Control of grubs and monitoring of the cockchafer, *Melolontha melolontha*

Laboratory and greenhouse experiments concerning control and field experiments concerning luring of *Melolontha melolontha*

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Monitoring of the cockchafer (*Melolontha melolontha* L.) and control of the grubs in outside crops

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1 Introduction

Grubs, larvae of chafers, as a group are a major pest throughout the world. In Europe, the major species attacking agricultural and horticultural crops, is probably the cockchafer (*Melolontha melolontha*). Cockchafer has a three-year life-cycle in most countries. Grubs of this species, as well as others, can cause serious attack to crops.
In Ukraine, damage caused by the grubs of the cockchafer is increasing in outside crops. Particularly strawberries, but also vegetable crops, meadows and ornamental plant production, like rose bushes, can be seriously attacked. Depending on the year losses of 20-25% can occur, resulting in substantial economic damage. The high infestation with grubs of Ukrainian fields is caused by the yearlong neglect of the pest: large areas of fellow, unused land provided the ideal place for propagation and increase of the insects.
In addition, the grubs can only be fought effectively in the period of May-June when they surface to the root level to cause the damage. So far fighting the grubs (larvae) proved to be very difficult. No effective crop protection product is available on the market in the Ukraine. Effective fighting will partly require specific equipment to apply insecticides, which currently is not available in Ukraine. No experiments have been done to find biological ways to control the pest.
Other possibly relevant beetles are the June beetle (*Amphimallon solstitialis*) which has a life cycle of two years and a flight activity in June / July and the garden chafer (*Phyllopertha horticola*) which has one year life cycle. The grubs of those beetles are very similar to the cockchafers.

Figure 1. Grub of Melolontha melolontha. *The species can be distinguished by the characteristics at the end of the abdomen.*

1.1 Aim of this research

The research should ultimately result in a strategy that is effective in reducing the damage as well as cost effective. It should also be environmentally sound and fitting into current Ukrainian regulations. New control methods will possibly require adaptation of existing regulations/laws. Project experts should prepare advice to Ukrainian authorities on how to adapt the current legislation to incorporate the new strategy to fight cockchafer damage (May beetle).

2 Experiments to control grubs

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2.1 Aim of the experiments

The experiments described below aim at finding possibilities to control larvae of *Melolontha melolontha*. Both biological and chemical control options are tested.

2.2 Laboratory experiments – biological control

Two laboratory experiments were conducted to establish whether a number of entomopathogenic nematodes and one bacterial compound would be able to kill *M. melolontha* larvae.

2.2.1 Experiment 1 – nematodes and a bacterium

2.2.1.1 Materials & methods

2.2.1.1.1 Treatments

Table 1 displays the factors used to control grubs in a laboratory experiment.

Table 1. Description of factors used to control grubs, 2006.

Factor	Code	Description	Concentration suspension
B (treatment)	B1	Untreated	0
	B2	Code Na*	3,500 nematodes/ml
	B3	Code Nb*	1,800 nematodes/ml
	B4	Code Nc*	2,900 nematodes/ml
	B5	Code Ba	0.1 mg/ml, 3,000 ITU/mg
T (application method)	T1	Apply on tray with grub in it	
	T2	Apply on grub, subsequently placement in tray	
	T3	Drench food source in suspension	
L (instar)	L1	1st instar	
	L2	2nd instar	
	L3	3rd instar	

* Nematodes were kindly provided by Becker Underwood Ltd. (UK)

The intended application rate was 2.5 billion nematodes per hectare in 1000 l. water per hectare (2,500/ml). Considering the tray size used this would result in 1 ml/tray. This was too little to spread properly. It was therefore decided to apply 2 ml of solution, with a subsequent doubling of the application rate.
Nematodes and Code Ba were applied using pipettes, which were replaced for each treatment. The untreated plots were treated first, using 2 ml of tap water.
The grubs were field-collected in a meadow on sandy soil. Previous to the experiment they had been stored in soil.
The intended concentration of the nematodes was 2,500/ml of water. To check the actual concentration of the prepared nematode suspensions, samples were taken. The numbers of nematodes in these samples were counted. The numbers of nematodes per ml of suspension are displayed in table 2.

Table 2. Numbers of nematodes per ml of nematode suspension (theoretically 2,500/ml) on 23 August 2006.

Factor	Code Na	Code Nb	Code Nc
Count 1	3,690	1,840	2,940
Count 2	3,270	1,800	2,830
Average	3,480	1,820	2,885

Numbers of the Code Nb counts were insufficient, since 2,500 nematodes per ml were expected. No clear reason for this could be found, since numbers of dead nematodes were at a similar (low) level as those of the other nematodes, and preparation had taken place in the same way as the other nematode batches. Levels of both Code Na and Code Nc were at a sufficient level.

2.2.1.1.2 Experimental data

Plots consisted of	:	One small tray (10 x 10 x 5 cm) with one soaked paper germination filter (about 1 mm thick) on the bottom. On this one grub and one piece of food were placed. For manual handling of the grubs, gloves were used. To cover each plot, a piece of soaked paper filter was used. Finally, the tray was covered with a lid.
Food source	:	Pieces of potato (∅ 2 cm, 1 cm thick)
Location of experiment	:	Laboratory. No artificial lighting during the experiment.
Temperature during experiment	:	20°C on average
Starting date	:	23 August 2006
Experimental layout	:	Randomized block design with 3 factors
Number of replicates	:	2 (I and II, annex 1). Through this, the treatment factor had 18 replicates and the application method and instar factors each had 30 replicates.

2.2.1.1.3 Assessments

Assessments focused on numbers of dead grubs, percentage of food source eaten and damage to the germination paper. Numbers of dead grubs per plot were counted on 24, 25, 28 and 30 August and on 1, 4, 7 and 11 September 2006. All dead grubs were visually assessed with binoculars for presence of nematodes; this was done with normal and back light, but grubs were not cut open. On 31 August and 11 September per plot the percentage of potato eaten was estimated. On these dates the damage to the germination paper was awarded a mark, 1 being no damage, 2 being slight damage and 3 being severe damage.

Figure 2. Grub inside small tray; large damage to germination paper.

2.2.1.1.4 Statistics

Data were analysed per factor using analysis of variance and Student's t-distribution with the PPAIR procedure. Means in the same column followed by different letters are significantly different. For useful parameters analysis of the complete data set was performed using analysis of variance.

2.2.1.2 Results

2.2.1.2.1 Per treatment

On 24 August no dead grubs were found. On 25 August no significant differences in the percentage of dead grubs were found between the treatments (table 3).
Both on 28 and 30 August treatment with Code Nb showed more dead grubs than the untreated plots. On 1 September, after nine days, no significant differences between treatments and the untreated plots were found.
Both on 4 and 7 September treatments with Code Na and Code Nb increased the percentage of dead grubs in comparison with the untreated plots.
On 11 September only Code Na showed a significantly higher percentage of dead grubs than the untreated plots.
Treatments with Code Nc and with Code Ba did not show different results from the untreated plots.

At assessing the exterior of the dead grubs, no nematodes were found. Furthermore, no typical signs were observed at dead grubs that could be ascribed to the nematode treatments rather than any other mortality factor. After dieing grubs in general first colour brown and later colour black. After a while they also shrivel and, at nematode treatments are eaten, only the chitin head capsule staying intact.

Figure 3. Dead grubs. Varying stages of decomposition.

Table 3. Percentage of dead grubs per factor (N=18) on seven dates, 2006.

Factor	25 August	28 August	30 August	1 September	4 September	7 September	11 September
Untreated	0.0	0.0 a	0.0 a	0.0	0.0 a	0.0 a	5.6 a
Code Na	0.0	5.6 a b	11.1 a b	16.7	22.2 b	27.8 c	27.8 b
Code Nb	5.6	16.7 b	16.7 b	16.7	22.2 b	22.2 b c	22.2 a b
Code Nc	0.0	0.0 a	5.6 a b	5.6	5.6 a b	5.6 a b	5.6 a
Code Ba	0.0	0.0 a	0.0 a	0.0	0.0 a	5.6 a b	5.6 a
LSD (α = 0.05)	7.0	13.3	16.5	17.6	19.4	21.1	22.2
F-prob.	0.412	0.062	0.204	0.135	0.034	0.043	0.120

In comparison with the untreated plots, none of the treatments resulted in a significant decrease of either the percentage of potato eaten or in the mark for damage to the germination paper (table 4).

Table 4. Percentage of potato eaten and mark for damage to germination paper (N=18) on two dates, 2006.

Factor	% eaten		Damage to paper	
	31 August	11 September	31 August	11 September
Untreated	17.5	38.6	2.33	2.44
Code Na	21.1	44.2	2.39	2.56
Code Nb	18.3	32.5	2.44	2.44
Code Nc	24.2	45.0	2.44	2.56
Code Ba	19.2	42.2	2.39	2.61
LSD (α = 0.05)	10.4	15.3	0.33	0.29
F-prob.	0.716	0.471	0.958	0.715

2.2.1.2.2 Per application method

On 24 August no dead grubs were found. Both on 25 and 28 August no significant differences in the percentage of dead grubs were found between the application methods of the treatments, as well as on 1 September (table 5).
Both on 30 August and 4 September treatment of both the tray and the grub showed a significantly higher percentage of dead grubs than treatment of the food only; treatment of only the grub, before placement in the tray, differed of neither one of the other application methods.
At the last two assessments on 7 and 11 September, after 15 and 19 days, treatment of tray and grub showed an increase of mortality over the remaining application methods.
At treatment of the grub only, the percentage of dead grubs stayed on the same level from 28 August through 11 September, whereas this kept increasing over this period at treatment of both tray and grub.

Table 5. Percentage of dead grubs per factor (N=30) on seven dates, 2006.

Factor	25 August	28 August	30 August	1 September	4 September	7 September	11 September
Tray + grub	0.0	6.7	13.3 . b	13.3	20.0 . b	23.3 . b	26.7 . b
Grub only	3.3	6.7	6.7 a b	6.7	6.7 a b	6.7 a .	6.7 a .
Drench food	0.0	0.0	0.0 a .	3.3	3.3 a .	6.7 a .	6.7 a .
LSD (α = 0.05)	5.4	10.6	12.8	13.9	15.3	16.6	17.0
F-prob.	0.372	0.359	0.123	0.350	0.078	0.076	0.030

Both on 31 August and 11 September the treatments showed no mutual differences in the percentage of potato eaten (table 6).
On 31 August treatment of only the grubs resulted in a significant decrease of the mark for damage to the germination paper in comparison with the remaining application methods. On 11 September no differences were found between the application methods.

Table 6. Percentage of potato eaten and mark for damage to germination paper (N=30) on two dates, 2006.

Factor	% eaten		Damage to paper	
	31 August	11 September	31 August	11 September
Tray + grub	22.0	40.3	2.47 . b	2.50
Grub only	20.8	37.7	2.20 a .	2.47
Drench food	17.3	43.5	2.53 . b	2.60
LSD (α = 0.05)	8.1	11.9	0.26	0.23
F-prob.	0.486	0.615	0.029	0.471

2.2.1.2.3 Per instar

Table 7 displays the percentage of dead grubs per instar on seven dates.

Table 7. Percentage of dead grubs per factor (N=30) on seven dates, 2006.

Factor	25 August	28 August	30 August	1 September	4 September	7 September	11 September
1st instar	0.0	10.0	16.7 . b	16.7 . b	20.0 . b	23.3 . b	23.3 . b
2nd instar	0.0	0.0	0.0 a .	3.3 a .	6.7 a b	10.0 a b	10.0 a b
3rd instar	3.3	3.3	3.3 a .	3.3 a .	3.3 a .	3.3 a .	6.7 a
LSD (α = 0.05)	5.4	10.5	12.5	13.7	15.3	16.5	17.3
F-prob.	0.372	0.164	0.024	0.087	0.078	0.055	0.134

On 24 August no dead grubs were found. On 25 and 28 August no significant differences in the percentage of dead grubs were found between the instars (table 7).
Both on 30 August and 1 September the 1st instar resulted in significantly more dead grubs than the 2nd and

3rd instars.
At the last three assessments, on 4, 7 and 11 September, the 1st instar showed an increase in mortality in comparison with the 3rd instar, but the 2nd instar differed from neither of the former.

All instars mutually differed significantly in the percentage of potato eaten on 31 August and 11 September (table 8), the first instar showing the lowest percentage of potato eaten and the third instar showing the highest.
On 31 August the same differences were visible in the marks given for damage to the germination paper.
On 11 September both the 2nd and 3rd instar showed a significantly higher mark than the 1st instar, but they did not differ mutually.

Table 8. Percentage of potato eaten and mark for damage to germination paper (N=30) on two dates, 2006.

