mortality

March 2001 application

- Larvanem: 9 % mortality
- N0093: 17% mortality

The results show that the winter application of *Steinernema kraussei*, strain N0093 is not effective for control of the vine weevil larvae. The autumn application, however, is giving good control of the larvae. The control for the higher application rate (0.5 million/m², 75% control) is significantly better than the lower rate (0.125 million/m², 66% control) (one-way analysis; $H_0 = P_B < P_A$).

Application of Larvanem at the rate of 0.5 million/m² applied in autumn and winter is giving no control.

4.2 ΡΗΥΤΟΤΟΧΙCITY

There were no symptoms of phytotoxicity found on any of the green plant parts.

4.3 CROP GROWTH AND DEVELOPMENT

not performed.

5. CONCLUSIONS

The results show that the winter application of *Steinernema kraussei*, strain N0093 is not effective for control of the vine weevil larvae. The autumn application, however, is giving good control of the larvae. The control for the higher application rate (0.5 million/m², 75% control) is significantly better than the lower rate (0.125 million/m², 66% control) (one-way analysis; $H_0 = P_B < P_A$). It is possible that a higher rate than 0.5 million/m² will give better control results but this has not been tested in this trial.

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There are no phytotoxic symptoms found on the test plants *Thuja occidentalis* 'Smaragd' at the tested application rates.

APPENDIX

Appendix 1. Data

1399 (2001): DATA FIELD TRIAL VINE WEEVIL LARVAE 2000/2001

Location: Boskoop, Rijneveld 153, The Netherlands

Harvest date: May 2001

Crop: Thuja occidentalis 'Smaragd'

O = untreated

S1 = Heterorhabditis megidis (Larvanem); 0.5×10^6 /m²; 3 October 2000 applied

S2 = Heterorhabditis megidis (Larvanem); 0.5×10^6 /m²; 26 March 2000 applied

October 2000 applied A = Steinernema

B = Steinernema kraussei (N0093); 0.5×10^6 /m²; 3 October 2000 applied C = Steinernema kraussei (N0093); 0.125×10^6 /m²; 26 March 2001 applied

D = Steinernema kraussei (N0093); 0.5×10^6 /m²; 26 March 2001 applied

block	treatm.	nlant	larvae total						damage root-collar
1	O O	1 1	4	1	stag.2	1	0	1	0
1	ŏ	2	2	ò	1	ò	õ	1	õ
1	ŏ	3	8	õ	6	1	1	ò	0
1	ŏ	4	2	õ	õ	2	ò	ŏ	õ
1	ŏ	5	6	õ	1	3	ŏ	2	0 0
1	S1	1	3	õ	0	õ	õ	3	Õ
1	S1	2	2	õ	õ	Õ	õ	2	Õ
1	S1	3	0	õ	õ	Õ	Õ	0	1
1	S1	4		Õ	Ō	0	Ō	2	0
1	S1	5	2 1	Ō	Ō	Ō	Õ	1	0
1	S2	1	7	Ō	1	1	Ō	5	1
1	S2	2	1	0	0	1	Ō	Õ	0
1	S2	3	2	0	0	2	0	0	0
1	S2	4	3	0	2	0	1	0	0
1	S2	5	10	0	3	7	0	0	0
1	Α	1	2	0	0	2	0	0	0
1	Α	2	1	0	0	0	0	1	0
1	Α	3	0	0	0	0	0	0	0
1	Α	4	1	0	0	0	0	1	0
1	Α	5	0	0	0	0	0	0	0
1	В	1	1	0	0	1	0	0	0
1	В	2	1	0	0	0	1	0	0
1	В	3	0	0	0	0	0	0	0
1	В	4	0	0	0	0	0	0	0
1	В	5	0	0	0	0	0	0	0
1	С	1	5	0	2	0	0	3	0
1	с с с с с с	2 3	13	0	0	6	0	7	1
1	C	3	1	0	0	0	0	1	0
1	Ç	4	5	0	0	5	0	0	0
1	Č	5	7	0	2	1	0	4	0
1	D	1	9	0	0	2	0	7	0
1	D	2	3	0	0	0	1	2	0
1	D	3	2 2	0	0	0	0	2 2	0
1	D	4	2	0	0	0	0	2	0
1	D	5	9	0	2	2 2	0	5	0
2	0	1	3	0	0	2	0	1	1

inis meginis (Laivalieni), 0.5×10/m ,
kraussei (N0093); 0.125x10 ⁶ /m ² ; 3 C
kraussei (N10003): 0 $5 \times 10^6 / m^2$: 3 Oct

8 0 2 6 0 0 1 0 5 1 0 4 2 0 0 0 0 2 1 0 5 2 1 1 0 0 0 2 1 0 5 2 1 1 0 0 0 2 1 51 1 5 0 1 0 0 0 2 0 51 4 0 2 0 0 0 1 0 52 2 2 0 0 0 0 0 0 0 52 1 4 0 0 0 0 0 0 0 52 1 1 0 1 0 0 0 0 0 0 52 5 0 0 0 0 0 0 0 0 0 52 5 0 0 0 0 <
$ \begin{array}{cccccccccccccccccccccccccccccccccccc$

Appendix 2. Statistic analysis

Values for larvae are analysed after square-root transformation of the original data, shown in appendix 1. The results shown in appendix 2 are therefore not presenting the average number of larvae found in the treatments.