Factor	% eaten		Damage to paper	
	31 August	11 September	31 August	11 September
1st instar	3.2 a . .	5.8 a . .	1.90 a . .	1.97 a .
2nd instar	15.0 . b .	29.7 . b .	2.47 . b .	2.73 b
3rd instar	42.0 . . c	86.0 . . c	2.83 . . c	2.87 b
LSD (α = 0.05)	8.1	11.9	0.26	0.23
F-prob.	< 0.001	< 0.001	< 0.001	< 0.001

2.2.1.2.4 Interactions grub mortality at final assessment

At the 1st instar, both Code Na and Code Nb resulted in a significant increase of grub mortality on 11 September (table 9). Code Na was the only treatment to show a significantly higher percentage of dead grubs than the untreated plots at the 2nd instar. At the 3rd instar no increase of mortality was found in comparison with the untreated plots.
All significant results found can be ascribed to the application to both tray and grub. Where significant differences between treatments and the untreated plots were found, this application method showed 100% mortality.
Treatment of tray and grub with Code Na resulted in significantly more dead 1st and 2nd instar grubs than 3rd instar grubs. Treatment with Code Nb in the same way resulted in more dead 1st instar grubs than 2nd or 3rd instar grubs.

Table 9. Percentage of dead grubs per combination of factors (N=2) on 11 September 2006.

Treatment	Application method		1st instar	2nd instar	3rd instar
Untreated	Tray + grub		0.0	0.0	50.0
	Grub only		0.0	0.0	0.0
	Drench food		0.0	0.0	0.0
	Average		***0.0***	***0.0***	***16.7***
Code Na	Tray + grub		100.0	100.0	0.0
	Grub only		0.0	0.0	0.0
	Drench food		0.0	50.0	0.0
	Average		***33.3***	***50.0***	***0.0***
Code Nb	Tray + grub		100.0	0.0	0.0
	Grub only		50.0	0.0	50.0
	Drench food		0.0	0.0	0.0
	Average		***50.0***	***0.0***	***16.7***
Code Nc	Tray + grub		50.0	0.0	0.0
	Grub only		0.0	0.0	0.0
	Drench food		0.0	0.0	0.0
	Average		***16.7***	***0.0***	***0.0***
Code Ba	Tray + grub		0.0	0.0	0.0
	Grub only		0.0	0.0	0.0
	Drench food		50.0	0.0	0.0
	Average		***16.7***	***0.0***	***0.0***
LSD (α = 0.05)	Treatment – application method – instar	52.0			
F-prob.		0.063			
LSD (α = 0.05)	***Treatment – instar***	***30.0***			
F-prob.		***0.017***			

2.2.1.3 Discussion and conclusions

- Treatments with Code Na at 3,500 nematodes per ml and Code Nb at 1,800 nematodes per ml show control of grubs (table 3). This was only visible by the percentage of dead grubs, rather than by the percentage of potato eaten or the mark for damage to the germination paper. This is probably explained by the fact that the smaller instars accounted for most of the mortality found, as displayed in table 9. This table also shows that mortality at the successful treatments can be ascribed to the 1st instar grubs. The 3rd instar, being the most ferocious, showed no significant increase in mortality. Therefore possible effects of the treatments on the smaller instars, showed by the percentage of potato eaten or the mark for damage to germination paper, cannot be made visible.
- Treatment with Code Nc at 2,900 nematodes per ml and Code Ba at 0,1 g per ml show no effects on grubs.
- Treatment of both grub and tray shows the highest mortality. This result was significantly better than that of only drenching the food (potato), on 30 August and 4, 7 and 11 September. Treatment of only the grub differed from neither of the other application methods on 30 August and 4 September. On 7 and 11 September it showed a decrease in comparison with treatment of grub and tray.
- The poor result of drenching may have two reasons. On the one hand drenching may result in a lower application rate per plot. It is not sure what the applied rate was, only the concentration of the suspension is known. On the other hand, this application rate is depending on the feeding pattern of the grubs, the smaller instars eating much less than the larger stages.
- The younger and smaller the grub, the more susceptible to the treatments. The effects of the effective treatments, as well as the effects of application method are largely accounted for by the 1st instar (table 9).

2.2.2 Experiment 2 – nematodes

2.2.2.1 Materials & methods

2.2.2.1.1 Treatments

Table 10 displays the factors used to control grubs in a laboratory experiment.

Table 10. Description of factors used to control grubs, 2006.

Factor	Code	Description	Concentration suspension
B (treatment)	B1	Untreated	0
	B2	Code Na*	11,000 nematodes/ml
	B3	Code Nc	5,230 nematodes/ml
	B4	Code Nc	6,200 nematodes/ml
L (instar)	L1	1st instar	
	L3	3rd instar	

* nematodes were kindly provided by Becker Underwood Ltd.

The intended application rate was 2.5 billion nematodes per hectare in 1000 l. water per hectare (2,500/ml). Considering the tray size used this resulted in 2.6 ml/tray. Nematodes were applied using pipettes, which were replaced for each treatment. The untreated plots were treated first, using 2.6 ml of tap water.
The grubs were field-collected in a meadow on sandy soil. The had been stored in soil.
The intended concentration of the nematodes was 2,500/ml of water. To check the actual concentration of the prepared nematode suspensions, samples were taken. The numbers of nematodes in these samples were counted. The numbers of nematodes per ml of suspension are displayed in table 11.

Table 11. Numbers of nematodes per ml of nematode suspension (theoretically 2500/ml) on 23 August 2006.

Factor	Code Na	Code Nc	Code Nc
Count 1	12,000	5,600	7,660
Count 2	10,000	4,860	4,700
Average	11,000	5,230	6,180

Numbers of the Code Na were at a very high level. This may well be the result of the fact that by accident the sample tipped over. The suspension probably had sagged somewhat. Both Code Nc and Code Nc showed sufficient numbers of nematodes in the samples.

2.2.2.1.2 Experimental data

Plots consisted of	:	Two small trays (13 x 20 x 4 cm) completely filled with clay soil (15% silt). In a superficial hole one grub was placed (figure 4). For manual handling of the grubs, gloves were used. After this treatment took place and the soil was moistened thoroughly. Finally, trays were covered with a lid.
Temperature during experiment	:	18°C on average
Location of experiment	:	Laboratory. No artificial lighting during the experiment.
Starting date	:	30 November 2006
Experimental layout	:	Randomized block design with 2 factors
Number of replicates	:	6 (I to VI, annex 1). Through this, the treatment factor had 8 replicates and the instar factor had 16 replicates.

Figure 4. Tray used to test nematodes on grubs. Left picture filled with soil and tunnels made by grub.

2.2.2.1.3 Assessments

On 8 and 21 December assessments were made. Of each tray mortality of the grub was assessed. On 8 December one tray of each plot was assessed, to leave the other tray undisturbed until the second assessment. Soil and grub after assessment were placed back into the tray. On 21 December the second tray of each plot was assessed. Also the first tray was assessed again. Dead grubs were visually assessed with binoculars for presence of nematodes; this was done with normal and back light, but grubs were not cut open. Of the nematodes found the species were not determined.

2.2.2.1.4 Statistics

Data were analysed per factor using analysis of variance and Student's t-distribution with the PPAIR procedure. Means in the same column followed by different letters are significantly different.

2.2.2.2 Results

No significant differences were found between the treatments and the untreated plots, at either of both assessments (table 12). In total, mortality in the untreated plots was equal to that of the treatments. Also nematodes were found on the dead grubs of the untreated plots.

Table 12. Percentage of dead grubs per factor at three assessments on two dates and total and percentage of grubs with nematodes present on two dates and total (N=8), 2006.

Factor	Dead grubs				Grubs with nematodes		
	Tray 1 after 10 days	Tray 1 after 23 days	Tray 2, after 23 days	Total	Tray 1	Tray 2	Total
Untreated	0	8	17	13	0	17	8
Code Na	8	25	8	17	8	8	8
Code Nc	17	17	8	13	0	8	4
Code Nc	17	17	17	17	8	8	8
Average	10	17	13	15	4	10	7
LSD (α = 0.05)	24	31	27	23	17	26	17
F-prob.	0.463	0.759	0.856	0.966	0.577	0.884	0.950

Ten days after starting the experiment, significantly more grubs of the 3rd instar were dead than grubs of the 1st instar (table 13). At the remaining assessments, no significant differences were found between the instars. Also no significant differences were found in the percentages of dead grubs with nematodes. Nevertheless, the percentage of grubs with nematodes was higher for 3rd instar at all assessments.

Table 13. Percentage of dead grubs per factor at three assessments on two dates and total and percentage of grubs with nematodes present (N=16) on two dates and total , 2006.

Factor	Dead grubs				Grubs with nematodes		
	Tray 1 after 10 days	Tray 1 after 23 days	Tray 2, after 23 days	Total	Tray 1	Tray 2	Total
1st instar	0 a .	8	8	8	0	4	2
3rd instar	21 . b	25	17	21	8	17	13
Average	10	17	13	15	4	10	7
LSD (α = 0.05)	17	31	27	16	12	26	12
F-prob.	0.019	0.133	0.386	0.132	0.165	0.170	0.096

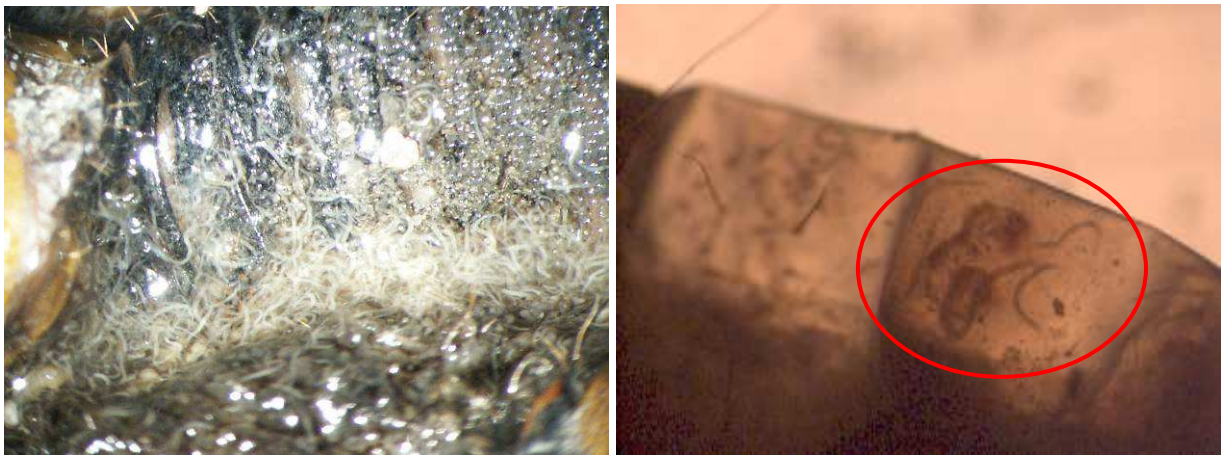

Figure 5. Nematodes outside (left) and inside grub corpse.

2.2.2.3 Discussion and conclusions

- The nematode strains tested – Code Na at 11,000/ml, Code Nc at 5,230/ml and Code Nc at 6,200/ml – showed no significant mortality after three weeks (table 12). A complete kill at either of the strains was not very realistic, considering the short period of time between treatment and final assessment.
- Mortality was at a high level in the untreated trays. This may well be the reason that no significant differences in comparison with the untreated plots were found. Also nematodes were found at some of the grubs from the untreated plots. The species of these nematodes is not clear, since this was not assessed. These nematodes may have been present in either the soil or the grub itself.
- At about 50% of the dead grubs, nematodes were found at assessment of the grubs – without cutting them open. Of the remaining half it is not sure what the cause of death was.
- Also at the untreated plots nematodes were found in the grubs. Of the untreated grubs in tray 2 even at all the grubs nematodes were found. It is very likely but not sure that these nematodes caused the killing of the grubs, since they were not determined. The nematodes found could only have been present in the soil used or already inside the grubs before starting the experiment. Contamination at the start of the experiment can be ruled out since pipettes were replaced at every treatment and the untreated plots were treated first.
- The 3rd instar is significantly more vulnerable to nematodes than the 1st instar. Already at the first assessment in more dead 3rd instar grubs were found than 1st instar grubs (table 13).

2.3 Greenhouse experiments – insecticides and biologicals

The following three experiments were carried out in the greenhouse. The aim of this was to screen a series of compounds and application rates under conditions that approximate field conditions, thus reducing the gap between experimental conditions and field conditions.

2.3.1 KAS585 – first screening

The aim of this experiment is to test a series of compounds at the N and 2N application rate. The N application rate was chosen based on application rates in practice and researcher's experience.

2.3.1.1 Materials & methods

2.3.1.1.1 Treatments

Table 14 displays the compounds and application rates used to control grubs in a greenhouse experiment.

Table 14. Description of compounds and application rates used to control grubs, 2006.

Treatment	Compound	Dose (N & 2N) formulated per hectare
A	Untreated – grubs	-
B	Untreated + grubs	-
C	Code A-b	1 kg
D	Code A-b	2 kg
E	Kali 60	1,500 kg
F	Kali 60	3,000 kg
G	Code B	6 l
H	Code B	12 l
I	Code C	30 kg
J	Code C	60 kg
K	Code D	20 l
L	Code D	40 l
M	Code E	1 kg
N	Code E	2 kg
O	Code F	0.5 l
P	Code F	1 l
Q	Code G	4 l
R	Code G	8 l
S	Code H	2 l
T	Code H	4 l
U	Code I	4 l
V	Code I	8 l
W	Code J	2 l
X	Code J	4 l
Y	Code K	4 l
Z	Code K	8 l
AA	Code L	2 l
BB	Code L	4 l
CC	Code M	2 l
DD	Code M	4 l
EE	Code N	10 kg
FF	Code N	20 kg
GG	Code O	1 l
HH	Code O	2 l
II	Code P	1 l
JJ	Code P	2 l
KK	Code Q	3 l
LL	Code Q	6 l
MM	Code R	2 l
NN	Code R	4 l
OO	Code S	2 l
PP	Code S	4 l
QQ	Code F + Code I	0.25 l + 0.5 l
RR	Code F + Code I	0.5 l + 1 l
SS	Code E + Code H	1 kg + 2 l
TT	Code E + Code H	2 kg + 4 l

2.3.1.1.2 Experimental data

Plots consisted of : - One 10 l. pot containing a clay soil.
- The lower half of each pot was untreated, the upper half was treated.
- The grubs were placed in the lower half of the pot, on top of each pot a piece of turf was placed.

Application method : Liquids were sprayed over the soil in a spraying cabin, other products were applied by hand.

Number of grubs per pot : 5

Temperature during experiment : No heating
Minimum ; 18°C
Maximum ; 28°C

Irrigation : The pots were irrigated regularly.

Starting date : 8 September 2006

Number of replicates : 4 (I to IV, annex 1)

2.3.1.1.3 Assessments

The pieces of turf were weighed before placement on the pots and at the end of the experiment. Assessment was made on 6 October. At this assessment, the percentage of the turf (roots) that was eaten by the grubs, was estimated (figure 6). Numbers of dead grubs found were counted as well as numbers of living grubs. Numbers of living grubs were counted separately for the turf, the upper (treated) half of the pot and the lower (untreated) half of the pot.

2.3.1.1.4 Statistics

Data were analysed using analysis of variance and Student's t-distribution with the PPAIR procedure. Means in the same column followed by different letters are significantly different.

2.3.1.2 Results

The untreated plots with grubs (B) showed only a low percentage of weight loss (table 15). This did not differ from the untreated plots without grubs (A). Treatments with 1 and 2 kg/ha Code A-b, 4 and 8 l/ha Code K and 2 l/ha Code R showed a significantly higher percentage of weight loss than the untreated plots without grubs. The remaining treatments showed the same weight loss as the untreated plots with out grubs. Application of 4 l/ha Code R showed a significant decrease of weight loss in comparison with 2 l/ha. Treatments with 1,500 and 3,000 kg of kali 60, 12 l/ha Code B, 30 and 60 kg/ha Code C, 40 l/ha Code D, 1 and 2 kg/ha Code E, 0.5 and 1 l/ha Code F, 2 and 4 l/ha Code H, 2 and 4 l/ha Code J, 4 l/ha Code K, 4 l/ha Code L, 2 and 4 l/ha Code M, 10 and 20 kg/ha Code N, 1 and 2 l/ha Code O, 2 l/ha Code P, 3 l/ha Code Q, 4 l/ha Code R, 2 and 4 l/ha Code S and both application rates of both Code F + Code I and Code E + Code H did not differ significantly from the untreated plots without grubs in the percentage of damage to the turf. The remaining treatments resulted in a significant increase of damage in comparison with the untreated plots without grubs. Application of 2 l/ha Code P showed a dose response in the percentage of damage in comparison with 1 l/ha. The untreated plots with grubs only resulted in 6.3% of visual damage to the turf.

Table 15. Percentage weight loss over the experiment period and estimated percentage of damage to the turf on 6 October 2006.

Treatment		Dose per hectare	Weight loss	Damage
A	Untreated – grubs	-	8.1 a	0.0 a . . . . .
B	Untreated + grubs	-	7.6 a	6.3 a b . . . . .
C	Code A-b	1 kg	29.3 . . . . e f	38.8 . . . . . g h
D	Code A-b	2 kg	26.1 . . c d e f	33.8 . . . d e f g h
E	Kali 60	1,500 kg	6.8 a	5.0 a b . . . . .
F	Kali 60	3,000 kg	6.6 a	2.5 a b . . . . .
G	Code B	6 l	14.5 a b c d . .	23.8 . b c d e f g h
H	Code B	12 l	12.2 a b c . . .	7.5 a b c . . . . .
I	Code C	30 kg	13.9 a b c d . .	2.5 a b . . . . .
J	Code C	60 kg	11.8 a b c . . .	5.0 a b . . . . .
K	Code D	20 l	19.0 a b c d e .	37.5 . . . . . f g h
L	Code D	40 l	15.1 a b c d e .	16.3 a b c d e f . .
M	Code E	1 kg	13.3 a b c . . .	6.3 a b . . . . .
N	Code E	2 kg	10.0 a	6.3 a b . . . . .
O	Code F	0.5 l	12.0 a b c . . .	0.0 a . . . . .
P	Code F	1 l	12.4 a b c . . .	1.3 a . . . . .
Q	Code G	4 l	14.9 a b c d . .	12.5 a b c d . . . .
R	Code G	8 l	10.8 a	17.5 a b c d e f g .
S	Code H	2 l	10.6 a	2.5 a b . . . . .
T	Code H	4 l	13.8 a b c d . .	1.3 a . . . . .
U	Code I	4 l	15.7 a b c d e .	16.3 a b c d e f . .
V	Code I	8 l	17.3 a b c d e .	23.8 . b c d e f g h
W	Code J	2 l	16.1 a b c d e .	1.3 a . . . . .
X	Code J	4 l	13.6 a b c d . .	0.0 a . . . . .
Y	Code K	4 l	37.4 . . . . . f	20.0 a b c d e f g h
Z	Code K	8 l	25.2 . b c d e f	40.0 . . . . . h
AA	Code L	2 l	18.2 a b c d e .	36.3 . . . . e f g h
BB	Code L	4 l	14.1 a b c d . .	15.0 a b c d e . . .
CC	Code M	2 l	13.2 a b c . . .	18.8 a b c d e f g h
DD	Code M	4 l	17.9 a b c d e .	17.5 a b c d e f g .
EE	Code N	10 kg	11.9 a b c . . .	2.5 a b . . . . .
FF	Code N	20 kg	12.6 a b c . . .	3.8 a b . . . . .
GG	Code O	1 l	8.9 a	5.0 a b . . . . .
HH	Code O	2 l	17.7 a b c d e .	6.3 a b . . . . .
II	Code P	1 l	13.9 a b c d . .	23.8 . b c d e f g h
JJ	Code P	2 l	16.6 a b c d e .	1.3 a . . . . .
KK	Code Q	3 l	15.2 a b c d e .	17.5 a b c d e f g .
LL	Code Q	6 l	16.7 a b c d e .	36.3 . . . . e f g h
MM	Code R	2 l	27.8 . . . d e f	28.8 . . c d e f g h
NN	Code R	4 l	13.2 a b c . . .	10.0 a b c . . . . .
OO	Code S	2 l	16.3 a b c d e .	17.5 a b c d e f g .
PP	Code S	4 l	16.7 a b c d e .	16.3 a b c d e f . .
QQ	Code F + Code I	0.25 l + 0.5 l	15.2 a b c d e .	0.0 a . . . . .
RR	Code F + Code I	0.5 l + 1 l	6.9 a	1.3 a . . . . .
SS	Code E + Code H	1 kg + 2 l	14.1 a b c d . .	2.5 a b . . . . .
TT	Code E + Code H	2 kg + 4 l	11.5 a b	0.0 a . . . . .
LSD (α = 0.05)			14.3	22.0
F-prob.			0.080	< 0.001

Figure 6. Undamaged (left) and heavily attacked pieces of turf.

At the untreated pots, on average one out of five (20%) of the grubs died (table 16). Both application rates of Code C and of Code N showed a significantly lower number living grubs than the untreated plots. Treatment with 12 l/ha Code B also showed a decrease of the number of living grubs, but 6 l/ha did not. Similarly 2 l/ha Code P showed fewer living grubs than the untreated plots, but 1 l/ha did not. At both compounds the application rates showed a dose response. Code C at 30 and 60 kg/ha showed significant mortality, but Code D at 20 and 40 l/ha, containing the same amounts of active ingredient, showed insufficient mortality. Both the higher and the lower application rate of either product differed significantly. The remaining treatments showed no significantly different numbers of living grubs in comparison with the untreated plots.

Table 16. Percentage and number of living grubs on 6 October 2006.

Treatment		Dose per hectare	Percentage	Number
B	Untreated + grubs	-	80	4.00 . . . . . g h i j
C	Code A-b	1 kg	100	5.00 . . . . . j
D	Code A-b	2 kg	95	4.75 . . . . . i j
E	Kali 60	1,500 kg	75	3.75 . . . . . f g h i .
F	Kali 60	3,000 kg	90	4.50 . . . . . h i j
G	Code B	6 l	90	4.50 . . . . . h i j
H	Code B	12 l	15	0.75 a b . . . . .
I	Code C	30 kg	45	2.25 . . c d . . . . .
J	Code C	60 kg	55	2.75 . . . d e f . . . .
K	Code D	20 l	85	4.25 . . . . . g h i j
L	Code D	40 l	75	3.75 . . . . . f g h i .
M	Code E	1 kg	95	4.75 . . . . . i j
N	Code E	2 kg	55	2.75 . . . d e f . . . .
O	Code F	0.5 l	80	4.00 . . . . . g h i j
P	Code F	1 l	70	3.50 . . . . e f g h . .
Q	Code G	4 l	90	4.50 . . . . . h i j
R	Code G	8 l	80	4.00 . . . . . g h i j
S	Code H	2 l	85	4.25 . . . . . g h i j
T	Code H	4 l	70	3.50 . . . . e f g h . .
U	Code I	4 l	100	5.00 . . . . . j
V	Code I	8 l	90	4.50 . . . . . h i j
W	Code J	2 l	85	4.25 . . . . . g h i j
X	Code J	4 l	90	4.50 . . . . . h i j
Y	Code K	4 l	85	4.25 . . . . . g h i j
Z	Code K	8 l	95	4.75 . . . . . i j
AA	Code L	2 l	95	4.75 . . . . . i j
BB	Code L	4 l	80	4.00 . . . . . g h i j
CC	Code M	2 l	95	4.75 . . . . . i j
DD	Code M	4 l	95	4.75 . . . . . i j
EE	Code N	10 kg	25	1.25 . b c . . . . .
FF	Code N	20 kg	10	0.50 a b . . . . .
GG	Code O	1 l	80	4.00 . . . . . g h i j
HH	Code O	2 l	90	4.50 . . . . . h i j
II	Code P	1 l	85	4.25 . . . . . g h i j
JJ	Code P	2 l	50	2.50 . . . d e . . . . .
KK	Code Q	3 l	95	4.75 . . . . . i j
LL	Code Q	6 l	85	4.25 . . . . . g h i j
MM	Code R	2 l	80	4.00 . . . . . g h i j
NN	Code R	4 l	80	4.00 . . . . . g h i j
OO	Code S	2 l	65	3.25 . . . d e f g . . .
PP	Code S	4 l	90	4.50 . . . . . h i j
QQ	Code F + Code I	0.25 l + 0.5 l	90	4.50 . . . . . h i j
RR	Code F + Code I	0.5 l + 1 l	65	3.25 . . . d e f g . . .
SS	Code E + Code H	1 kg + 2 l	90	4.50 . . . . . h i j
TT	Code E + Code H	2 kg + 4 l	70	3.50 . . . . e f g h . .
LSD (α = 0.05)				1.22
F-prob.				< 0.001

Treatments with 2 kg/ha Code E, 0.5 and 1 l/ha Code F, 4 l/ha Code J, 20 kg/ha Code N and both application rates of either Code F + Code I and Code E + Code H showed a decrease of the numbers of grubs in the turf(table 17). The same went for12 l/ha Code B, whereas 6 l/ha showed a significant increase of grubs in the turf in comparison with the untreated plots. Also at application of 1 kg/ha Code A-b, 20 l/ha Code D, 8 l/ha Code I, 4 l/ha Code K, 4l/ha Code L, 4 l/ha Code M and 3 l/ha Code Q significantly more grubs were found in the turf than at the untreated plots. Both at treatment with Code B and Code P the higher application rate showed significantly fewer grubs in the turf than the lower rate.
No significant differences in comparison with the untreated plots were found at the numbers of grubs in either the upper or lower half of the pots.

Table 17. Numbers of living grubs in turf, top half of pots and lower half of pots on 6 October 2006.