Genstat 5 Release 4.1 (PC/Windows 98) 29 May 2001 16:18:59 Copyright 1998, Lawes Agricultural Trust (Rothamsted Experimental Station)

3 OUTPUT[WIDTH=80] 1 4 UNITS [NVALUES = 140] 5 FACTOR [LABELS = !T(O, S1, S2, A, B, C, D)] beh 6 FACTOR [LEVELS = !(1...4)] blok 7 factor[lev=5]plant 8 OPEN 'DATA1399.txt'; CHANNEL = 2; width=132 9 "SKIP [CHANNEL = 2] 21" 10 READ [CHANNEL = 2] blok, beh, plant, larv, sta1, sta2, sta3,\ sta4, sta5, schadewrt:\ 11 12 FREP = levels, labels, levels, *, *, *, *, *, *, *, * Mean Maximum Values Missing Identifier Minimum 1 Skew 0.000 3.115 13.000 140 larv sta1 0.00000 0.06475 2.00000 140 Skew 1 sta2 0.0000 0.3741 6.0000 140 Skew 1 140 Skew sta3 0.0000 0.8058 7.0000 1 sta4 0.00000 0.06475 3.00000 140 1 Skew 140 1.806 8.000 Skew sta5 0.000 1 schadewr 0.0000 0.1511 3.0000 140 1 Skew Identifier Values Missing Levels blok 140 0 4 7 beh 140 0 plant 140 0 5 13 close 2 14 CALC Wlarv = SQRT(larv) 15 CALC WL1 = SQRT (sta1) 16 CALC WL2 = SQRT (sta2) 17 CALC WL3 = SQRT (sta3) 18 CALC WL4 = SQRT (sta4) 19 CALC WL5 = SQRT (sta5) 20 21 BLOCKS blok 22 TREATMENTS beh

41 ANOVA [FPROB = yes] Wlarv

41..... ***** Analysis of variance ***** Variate: Wlarv Source of variation d.f.(m.v.) m.s. v.r. F pr. S.S. blok stratum 3 0.5469 0.1823 0.36 blok.*Units* stratum 5.9421 11.58 <.001 beh 6 35.6528 Residual 129(1) 66.1718 0.5130 Total 138(1) 102.2908 * MESSAGE: the following units have large residuals. blok 1.00 *units* 27 1.932 s.e. 0.687 ***** Tables of means ***** Variate: Wlarv Grand mean 1.545 beh O **S**1 S2 В С D А 1.873 0.896 0.650 1.694 2.082 1.754 1.861 *** Standard errors of differences of means *** Table beh 20 rep. 129 d.f. 0.2265 s.e.d. (Not adjusted for missing values) ***** Missing values ***** Variate: Wlarv Unit estimate 115 1.827 Max. no. iterations 2 42 ANOVA [FPROB = yes] WL1

42..... ***** Analysis of variance ***** Variate: WL1 Source of variation d.f.(m.v.) m.s. v.r. Fpr. S.S. 0.23214 0.07738 blok stratum 3 1.27 blok.*Units* stratum 0.45330 0.07555 1.24 0.291 beh 6 Residual 129(1) 7.87403 0.06104 Total 8.55911 138(1) * MESSAGE: the following units have large residuals. blok 1.00 *units* 1 0.878 s.e. 0.237 blok 2.00 *units* 5 0.878 s.e. 0.237 blok 3.00 *units* 1 0.780 s.e. 0.237 blok 3.00 *units* 6 0.878 s.e. 0.237 blok 3.00 *units* 31 0.809 s.e. 0.237 blok 3.00 *units* 33 1.224 s.e. 0.237 blok 4.00 *units* 19 1.358 s.e. 0.237 ***** Tables of means ***** Variate: WL1 Grand mean 0.056 beh O **S1** S2 D В С Α 0.052 0.000 0.071 0.000 0.000 0.121 0.150 *** Standard errors of differences of means *** Table beh rep. 20 d.f. 129 0.0781 s.e.d. (Not adjusted for missing values) ***** Missing values ***** Variate: WL1 Unit estimate 0.037 115

Max. no. iterations 2

43 ANOVA [FPROB = yes] WL2

43..... ***** Analysis of variance ***** Variate: WL2 Source of variation d.f.(m.v.) m.s. v.r. Fpr. S.S. 0.2419 0.81 blok stratum 3 0.7256 blok.*Units* stratum 1.1417 0.1903 0.64 0.701 beh 6 Residual 129(1) 38.5938 0.2992 Total 138(1) 40.4571 * MESSAGE: the following units have large residuals. blok 1.00 *units* 3 1.919 s.e. 0.525 blok 4.00 *units* 25 1.602 s.e. 0.525 ***** Tables of means ***** Variate: WL2 Grand mean 0.288 beh O S1 S2 С D А В 0.422 0.303 0.399 0.150 0.207 0.262 0.271 *** Standard errors of differences of means *** Table beh 20 rep. 129 d.f. 0.1730 s.e.d. (Not adjusted for missing values) ***** Missing values ***** Variate: WL2 Unit estimate 0.226 115 Max. no. iterations 2 44 ANOVA [FPROB = yes] WL3