Treatment		Dose per hectare	Turf	Upper half	Lower half
B	Untreated + grubs	-	1.75 . b c d e f . . .	0.75 a b c	1.50 a b c d e f
C	Code A-b	1 kg	3.75 i	0.50 a b c	0.75 a b c . . .
D	Code A-b	2 kg	2.75 . . . . . f g h i	0.50 a b c	1.50 a b c d e f
E	Kali 60	1,500 kg	1.25 a b c d e	0.50 a b c	2.00 . . c d e f
F	Kali 60	3,000 kg	2.50 . . . . e f g h i	0.00 a . .	2.00 . . c d e f
G	Code B	6 l	3.75 i	0.00 a . .	0.75 a b c . . .
H	Code B	12 l	0.25 a	0.25 a b .	0.25 a b . . . .
I	Code C	30 kg	1.25 a b c d e	0.25 a b .	0.75 a b c . . .
J	Code C	60 kg	1.25 a b c d e	0.25 a b .	1.25 a b c d e .
K	Code D	20 l	3.50 . . . . . h i	0.75 a b c	0.00 a . . . . .
L	Code D	40 l	2.25 . . . d e f g h .	0.25 a b .	1.25 a b c d e .
M	Code E	1 kg	1.25 a b c d e	0.50 a b c	3.00 . . . . . f
N	Code E	2 kg	0.25 a	1.00 a b c	1.50 a b c d e f
O	Code F	0.5 l	0.00 a	1.50 . b c	2.50 . . . d e f
P	Code F	1 l	0.00 a	0.50 a b c	3.00 . . . . . f
Q	Code G	4 l	2.00 . . c d e f g . .	1.25 a b c	1.25 a b c d e .
R	Code G	8 l	2.25 . . . d e f g h .	0.50 a b c	1.25 a b c d e .
S	Code H	2 l	1.00 a b c d	0.25 a b .	3.00 . . . . . f
T	Code H	4 l	0.50 a b	1.50 . b c	1.50 a b c d e f
U	Code I	4 l	2.50 . . . . e f g h i	0.75 a b c	1.75 . b c d e f
V	Code I	8 l	3.50 . . . . . h i	0.75 a b c	0.25 a b . . . .
W	Code J	2 l	0.75 a b c	1.75 . . c	1.75 . b c d e f
X	Code J	4 l	0.25 a	1.50 . b c	2.75 . . . . e f
Y	Code K	4 l	3.25 . . . . . g h i	1.00 a b c	0.00 a . . . . .
Z	Code K	8 l	2.50 . . . . e f g h i	1.25 a b c	1.00 a b c d . .
AA	Code L	2 l	3.00 . . . . . f g h i	0.50 a b c	1.25 a b c d e .
BB	Code L	4 l	3.50 . . . . . h i	0.25 a b .	0.25 a b . . . .
CC	Code M	2 l	2.75 . . . . . f g h i	0.50 a b c	1.50 a b c d e f
DD	Code M	4 l	3.50 . . . . . h i	0.75 a b c	0.50 a b c . . .
EE	Code N	10 kg	0.75 a b c	0.25 a b .	0.25 a b . . . .
FF	Code N	20 kg	0.00 a	0.50 a b c	0.00 a . . . . .
GG	Code O	1 l	1.75 . b c d e f . . .	0.50 a b c	1.75 . b c d e f
HH	Code O	2 l	1.25 a b c d e	1.25 a b c	2.00 . . c d e f
II	Code P	1 l	2.50 . . . . e f g h i	0.50 a b c	1.25 a b c d e .
JJ	Code P	2 l	0.75 a b c	0.25 a b .	1.50 a b c d e f
KK	Code Q	3 l	3.25 . . . . . g h i	0.25 a b .	1.25 a b c d e .
LL	Code Q	6 l	2.75 . . . . . f g h i	0.25 a b .	1.25 a b c d e .
MM	Code R	2 l	3.00 . . . . . f g h i	0.00 a . .	1.00 a b c d . .
NN	Code R	4 l	1.75 . b c d e f . . .	1.00 a b c	1.25 a b c d e .
OO	Code S	2 l	3.00 . . . . . f g h i	0.00 a . .	0.25 a b . . . .
PP	Code S	4 l	3.00 . . . . . f g h i	0.50 a b c	1.00 a b c d . .
QQ	Code F + Code I	0.25 l + 0.5 l	0.00 a	1.50 . b c	3.00 . . . . . f
RR	Code F + Code I	0.5 l + 1 l	0.00 a	1.50 . b c	1.75 . b c d e f
SS	Code E + Code H	1 kg + 2 l	0.00 a	1.50 . b c	3.00 . . . . . f
TT	Code E + Code H	2 kg + 4 l	0.00 a	0.75 a b c	2.75 . . . . e f
LSD (α = 0.05)			1.79	0.68	1.41
F-prob.			< 0.001	0.303	< 0.001

The total numbers of living grubs and spatial division, as shown in table 17, are also displayed in figure 7. At treatments with Code F at rates of 0.5 and 1 l/ha and the combined treatments with Code F + Code I and Code E + Code H showed no grubs in the turf at all. Treatment with 20 kg/ha Code N did as well, but 10 kg/ha Code N did not. Proportionally the highest numbers of grubs were found in the lower half of the pots at treatments with 1,500 kg/ha Kali 60, 1 and 2 kg/ha Code E, 0.5 and 1 l/ha Code F, 2 l/ha Code H, 8 l/ha Code J, 2 l/ha Code P and the combined treatments with Code F + Code I and Code E + Code H.

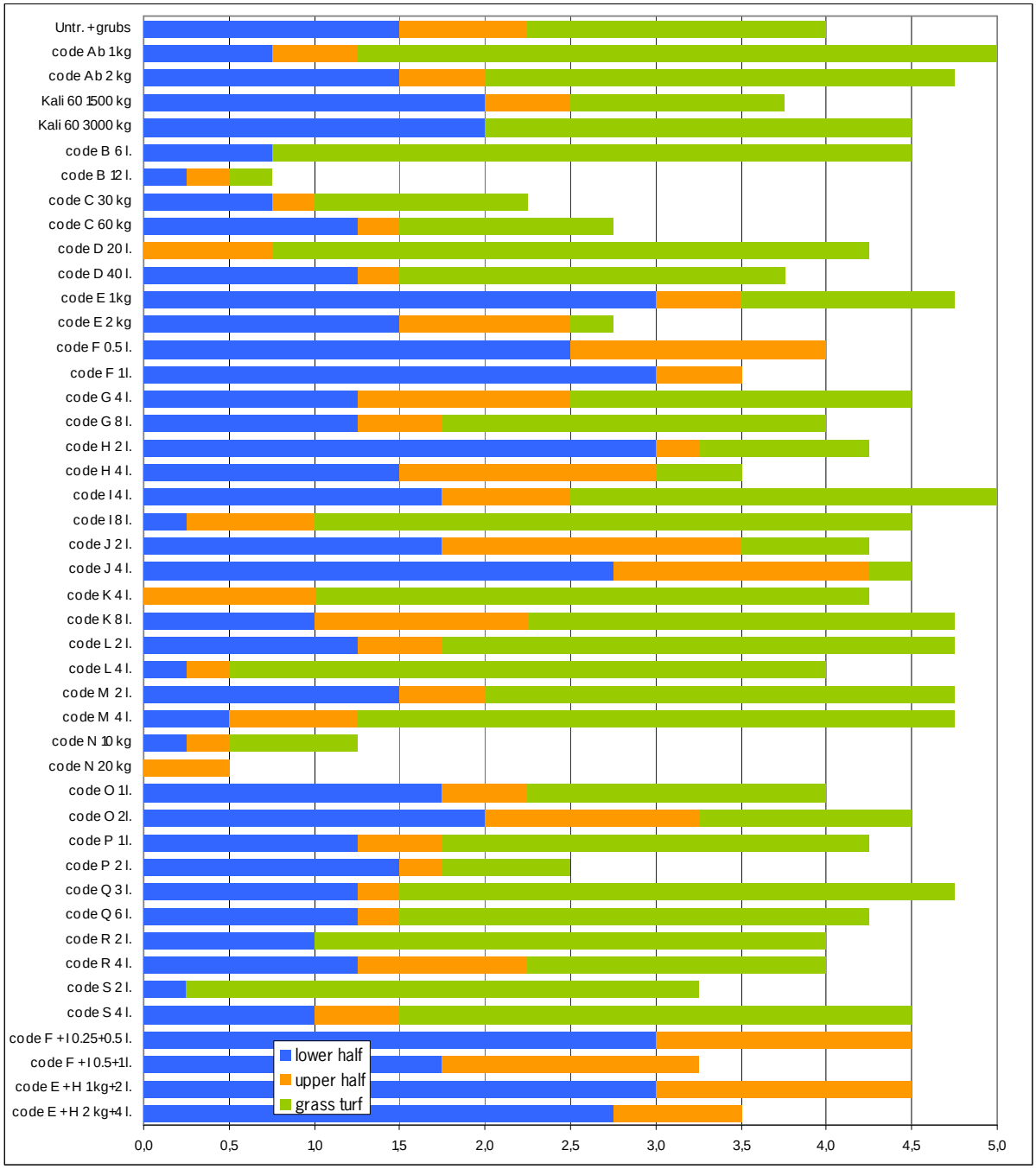

Figure 7. Division of the living grubs within the pots, 6 October 2006.

2.3.1.3 Discussion & conclusions

- Assessment of weight loss showed large variation between individual results of the pots (F-prob. = 0.080, table 15). Weight loss of the untreated plots without grubs was 8%, representing the effects of dehydration. It is safe to state that these effects are fairly equal for all the plots. Overall, weight loss of the turf seems to be a useful parameter to measure treatment effects.
- The untreated plots with grubs showed only 7.6% of weight loss and 6.3% of attack to the turf, which is very little. Such damage results were not expected, particularly because 44% of the grubs were retrieved in the turf and 19% and 37% in the top and bottom half respectively.
- Treatment with Code A-b at 1 and 2 kg/ha significantly increased the weight loss of the turf. No dose response was observed.

- Treatment with 1,500 or 3,000 kg/ha Kali 60 (60% K_2O, 46% Cl) showed insufficient protection against grubs. Although only little damage to the turf was recorded, 80% of the grubs were retrieved alive.
- Treatment with Code B at a rate of 12 l/ha showed good control of grubs. The lower application rate of 6 l/ha showed no control. The contrast between the application rates is also shown by the fact that the higher dose resulted in a significant decrease of living grubs in the turf, whereas the lower dose showed an increase.
- Application of Code C at rates of 30 and 60 kg/ha showed significant control of grubs, on average resulting of 50% living grubs remaining. Also damage to the turf did not differ from the untreated plots without grubs. The application rates did not differ mutually.
- At treatments with Code D, both application rates show insufficient protection. Both rates did not decrease the number of living grubs and high numbers of grubs were retrieved in the turf.
- Code E at rates of 1 and 2 kg/ha showed a repellent effect, indicated by a high portion of the grubs in the lower half of the pots and few damage to the turf, treatment with 2 kg/ha also showed sufficient control of grubs, indicated by significantly fewer living grubs. The higher application rate also showed a significant decrease of the numbers of grubs in the turf.
- Treatment with Code F did not result in a decrease of the numbers of living grubs. However, no grubs at all were found in the turf and almost no damage to the turf was observed. No dose response was found between the application rates.
- Treatment with Code G at 4 and 8 l/ha showed insufficient protection against grubs. Although only little damage to the turf was recorded, at least 80% of the grubs were retrieved alive. Grubs were retrieved throughout the pots.
- Also Code H at 2 and 4 l/ha showed insufficient protection against grubs. Damage to the turf was minimal, but 80% of the grubs were retrieved alive. At the highest application rate, a great deal of the grubs was even found in the treated soil layer.
- Treatment with 4 and 8 l/ha Code I showed no protection against grubs.
- Code J may have a repellent effect. Weight loss and damage to the turf did not differ from the untreated plots, but almost 90% of the grubs were found to be alive. About half of the living grubs were found in the untreated lower half of the pots.
- Application of 4 and 8 l/ha Code K showed an increased weight loss of the turf. All grubs were able to cross the treated soil layer and do significant damage to the turf. In conclusion, Code K shows insufficient protection.
- Treatments with Code L at application rates of 2 and 4 l/ha showed no indications of efficacy on grubs. Increased damage to the turf (lowest rate) was found, as well as high numbers of living grubs in the turf and overall.
- Also application of 2 and 4 l/ha Code M showed no indications of efficacy on grubs. Of the grubs 95% was retrieved alive, the main part of them in the turf.
- Application of Code N showed an excellent effect on grubs, resulting in only 10 to 25% of living grubs for 20 and 10 kg/ha respectively. The grubs that were found at treatment with 20 kg/ha all were found in the top (treated) half of the pot.
- Treatments with Code O resulted in only little damage to the turf, although 25-45% of the grubs were retrieved in the turf. Nevertheless they did not damage the turf significantly.
- Application of Code P at a rate of 2 l/ha shows protection against grubs. The damage to the turf was equal to the untreated plots without grubs and the numbers of living grubs halved. Treatment with 1 l/ha showed insufficient protection, with significantly more damage to the turf, more living grubs and more grubs in the turf than 2 l/ha.
- Treatment with 3 and 6 l/ha Code Q showed no protection against grubs. Almost all grubs were retrieved alive and were present throughout the pots.
- Treatment with 2 l/ha Code R showed insufficient control; the weight loss of the turf increased as well as numbers of grubs in the turf, not reducing numbers of living grubs (80% alive). Grubs were retrieved throughout the pots. Application of 4 l/ha did not increase weight loss and damage to the turf, but also did not reduce numbers of living grubs, so control of this application rate was also insufficient.
- Treatment with 2 and 4 l/ha Code S showed no protection against grubs. Most grubs were

retrieved alive, most of them in the turf.

- Application of Code F + Code I and Code E + Code H showed no grubs in the turf at all. Nevertheless about one third of the living grubs were found in the treated top half of the pots. For treatment with Code E + Code H the efficacy seems to be a combined effect of both products.

2.3.2 KAS589 – second screening

The aim of the second screening is to optimise application rates of the most promising compounds tested in the first screening experiment, as well as testing of a few other chemicals and biologicals.

2.3.2.1 Materials & methods

2.3.2.1.1 Treatments

Table 18 displays the compounds and application rates used to control grubs in a greenhouse experiment. This list was put together based on the results of the first screening experiment.

Table 18. Description of compounds and application rates used to control grubs, 2006.

Treatment	Compound	Dose (N & 2N) formulated per hectare
A	Untreated – grubs	-
B	Untreated + grubs	-
C	Code T-b	50 kg
D	Code U-b	35 kg
E	Code U-b	70 kg
F	Code V-b	35 kg
G	Code V-b	70 kg
H	Code W	6 l
I	Code W	12 l
J	Code B	12 l
K	Code C	15 kg
L	Code C	30 kg
M	Code E	1 kg
N	Code E	2 kg
O	Code E	3 kg
P	Code H	2 l
Q	Code H	4 l
R	Code J	4 l
S	Code J	8 l
T	Code N	2,5 kg
U	Code N	5 kg
V	Code N	10 kg
W	Code N	20 kg
X	Code P	2 l
Y	Code P	4 l
Z	Code F + Code I	0,5 l + 1 l
AA	Code F + Code I	1 l + 2 l
BB	Code E + Code H	0,5 kg + 1 l
CC	Code E + Code H	1 kg + 2 l
DD	Code E + Code H	2 kg + 4 l
EE	Code E + Code H	4 kg + 8 l

2.3.2.1.2 Experimental data

Plots consisted of : - One 10 l. pot containing a clay soil.
- The lower half of each pot was untreated, the upper half was treated.
- The grubs were placed in the lower half of the pot, on top of each pot a piece of turf was placed.

Application method : Liquids were sprayed over the soil in a spraying cabin, other products were applied by hand.

Number of grubs per pot : 5

Temperature during experiment : Minimum temperature set at 16°C
Minimum ; 16°C
Maximum ; 21°C

Starting date : 30 October 2006
Experimental design : Replicated block design
Number of replicates : 4 (I to IV, annex 1)

2.3.2.1.3 Assessments

Replicates I and II were assessed on 23 November, replicate III on 30 November and replicate IV on 7 December. The percentage of the turf that was eaten by the grubs was estimated. Numbers of dead grubs found were counted as well as numbers of living grubs. Numbers of living grubs were counted separately for the turf, the upper (treated) half of the pot, the lower (untreated) half of the pot and the bottom of the pot.

2.3.2.1.4 Statistics

Data were analysed using analysis of variance and Student's t-distribution with the PPAIR procedure. Means in the same column followed by different letters are significantly different.

2.3.2.2 Results

Damage to the turf was at a very low level, not exceeding 5% off turf surface (table 19). The treatments showed no differences in comparison with the untreated plots with grubs.

Table 19. Estimated percentage of damage to the turf, assessment on 23 and 30 November and 7 December 2006.

Treatment		Dose per hectare	Percentage damage
A	Untreated – grubs	-	0.0 a .
B	Untreated + grubs	-	2.5 a b
C	Code T-b	50 kg	3.8 . b
D	Code U-b	35 kg	2.5 a b
E	Code U-b	70 kg	2.5 a b
F	Code V-b	35 kg	1.3 a b
G	Code V-b	70 kg	1.3 a b
H	Code W	6 l	0.0 a .
I	Code W	12 l	0.0 a .
J	Code B	12 l	0.0 a .
K	Code C	15 kg	1.3 a b
L	Code C	30 kg	1.3 a b
M	Code E	1 kg	0.0 a .
N	Code E	2 kg	0.0 a .
O	Code E	3 kg	0.0 a .
P	Code H	2 l	0.0 a .
Q	Code H	4 l	0.0 a .
R	Code J	4 l	0.0 a .
S	Code J	8 l	0.0 a .
T	Code N	2,5 kg	2.5 a b
U	Code N	5 kg	0.0 a .
V	Code N	10 kg	1.3 a b
W	Code N	20 kg	1.3 a b
X	Code P	2 l	2.5 a b
Y	Code P	4 l	1.3 a b
Z	Code F + Code I	0,5 l + 1 l	0.0 a .
AA	Code F + Code I	1 l + 2 l	0.0 a .
BB	Code E + Code H	0,5 kg + 1 l	0.0 a .
CC	Code E + Code H	1 kg + 2 l	0.0 a .
DD	Code E + Code H	2 kg + 4 l	0.0 a .
EE	Code E + Code H	4 kg + 8 l	0.0 a .
LSD (α = 0.05)			2.8
F-prob.			0.276

Of the untreated pots, only 70% of living grubs were retrieved at the end of the experiment (table 20). No significant differences were found between the treatments and the untreated plots.

Table 20. Percentage and number of living grubs, assessment on 23 and 30 November and 7 December 2006.

Treatment		Dose per hectare	Percentage	Number
B	Untreated + grubs	-	70	3.5 a b c d
C	Code T-b	50 kg	70	3.5 a b c d
D	Code U-b	35 kg	90	4.5 . . c d
E	Code U-b	70 kg	60	3.0 a b c .
F	Code V-b	35 kg	50	2.5 a . . .
G	Code V-b	70 kg	65	3.3 a b c d
H	Code W	6 l	65	3.3 a b c d
I	Code W	12 l	55	2.8 a b . .
J	Code B	12 l	85	4.3 . b c d
K	Code C	15 kg	95	4.8 . . . d
L	Code C	30 kg	80	4.0 a b c d
M	Code E	1 kg	70	3.5 a b c d
N	Code E	2 kg	55	2.8 a b . .
O	Code E	3 kg	65	3.3 a b c d
P	Code H	2 l	85	4.3 . b c d
Q	Code H	4 l	70	3.5 a b c d
R	Code J	4 l	90	4.5 . . c d
S	Code J	8 l	70	3.5 a b c d
T	Code N	2.5 kg	90	4.5 . . c d
U	Code N	5 kg	65	3.3 a b c d
V	Code N	10 kg	65	3.3 a b c d
W	Code N	20 kg	75	3.8 a b c d
X	Code P	2 l	80	4.0 a b c d
Y	Code P	4 l	60	3.0 a b c .
Z	Code F + Code I	0.5 l + 1 l	90	4.5 . . c d
AA	Code F + Code I	1 l + 2 l	90	4.5 . . c d
BB	Code E + Code H	0.5 kg + 1 l	95	4.8 . . . d
CC	Code E + Code H	1 kg + 2 l	80	4.0 a b c d
DD	Code E + Code H	2 kg + 4 l	60	3.0 a b c .
EE	Code E + Code H	4 kg + 8 l	75	3.8 a b c d
LSD (α = 0.05)				1.5
F-prob.				0.092

Of the living grubs retrieved at the untreated plots, a little over 60% was found in the lower half of the pots, including almost 30% entirely at the bottom (table 21).
Treatments with Code W, Code B, Code E at a rate of 2 kg/ha, Code H, Code J, Code P at a rate of 4 l/ha, Code F + Code I and Code E + Code H, except for 1 kg + 2 l/ha, all showed no grubs at all in the turf; significantly fewer than the untreated plots.
At treatments with 6 and 12 l/ha Code W and Code P at 4 l/ha, no grubs at all were found in the upper half of the pots, including the turf.
Treatment with Code F + Code I at a rate of 1 + 2 l/ha showed significantly more grubs in the upper (treated) half of the pots than the untreated plots and the remaining treatments.
Application of 0.5 kg + 1 l/ha Code E + Code H was the only treatment to result in significantly more grubs in the lower half of the pots than the untreated plots.
Numbers of grubs on the bottom of the pots did not show significant differences in comparison with the untreated plots.
Numbers of grubs in the lower half op the pots added to numbers on the bottom showed higher numbers for treatment with Code H at 2 l/ha, Code J at 4 l/ha, Code F + Code I at 0.5 + 1 l/ha and Code E + Code H at 0.5 kg + 1 l/ha.

Table 21. Numbers of living grubs in turf, top half of pots and bottom half of pots, assessment on 23 and 30 November and 7 December 2006.

Treatment		Dose per hectare	Turf	Upper half	Lower half	Bottom	Lower half & bottom
B	Untreated + grubs	-	1.0 . b c d	0.3 a .	1.0 a b c .	1.3 a b c d	2.3 a b c d e . . .
C	Code T-b	50 kg	1.0 . b c d	0.8 a .	0.3 a . . .	1.5 a b c d	1.8 a b c . . . . .
D	Code U-b	35 kg	1.0 . b c d	0.5 a .	1.0 a b c .	2.0 a b c d	3.0 . . c d e f g .
E	Code U-b	70 kg	1.3 . . c d	0.3 a .	0.8 a b c .	0.8 a b . .	1.5 a b . . . . .
F	Code V-b	35 kg	0.5 a b c .	0.0 a .	0.5 a b . .	1.5 a b c d	2.0 a b c d . . . .
G	Code V-b	70 kg	1.3 . . c d	0.0 a .	0.5 a b . .	1.5 a b c d	2.0 a b c d . . . .
H	Code W	6 l	0.0 a . . .	0.0 a .	1.8 a b c d	1.5 a b c d	3.3 . . . d e f g h
I	Code W	12 l	0.0 a . . .	0.0 a .	2.3 . . c d	0.5 a . . .	2.8 . b c d e f g .
J	Code B	12 l	0.0 a . . .	0.8 a .	1.3 a b c d	2.3 . b c d	3.5 . . . . e f g h
K	Code C	15 kg	1.3 . . c d	0.5 a .	0.5 a b . .	2.5 . . c d	3.0 . . c d e f g .
L	Code C	30 kg	0.5 a b c .	0.0 a .	1.3 a b c d	2.3 . b c d	3.5 . . . . e f g h
M	Code E	1 kg	0.3 a b . .	0.5 a .	1.8 a b c d	1.0 a b c .	2.8 . b c d e f g .
N	Code E	2 kg	0.0 a . . .	0.8 a .	0.5 a b . .	1.5 a b c d	2.0 a b c d . . . .
O	Code E	3 kg	0.3 a b . .	0.5 a .	0.5 a b . .	2.0 a b c d	2.5 a b c d e f . .
P	Code H	2 l	0.0 a . . .	0.5 a .	1.0 a b c .	2.8 . . . d	3.8 . . . . . f g h
Q	Code H	4 l	0.0 a . . .	0.8 a .	1.3 a b c d	1.5 a b c d	2.8 . b c d e f g .
R	Code J	4 l	0.0 a . . .	0.5 a .	2.3 . . c d	1.8 a b c d	4.0 . . . . . g h
S	Code J	8 l	0.0 a . . .	0.3 a .	1.8 a b c d	1.5 a b c d	3.3 . . . d e f g h
T	Code N	2.5 kg	1.3 . . c d	0.0 a .	0.8 a b c .	2.5 . . c d	3.3 . . . d e f g h
U	Code N	5 kg	1.5 . . . d	0.5 a .	0.5 a b . .	0.8 a b . .	1.3 a . . . . .
V	Code N	10 kg	0.8 a b c d	0.0 a .	1.0 a b c .	1.5 a b c d	2.5 a b c d e f . .
W	Code N	20 kg	0.8 a b c d	0.5 a .	1.3 a b c d	1.3 a b c d	2.5 a b c d e f . .
X	Code P	2 l	0.3 a b . .	0.8 a .	1.3 a b c d	1.8 a b c d	3.0 . . c d e f g .
Y	Code P	4 l	0.0 a . . .	0.0 a .	1.3 a b c d	1.8 a b c d	3.0 . . c d e f g .
Z	Code F + Code I	0.5 l + 1 l	0.0 a . . .	0.5 a .	2.3 . . c d	1.8 a b c d	4.0 . . . . . g h
AA	Code F + Code I	1 l + 2 l	0.0 a . . .	1.8 . b	1.5 a b c d	1.3 a b c d	2.8 . b c d e f g .
BB	Code E + Code H	0.5 kg + 1 l	0.0 a . . .	0.3 a .	2.8 . . . d	1.8 a b c d	4.5 . . . . . h
CC	Code E + Code H	1 kg + 2 l	0.3 a b . .	0.3 a .	2.0 . b c d	1.5 a b c d	3.5 . . . . e f g h
DD	Code E + Code H	2 kg + 4 l	0.0 a . . .	0.3 a .	0.5 a b . .	2.3 . b c d	2.8 . b c d e f g .
EE	Code E + Code H	4 kg + 8 l	0.0 a . . .	0.8 a .	1.0 a b c .	2.0 a b c d	3.0 . . c d e f g .
LSD (α = 0.05)			0.8	1.0	1.6	1.6	1.4
F-prob.			< 0.001	0.295	0.126	0.628	0.002

Figure 8. Grubs beneath the turf, after removing the turf (left) and at the bottom of the pots, after turning the pot over.