44..... ***** Analysis of variance ***** Variate: WL3 Source of variation d.f.(m.v.) s.s. m.s. v.r. F pr. blok stratum 3 2.3451 0.7817 1.44 blok.*Units* stratum 1.9329 0.3221 0.59 0.736 beh 6 Residual 129(1) 70.1846 0.5441 Total 138(1) 74.4193 * MESSAGE: the following units have large residuals. blok 1.00 *units* 27 1.931 s.e. 0.708 blok 2.00 *units* 9 2.078 s.e. 0.708 blok 4.00 *units* 14 1.940 s.e. 0.708 ***** Tables of means ***** Variate: WL3 Grand mean 0.518 beh O **S1** S2 Α В С D 0.719 0.469 0.667 0.491 0.387 0.405 0.491 *** Standard errors of differences of means *** Table beh 20 rep. 129 d.f. 0.2333 s.e.d. (Not adjusted for missing values) ***** Missing values ***** Variate: WL3 Unit estimate 115 0.311 Max. no. iterations 2 45 ANOVA [FPROB = yes] WL4

45..... ***** Analysis of variance ***** Variate: WL4 Source of variation d.f.(m.v.) m.s. v.r. Fpr. s.s. blok stratum 3 0.38087 0.12696 2.08 blok.*Units* stratum 0.40559 0.06760 1.11 0.360 beh 6 129(1) Residual 7.85738 0.06091 Total 138(1) 8.63260 * MESSAGE: the following units have large residuals. blok 1.00 *units* 3 0.816 s.e. 0.237 blok 1.00 *units* 14 0.886 s.e. 0.237 blok 1.00 *units* 22 0.886 s.e. 0.237 blok 1.00 *units* 32 0.800 s.e. 0.237 blok 3.00 *units* 1 1.254 s.e. 0.237 blok 3.00 *units* 33 1.556 s.e. 0.237 ***** Tables of means ***** Variate: WL4 Grand mean 0.051 beh O D **S**1 S2 В С А 0.000 0.050 0.000 0.137 0.121 -0.003 0.050 *** Standard errors of differences of means *** Table beh rep. 20 129 d.f. 0.0780 s.e.d. (Not adjusted for missing values) ***** Missing values ***** Variate: WL4 Unit estimate 115 -0.055 Max. no. iterations 2

46 ANOVA [FPROB = yes] WL5

46..... ***** Analysis of variance ***** Variate: WL5 Source of variation d.f.(m.v.) m.s. v.r. F pr. S.S. 2.2431 4.66 blok stratum 3 6.7293 blok.*Units* stratum beh 35.9580 5.9930 12.44 <.001 6 129(1) Residual 62.1533 0.4818 Total 138(1) 104.4484 ***** Tables of means ***** Variate: WL5 Grand mean 1.031 beh O **S1** S2 Α В С 1.177 1.379 1.226 0.371 0.150 1.315 1.600 *** Standard errors of differences of means *** Table beh 20 rep. 129 d.f. 0.2195 s.e.d. (Not adjusted for missing values) ***** Missing values ***** Variate: WL5 Unit estimate 115 1.655 Max. no. iterations 2

47 ANOVA [FPROB = yes] schadewrt

D

47..... ***** Analysis of variance ***** Variate: damage root-collar Source of variation d.f.(m.v.) m.s. v.r. F pr. s.s. 0.3062 blok stratum 3 0.9186 1.58 blok.*Units* stratum beh 1.9026 0.3171 1.64 0.142 6 Residual 129(1) 0.1939 25.0152 Total 138(1) 27.8273 * MESSAGE: the following units have large residuals. blok 3.00 *units* 26 1.895 s.e. 0.423 1.566 s.e. 0.423 blok 4.00 *units* 3 blok 4.00 *units* 32 2.666 s.e. 0.423 ***** Tables of means ***** Variate: schadewr Grand mean 0.152 beh O **S1** S2 В С D Α 0.350 0.162 0.050 0.050 0.000 0.200 0.250 *** Standard errors of differences of means *** Table beh rep. 20 d.f. 129 s.e.d. 0.1393 (Not adjusted for missing values) ***** Missing values ***** Variate: schadewr Unit estimate 115 0.246 Max. no. iterations 2 48 STOP

******** End of 1399-01; bestrijding keverlarven in de vollegrond 2001. Maximum of 7364 data units used at line 48 (81654 left) Genstat 5 Release 4.1 (PC/Windows 98)29 May 2001 16:19:00Copyright 1998, Lawes Agricultural Trust (Rothamsted Experimental Station)

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