From figure 9 it is visible that a large proportion of the grubs was retrieved on the bottom of the pots. Another large proportion, although smaller, was retrieved in the bottom half of the pots. At maximum about one quarter of the grubs were retrieved in the turf.

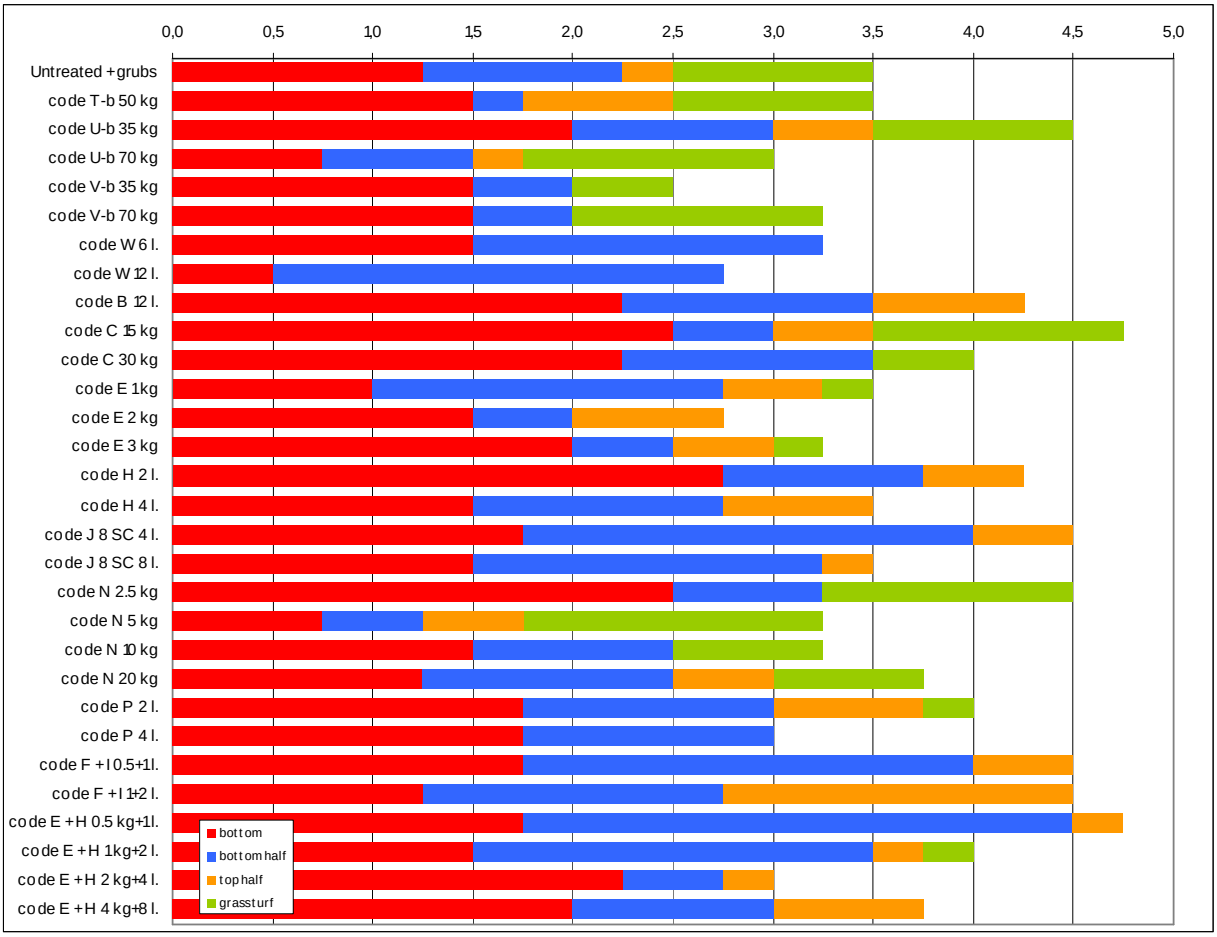

Figure 9. Division of the living grubs within the pots, assessment on 23 and 30 November and 7 December 2006.

2.3.2.3 Discussion and conclusions

- A large proportion of the grubs were retrieved at the bottom of the pots. Apparently they were (almost) inactive, which is shown by a very low percentage of damage to the turf (table 19). The same soil and turf were used and also the grubs had been collected in the same field.
- A number of treatments show good protection of the turf against grubs, regardless of application rate. At these applications no living grubs were found in the turf, which was significantly different from the untreated plots (table 21). Also no damage to the turf was recorded. These treatments are:
    - Code W at rates of 6 and 12 l/ha
    - Code B at 12 l/ha
    - Code H at rates of 2 and 4 l/ha
    - Code J at rates of 2 and 4 l/ha
    - Code F + Code I at rates of 0.5 kg+1 l/ha and 1 kg+2 l/ha
- Treatments with 6 and 12 l/ha Code W and 4 l/ha Code P showed no grubs at all in the top half of the pots.
- Of treatments with Code E only 2 kg/ha showed the same result as mentioned above. The lower and higher rates, 1 and 3 kg/ha showed no significantly different numbers of living grubs in the turf.
- The same was found at treatments with Code E and Code H. Application rates of 0.5 + 1 l/ha, 2 + 4 l/ha and 4 + 8 l/ha showed no grubs in the turf as well as no visual damage, but at treatment with 1 + 2 l/ha living grubs were found in the turf.
- The remaining treatments, including the entomopathogenic fungi, did not result in a significant

protection against grubs. However, treatment with Code V-b at a rate of 35 kg/ha did show the lowest numbers of living grubs.
- The absence of protection effects of the entomopathogenic fungi may be a consequence of duration of the experiment – five weeks.

2.3.3 KAS582 – third screening, using strawberry plants

The aim of the third screening is to test the most promising treatments from the first and second screening experiments in an experiment with strawberry plants.

2.3.3.1 Materials & methods

2.3.3.1.1 Treatments

Table 22 displays the compounds and application rates used to control grubs in a greenhouse experiment. This list was put together based on the results of the first and second screening experiment.

Table 22. Description of compounds and application rates used to control grubs, 2006.

Treatment	Compound	Dose (N & 2N) formulated per hectare
A	Untreated – grubs	-
B	Untreated + grubs (6x)	-
C	Code T-b	50 kg
D	Code U-b	35 kg
E	Code U-b	70 kg
F	Code V-b	35 kg
G	Code V-b	70 kg
H	Code W	6 l
I	Code W	12 l
J	Code B	12 l
K	Code C	30 kg
L	Code C	60 kg
M	Code E	2 kg
N	Code E	3 kg
O	Code J	2 l
P	Code J	4 l
Q	Code N	2.5 kg
R	Code N	5 kg
S	Code N	10 kg
T	Code N	20 kg
U	Code P	2 l
V	Code P	4 l
W	Code F + Code I	0.25 l + 0.5 l
X	Code F + Code I	0.5 l + 1 l
Y	Code F + Code I	1 l + 2 l
Z	Code E + Code H	0.5 kg + 1 l
AA	Code E + Code H	1 kg + 2 l
BB	Code E + Code H	2 kg + 4 l
CC	Code E + Code H	4 kg + 8 l
DD	Code X	0.45 l
EE	Code X	0.90 l

2.3.3.1.2 Experimental data

Plots consisted of :
- One 10 l. pot containing a clay soil.
- The lower of each pot quarter was untreated, the upper three quarters were treated.
- The grubs were placed on the soil surface at one quarter from the bottom of the pot, after which the pots were filled per treatment.
- In each pot a strawberry plant (cv. Elsanta, 3-4 leaves per plant) was planted as an attractant and food source.

Application method : Liquids were sprayed over the soil in a spraying cabin, other products were applied by hand.

Number of grubs per pot : 3

Temperature during experiment	:	Minimum Maximum	; ;	17°C 20°C
Starting date	:	29 November 2006		
Experimental design	:	Replicated block design		
Number of replicates	:	4 (I to IV, annex 1)		

Figure 10. Manual planting of strawberry plant (left) and crop directly after the start of the experiment.

2.3.3.1.3 Assessments

Assessment was made on 18 December. Numbers of green leaves, brownish leaves and dead leaves were counted per plant. The percentage of strawberry plant roots that had suffered attack by the grubs was estimated. Numbers of dead grubs found were counted as well as numbers of living grubs. Numbers of living grubs were counted separately for the root zone of the strawberry plants, the lower (untreated) quarter of the pot and the bottom of the pot. Total numbers of living grubs as well as total numbers of dead grubs (complementary) were calculated.

2.3.3.1.4 Statistics

Data were analysed using analysis of variance and Student's t-distribution with the PPAIR procedure. Means in the same column followed by different letters are significantly different.

2.3.3.2 Results

On 18 December none of the treatments showed a significant difference in the percentage of green leaves in comparison with the untreated plots, both with and without grubs (table 23).
Application of Code X at 0.45 l/ha showed an increase in the percentage of brownish leaves, but 0.90 l/ha did not. Also 2 kg + 4 l/ha Code E + Code H showed more brownish leaves than the untreated plots, but the remaining doses did not.
Addition of grubs to the untreated did not significantly increase the percentage of dead leaves. Also the treatments showed no difference in comparison with the untreated plots. Nevertheless, treatments with Code B, Code J, Code N and Code E + Code H showed no dead leaves at all, like the untreated plots without grubs, regardless of application rate. Treatment with 70 kg/ha Code U-b, 30 kg/ha Code C, 4 l/ha Code P, 0.5 + 1 l/ha Code F + Code I and 0.45 l/ha Code P showed the same result.

Table 23. Percentages of green, brownish and dead leaves on 18 December 2006.

Treatment		Dose per hectare	Green	Brownish	Dead
A	Untreated – grubs	-	51.7 a b c d	48.3 . b c d e	0.0 a .
B	Untreated + grubs	-	48.3 a b c d	41.2 a b c d .	10.6 a b
C	Code T-b	50 kg	43.3 a b c d	52.5 . b c d e	4.2 a b
D	Code U-b	35 kg	61.9 . . . d	35.0 a b c . .	3.1 a b
E	Code U-b	70 kg	51.8 a b c d	48.2 . b c d e	0.0 a .
F	Code V-b	35 kg	54.3 a b c d	42.1 a b c d e	3.6 a b
G	Code V-b	70 kg	50.0 a b c d	43.8 a b c d e	6.3 a b
H	Code W	6 l	47.5 a b c d	47.5 . b c d e	5.0 a b
I	Code W	12 l	54.9 a b c d	32.6 a b . . .	12.5 a b
J	Code B	12 l	39.6 a b . .	60.4 . . . d e	0.0 a .
K	Code C	30 kg	40.0 a b c .	60.0 . . . d e	0.0 a .
L	Code C	60 kg	60.0 . . c d	35.8 a b c . .	4.2 a b
M	Code E	2 kg	56.7 . b c d	38.3 a b c d .	5.0 a b
N	Code E	3 kg	57.5 . b c d	42.5 a b c d e	0.0 a .
O	Code J	2 l	45.8 a b c d	54.2 . b c d e	0.0 a .
P	Code J	4 l	46.3 a b c d	53.7 . b c d e	0.0 a .
Q	Code N	2.5 kg	61.7 . . . d	38.3 a b c d .	0.0 a .
R	Code N	5 kg	47.5 a b c d	52.5 . b c d e	0.0 a .
S	Code N	10 kg	42.1 a b c d	57.9 . . c d e	0.0 a .
T	Code N	20 kg	57.4 . b c d	42.6 a b c d e	0.0 a .
U	Code P	2 l	51.8 a b c d	44.6 a b c d e	3.6 a b
V	Code P	4 l	58.7 . b c d	41.3 a b c d e	0.0 a .
W	Code F + Code I	0.25 l + 0.5 l	54.9 a b c d	41.6 a b c d e	3.6 a b
X	Code F + Code I	0.5 l + 1 l	50.0 a b c d	50.0 . b c d e	0.0 a .
Y	Code F + Code I	1 l + 2 l	44.1 a b c d	43.5 a b c d e	12.5 a b
Z	Code E + Code H	0.5 kg + 1 l	58.2 . b c d	41.8 a b c d e	0.0 a .
AA	Code E + Code H	1 kg + 2 l	51.7 a b c d	48.3 . b c d e	0.0 a .
BB	Code E + Code H	2 kg + 4 l	36.3 a . . .	63.8 . . . . e	0.0 a .
CC	Code E + Code H	4 kg + 8 l	60.0 . . c d	40.0 a b c d .	0.0 a .
DD	Code X	0.45 l	35.4 a . . .	64.6 . . . . e	0.0 a .
EE	Code X	0.90 l	61.7 . . . d	21.7 a	16.7 . b
LSD (α = 0.05) treatm.			20.2	23.3	16.6
LSD (α = 0.05) untreated - treatm.			15.6	18.0	12.9
F-prob.			0.295	0.126	0.785

Damage to the plant roots varied greatly between treatments (table 24). Application of 35 kg/ha Code U-b decreased the percentage of damaged roots significantly, but 70 kg/ha did not. Treatment with Code W, Code B, Code C, Code E, Code J, Code P, Code F + Code I and Code E + Code H all resulted in a significant decrease of the percentage of damaged roots in comparison with the untreated plots. Treatment with 2.5 kg/ha Code N showed a significantly lower percentage of damage to the roots than the higher application rates of 5 and 20 kg/ha.

Table 24. Estimated percentage of damage to the strawberry plant roots on 18 December 2006.

Treatment		Dose per hectare	Percentage damage	
A	Untreated – grubs	-	0.0	a . . .
B	Untreated + grubs	-	55.8	. . c d
C	Code T-b	50 kg	70.0	. . . d
D	Code U-b	35 kg	12.5	a b . .
E	Code U-b	70 kg	46.3	. b c d
F	Code V-b	35 kg	60.0	. . c d
G	Code V-b	70 kg	67.5	. . . d
H	Code W	6 l	0.0	a . . .
I	Code W	12 l	5.0	a . . .
J	Code B	12 l	0.0	a . . .
K	Code C	30 kg	1.3	a . . .
L	Code C	60 kg	2.5	a . . .
M	Code E	2 kg	2.5	a . . .
N	Code E	3 kg	2.5	a . . .
O	Code J	2 l	0.0	a . . .
P	Code J	4 l	0.0	a . . .
Q	Code N	2.5 kg	22.5	a b c .
R	Code N	5 kg	68.8	. . . d
S	Code N	10 kg	53.8	. . c d
T	Code N	20 kg	62.5	. . . d
U	Code P	2 l	5.0	a . . .
V	Code P	4 l	0.0	a . . .
W	Code F + Code I	0.25 l + 0.5 l	2.5	a . . .
X	Code F + Code I	0.5 l + 1 l	1.3	a . . .
Y	Code F + Code I	1 l + 2 l	2.5	a . . .
Z	Code E + Code H	0.5 kg + 1 l	1.3	a . . .
AA	Code E + Code H	1 kg + 2 l	0.0	a . . .
BB	Code E + Code H	2 kg + 4 l	2.5	a . . .
CC	Code E + Code H	4 kg + 8 l	0.0	a . . .
DD	Code X	0.45 l	53.8	. . c d
EE	Code X	0.90 l	45.0	. b c d
LSD (α = 0.05) treatm.			38.9	
LSD (α = 0.05) untreated - treatm.			30.2	
F-prob.			< 0.001	

Treatment with 12 l/ha Code W showed a significant decrease of the numbers of living grubs, but 6 l/ha did not (table 25). Application of Code P at both rates also resulted in a significantly lower number of living grubs than the untreated plots. Of treatment with Code F + Code I, 0.5 + 1 and 1 +2 l/ha also showed a decrease, but 0.25 + 0.5 l/ha did not result in a significantly lower number of licing grubs than the untreated plots.

Figure 11. Severe damage to strawberry plant and well protected plant; full grown roots (left) and detail of damage. Right:detail

Table 25. Percentage and number of living grubs on 18 December 2006.

Treatment		Dose per hectare	Percentage	Number
B	Untreated + grubs	-	88	2.6 . . . d e
C	Code T-b	50 kg	100	3.0 . . . . e
D	Code U-b	35 kg	75	2.3 . b c d e
E	Code U-b	70 kg	83	2.5 . . c d e
F	Code V-b	35 kg	75	2.3 . b c d e
G	Code V-b	70 kg	100	3.0 . . . . e
H	Code W	6 l	75	2.3 . b c d e
I	Code W	12 l	58	1.8 a b c . .
J	Code B	12 l	67	2.0 a b c d .
K	Code C	30 kg	83	2.5 . . c d e
L	Code C	60 kg	83	2.5 . . c d e
M	Code E	2 kg	92	2.8 . . . d e
N	Code E	3 kg	100	3.0 . . . . e
O	Code J	2 l	75	2.3 . b c d e
P	Code J	4 l	83	2.5 . . c d e
Q	Code N	2.5 kg	92	2.8 . . . d e
R	Code N	5 kg	75	2.3 . b c d e
S	Code N	10 kg	92	2.8 . . . d e
T	Code N	20 kg	100	3.0 . . . . e
U	Code P	2 l	42	1.3 a . . . .
V	Code P	4 l	58	1.8 a b c . .
W	Code F + Code I	0.25 l + 0.5 l	92	2.8 . . . d e
X	Code F + Code I	0.5 l + 1 l	50	1.5 a b . . .
Y	Code F + Code I	1 l + 2 l	58	1.8 a b c . .
Z	Code E + Code H	0.5 kg + 1 l	67	2.0 a b c d .
AA	Code E + Code H	1 kg + 2 l	100	3.0 . . . . e
BB	Code E + Code H	2 kg + 4 l	100	3.0 . . . . e
CC	Code E + Code H	4 kg + 8 l	92	2.8 . . . d e
DD	Code X	0.45 l	83	2.5 . . c d e
EE	Code X	0.90 l	83	2.5 . . c d e
LSD (α = 0.05) treatm.				0.9
LSD (α = 0.05) untreated - treatm.				0.7
F-prob.				0.001

Of the living grubs retrieved at the untreated pots, a little over 20% was found in the lower quarter of the pots, including almost 7% entirely at the bottom (table 26).
Treatments with Code W, Code B, Code C at a rate of 30 kg/ha, Code E, Code J at a rate of 4 l/ha, Code P, Code F + Code I and Code E + Code H showed significantly fewer grubs in the root zone (three quarters of the pots) than the untreated plots.
Application of 2 kg/ha Code E, 0.25 + 0.5 and 1 + 2 l/ha Code F + Code I and 1 kg + 2 l and 4 kg + 8 l/ha Code E + Code H showed significantly more grubs in the lower quarter of the pots than the untreated plots.
Numbers of grubs on the bottom of the pots only at treatment with 4 l/ha Code J showed significantly more living grubs than the untreated plots.

Table 26. Numbers of living grubs in turf, top half of pots and bottom half of pots on 18 December 2006.

Treatment		Dose per hectare	Root zone	Lower quarter	Bottom	Lower quarter & bottom
B	Untreated + grubs	-	1.8 . . . . e f	0.5 a . . .	0.3 a b . .	0.8 a b . . . .
C	Code T-b	50 kg	2.3 . . . . . f	0.3 a . . .	0.5 a b c .	0.8 a b . . . .
D	Code U-b	35 kg	1.5 . . c d e f	0.3 a . . .	0.5 a b c .	0.8 a b . . . .
E	Code U-b	70 kg	1.8 . . . d e f	0.5 a b . .	0.3 a b . .	0.8 a b . . . .
F	Code V-b	35 kg	1.3 . b c d e f	0.3 a . . .	0.8 a b c d	1.0 a b c . . .
G	Code V-b	70 kg	2.0 . . . . e f	0.5 a b . .	0.5 a b c .	1.0 a b c . . .
H	Code W	6 l	0.8 a b c d . .	1.0 a b c .	0.5 a b c .	1.5 a b c d e .
I	Code W	12 l	0.3 a b	0.5 a b . .	1.0 . b c d	1.5 a b c d e .
J	Code B	12 l	0.8 a b c d . .	1.0 a b c .	0.3 a b . .	1.3 a b c d . .
K	Code C	30 kg	0.5 a b c . . .	1.3 a b c .	0.8 a b c d	2.0 . . c d e f
L	Code C	60 kg	1.5 . . c d e f	0.8 a b c .	0.3 a b . .	1.0 a b c . . .
M	Code E	2 kg	0.3 a b	1.8 . . c d	0.8 a b c d	2.5 . . . . e f
N	Code E	3 kg	0.3 a b	1.3 a b c .	1.5 . . . d	2.8 . . . . . f
O	Code J	2 l	1.0 a b c d e .	1.0 a b c .	0.3 a b . .	1.3 a b c d . .
P	Code J	4 l	0.5 a b c . . .	0.8 a b c .	1.3 . . c d	2.0 . . c d e f
Q	Code N	2.5 kg	1.0 a b c d e .	1.3 a b c .	0.5 a b c .	1.8 . b c d e f
R	Code N	5 kg	1.5 . . c d e f	0.5 a b . .	0.3 a b . .	0.8 a b . . . .
S	Code N	10 kg	1.8 . . . d e f	0.5 a b . .	0.5 a b c .	1.0 a b c . . .
T	Code N	20 kg	2.0 . . . . e f	0.8 a b c .	0.3 a b . .	1.0 a b c . . .
U	Code P	2 l	0.0 a	1.3 a b c .	0.0 a . . .	1.3 a b c d . .
V	Code P	4 l	0.5 a b c . . .	1.0 a b c .	0.3 a b . .	1.3 a b c d . .
W	Code F + Code I	0.25 l + 0.5 l	0.0 a	1.8 . . c d	1.0 . b c d	2.8 . . . . . f
X	Code F + Code I	0.5 l + 1 l	0.3 a b	0.8 a b c .	0.5 a b c .	1.3 a b c d . .
Y	Code F + Code I	1 l + 2 l	0.3 a b	1.5 . b c d	0.0 a . . .	1.5 a b c d e .
Z	Code E + Code H	0.5 kg + 1 l	0.5 a b c . . .	1.0 a b c .	0.5 a b c .	1.5 a b c d e .
AA	Code E + Code H	1 kg + 2 l	0.5 a b c . . .	1.5 . b c d	1.0 . b c d	2.5 . . . . e f
BB	Code E + Code H	2 kg + 4 l	0.8 a b c d . .	1.3 a b c .	1.0 . b c d	2.3 . . . d e f
CC	Code E + Code H	4 kg + 8 l	0.3 a b	2.5 . . . d	0.0 a . . .	2.5 . . . . e f
DD	Code X	0.45 l	2.0 . . . . e f	0.5 a b . .	0.0 a . . .	0.5 a . . . . .
EE	Code X	0.90 l	1.3 . b c d e f	1.3 a b c .	0.0 a . . .	1.3 a b c d . .
LSD (α = 0.05) treatm.			1.1	1.1	0.9	1.4
LSD (α = 0.05) untreated - treatm.			0.9	0.9	0.7	1.2
F-prob.			< 0.001	0.014	0.030	0.889

The proportional division of the grubs in the pots is displayed again in figure 12. Overall, most grubs were retrieved in the root zone, which is three quarters of the soil contents.
At treatments with 2 l/ha Code P and 0.25 + 0.5 l/ha Code F + Code I, no grubs were found in the root zone.

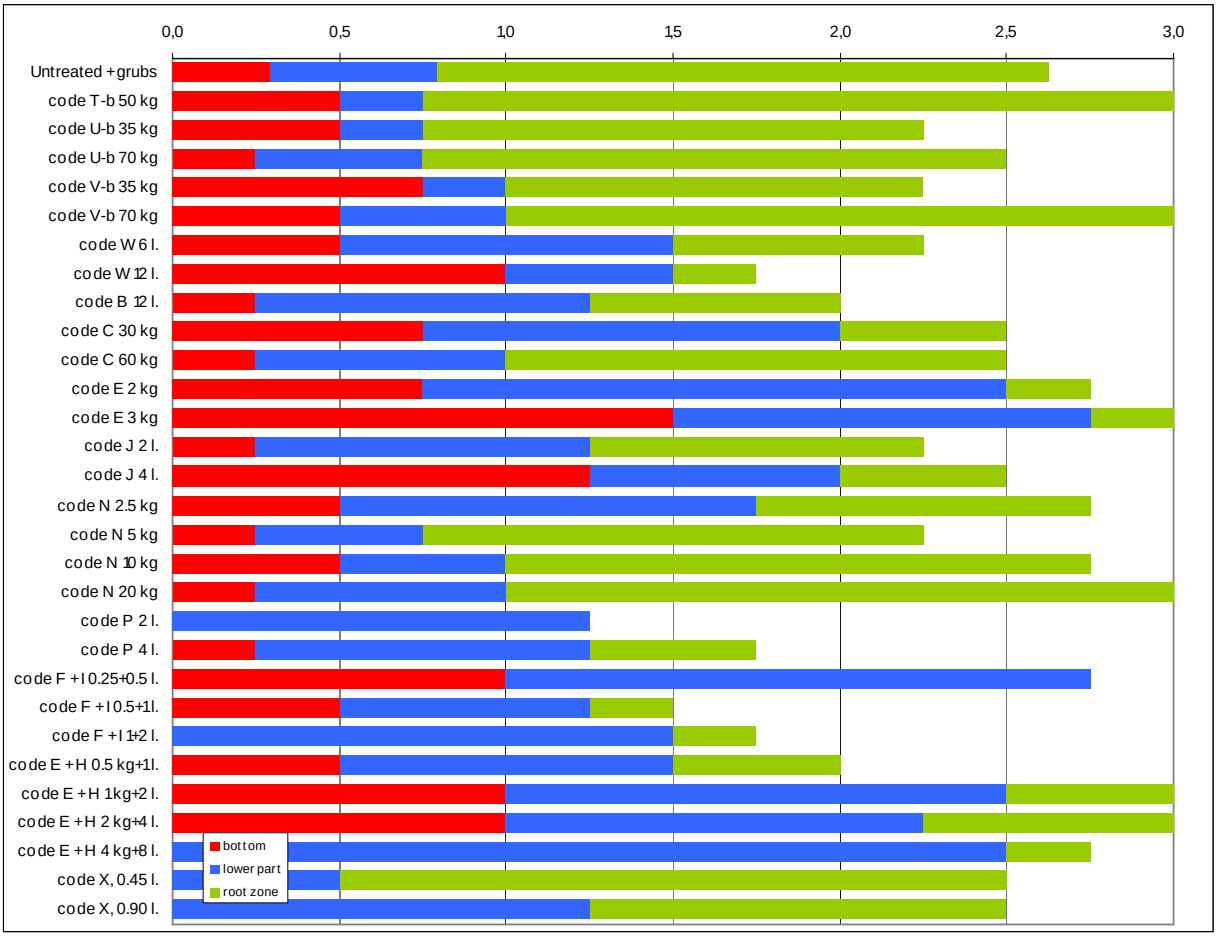

Figure 12. Division of the living grubs within the pots, 18 December 2006.

2.3.3.3 Conclusions & discussion

- Damage to the strawberry roots was at quite a high level in the untreated plots, with an estimation of 56% of the roots having suffered damage by grubs in a period of three weeks (table 24).
- At treatment with Code T-b at a rate of 50 kg/ha no grubs were killed at all and 70% of the plants were damaged (table 25, figure 16).
- Application of Code U-b at rates of 35 and 70 kg/ha shows insufficient control of grubs, although the lower rate did decrease the percentage of damage to the plant roots. Division of the grubs hardly differed from the untreated plots.
- Treatment with 35 or 70 kg/ha Code V-b did not protect the strawberry crop from damage, nor did it show significant killing of grubs. Also no influence was found on the location of the grubs in the pots.
- Code W at rates of 6 and 12 l/ha shows excellent results. Both application rates resulted in a significant decrease in the percentage of damage to the roots. Although 6 l/ha did not, at 12 l/ha Code W resulted in a decrease of the percentage of living grubs.
- Treatment with Code B at a rate of 12 l/ha showed good control of grubs. No grub damage to the strawberry roots was found. Dead leaves were absent as well, but the percentage of brownish leaves was high (table 23). The highest proportion of grubs was retrieved in the lower quarter of the pots.
- Application of Code C at rates of 30 and 60 kg/ha showed significant protection of the crop. The percentage of living grubs did not differ from the untreated plots. At treatment with 30 kg/ha 20% of the living grubs was found in the root zone, but at 60 kg/ha this was 60%.
- At treatment with Code E at rates of 2 and 3 kg/ha only 2.5% of root damage was found, but

almost no dead grubs were found. Also almost all grubs were retrieved in the lower quarters. No dose response was found.

- Code J at 2 and 4 l/ha probably has a repellent effect. No damage to the roots was found at all, although at 2 l/ha almost half of the grubs were retrieved in the root zone; at 4 l/ha this was only 20%.
- Application of Code N showed no protection against grubs, which is shown be equal percentages of root damage as the untreated plots with grubs.
- Application of Code P at rates of 2 and 4 l/ha shows excellent protection against grubs. The damage to the roots was significantly lower than at the untreated plots and hardly any grubs were retrieved in the root zone. Total numbers of living grubs were significantly decreased by either application rate.
- Application of Code F + Code I showed hardly any damage to the roots. Also hardly any grubs were retrieved in the root zone, regardless of application rate. At 0.25 + 0.5 l/ha even no grubs were found in the upper three quarters of the pots, although over 90% of the grubs stayed alive. At the higher rates, 0.5 + 1 and 1 + 2 l/ha, significantly fewer living grubs were found than at treatment with 0.25 + 0.5 l/ha and the untreated plots.
- Application of Code E + Code H showed significantly fewer damage to the strawberry roots than the untreated plots, although hardly any grubs were killed. Most of the grubs were found in the lower quarter of the pots. Dose responses were not found.
- Treatment with Code X did not protect the strawberry plants against grubs damage. 50 to 80% of the living grubs were retrieved in the root zone, but no grubs were found on the bottom of the pots.

2.4 Overall conclusion control experiments

2.4.1 Nematodes and a bacterium

- Treatments with Code Na show promising results. In experiment 1, at a dose of 3,500 nematodes per ml, a significant increase of dead grubs was found. In experiment 2, at 11,000 nematodes per ml, this could not be reproduced.
- Treatment with Code Nb, only tested in experiment 1 and at a dose of 1,800 nematodes per ml, shows control of grubs. This was only visible by the percentage of dead grubs, rather than by the percentage of potato eaten or the mark for damage to the germination paper.
- Treatment with Code Nc shows no effects on grubs. Both in experiment 1 at 2,900 and 2 at 6,200 nematodes per ml no efficacy could be established.
- Treatment with Code Ba showed no effects on grubs, only tested in experiment 1.
- No significant effects of treatment with Code Nc at 5,230 nematodes per ml could be established after three weeks in experiment 2.
- In experiment 1 the smaller instars accounted for most of the mortality found. Mortality at the successful treatments could be ascribed to the 1st instar grubs. The 3rd instar, being the most ferocious, showed no significant increase in mortality. In experiment 2 however, the 3rd instar showed to be significantly more vulnerable to nematodes than the 1st instar. Already at the first assessment in more dead 3rd instar grubs were found than 1st instar grubs.
- In experiment 1, treatment of both grub and tray shows the highest mortality. This result was significantly better than that of only drenching the food (potato). Treatment of only the grub differed from neither of the other application methods.

2.4.2 Insecticides and biologicals

- Using turf as an attractant for grubs was very successful in experiment 1. However, in experiment 2 a large portion of the grubs were retrieved on the bottom of the pots. In experiment 3, when strawberry plants were used as attractant, clear effects of the treatments were obtained.
- At treatment with Code T-b at a rate of 50 kg/ha no grubs were killed at all in two experiments (table 25, figure 16). This poor result of a treatment that is use in practice in Central Europe may be the result of the short experiment duration.
- Application of Code U-b at rates of 35 and 70 kg/ha shows insufficient control of grubs, in the second and third experiment, although the lower rate did decrease the percentage of damage to the plant roots.
- Treatment with 35 or 70 kg/ha Code V-b did not protect the strawberry crop from damage, nor did it show significant killing of grubs, in the second and third experiment. Also no influence was found on the location of the grubs in the pots.
- Code W at rates of 6 and 12 l/ha shows excellent results. Both application rates resulted in a significant decrease in the percentage of damage to the roots, of the turf and strawberry plants. Although 6 l/ha did not, at 12 l/ha Code W resulted in a decrease of the percentage of living grubs.
- Treatment with Code B at a rate of 12 l/ha showed good control of grubs. No damage to the strawberry plant roots was found and dead leaves were absent as well (table 23).
- Application of Code C at rates of 30 and 60 kg/ha showed significant control of grubs. At treatment with 30 kg/ha 20% of the living grubs was found in the root zone, but at 60 kg/ha this was 60%. The application rates did not differ mutually.
- At treatment with Code E at rates of 2 and 3 kg/ha only 2.5% of root damage was found, but almost no dead grubs were found. In the second experiment this was also found at 1 kg/ha. No dose response was found.
- Code J at 2 and 4 l/ha probably has a repellent effect. No damage to the roots of either turf or strawberry plants was found at all. In experiment 2 at treatment with 4 and 8 l/ha nearly all grubs were found in the untreated part of the pots.
- Application of Code N showed no protection against grubs, which is shown be equal percentages

of turf and strawberry root damage as the untreated plots with grubs. Strikingly the lowest application rate, 2.5 kg/ha, showed the best result, significantly better than 5 and 20 kg/ha.
- Application of Code P at rates of 2 and 4 l/ha shows excellent protection against grubs in experiment 2 and 3. The damage to the plant roots was significantly lower than at the untreated plots and hardly any grubs were retrieved in the root zone. Total numbers of living grubs were significantly decreased by either application rate.
- Application of Code F + Code I showed hardly any damage to the plant roots. At 0.25 + 0.5 l/ha even no grubs were found in the upper three quarters of the pots, although over 90% of the grubs stayed alive. At the higher rates, 0.5 + 1 and 1 + 2 l/ha, significantly fewer living grubs were found than at the untreated plots.
- Application of Code E + Code H at application rates of 0.5 kg + 1 l, 1 kg + 2 l, 2 kg + 4 l and 4 kg + 8 l/ha showed significantly fewer damage to the strawberry plant roots than the untreated plots, although hardly any grubs were killed.
- Treatment with Code X did not protect the strawberry plants against grubs damage. 50 to 80% of the living grubs were retrieved in the root zone.

Code X showed insufficient protection against grubs. Other products that did not result in sufficient protection are Code A-b, Kali 60 (60% K_2O and 46% Cl), Code G and Code L. Code M, Code O, Code Q, Code R and Code S, all tested in experiment 1. Code H was discarded after experiment 2.

The compounds that are worthwhile testing in practice are:
- Code W
- Code B
- Code C
- Code E
- Code J
- Code N – although results fell short in experiment 3.
- Code P
- Code F
- Code E + Code H

Code T-b did not meet with expectations. Also the other entomopathogenic fungi that were tested, did not result in protection against grubs. This may be the result of test conditions, including the duration of the experiments. Therefore it is useful to test these products in practice as well:
- Code T-b
- Code U-b
- Code V-b

3 Pheromones and *Melolontha melolontha* – practical uses and concerns

Dr.ir. F.C. Griepink

3.1 Introduction

Figure 13. Melolontha melolontha *mating in trees*

Pheromones are widely used for the trapping of insects. With traps placed at strategic positions it is possible to see in an early stage if the pest is present and to predict the population density. The trap catch numbers can be used to determine the optimum time for conventional insecticide application and to determine, afterwards, if the application had the anticipated effect. If the pesticide has been applied properly you would expect no more catches in the trap. Also traps are commonly used as an aid for the identification of an unknown pest (mostly lepidopterans). One of the conditions for a pheromone trap to be used as early warning device is that the trap plus pheromone dispenser must be very attractive and that the incoming insect is trapped. If a trap is only moderately effective the insect is caught when their appearance is already obvious (insects flying round or damage). For monitoring the population growth early in the season the trap does not necessarily needs to be extremely attractive as long as it is attractive for the pest. It is very important that the device is performing constantly over time and that the (commercial) pheromone dispensers are comparably attractive over the years. Only then the population density can be judged in a reliable way and over the years a good view is obtained about the efficacy of taken measurements.

To be able to determine if the grubs that have been found, were indeed *Melolontha melolontha* and not, for example, *Phyllopertha horticola*, for both species traps with different pheromones have been used. However, the primary objective is to see *if* adult *Melolontha* beetles could be trapped, in what relative numbers and to see if there is a correlation with the estimated population density at that time of the season.

3.2 Experimental

The traps that have been used were yellow UNITRAPS with vertical barrier crosses (PHEROBANK, Wageningen) (see figures 14 and 15). The protocols for trap application for these insects are presented in annex 3 and 4.

Figure 15 and 15. Yellow UNITRAPS with barrier cross

For *Melolontha melolontha* dispensers have been used containing a mixture 75 mg toluquinone plus 425 mg Z3-hexenol. PRI has obtained good results with this dispenser type, ratio and quantity in earlier tests in The Netherlands.

For *Phyllopertha horticola* dispensers have been used containing a (patented) mixture of 650 mg Z3-hexenol, 350 mg geraniol, 150 mg eugenol plus 150 mg phenylethyl propionate. These dispensers have been used in the The Netherlands with great success to trap *P. horticola* beetles in golf courts.

The protocol for installing the lures in the traps is presented in annex 5.

Traps have been placed near Kiev, and in the Vicina region. A third set has been sent to a local advisor but no results have been received from him.

3.3 Results, conclusions and remarks

The traps that have been placed in the Vinica region did not trap any insects. After an evaluation it appeared that these traps were probably hung in trees unsuitable for reproduction. There where beetles present but they did not feed on these trees. These traps haven't been relocated to other trees.

The traps that were hung near Kiev also initially did not catch any insects. However, after repositioning them to a nearby row were we noticed that insects were eating these traps indeed caught substantial numbers of beetles. Trap numbers 1-3 were traps with pheromone for *Melolontha melolontha* and trap numbers 4-6 were traps with attractants for *Phyllopertha horticola*. The trap results are summarized in figure 4 (raw data in annex 6). As can be observed traps 1 and 2 appeared to be attractive for *M. melolontha* males. The

lesser performance of similar trap number 3 can not be explained. No *Phyllopertha horticola* have been found in either the traps.

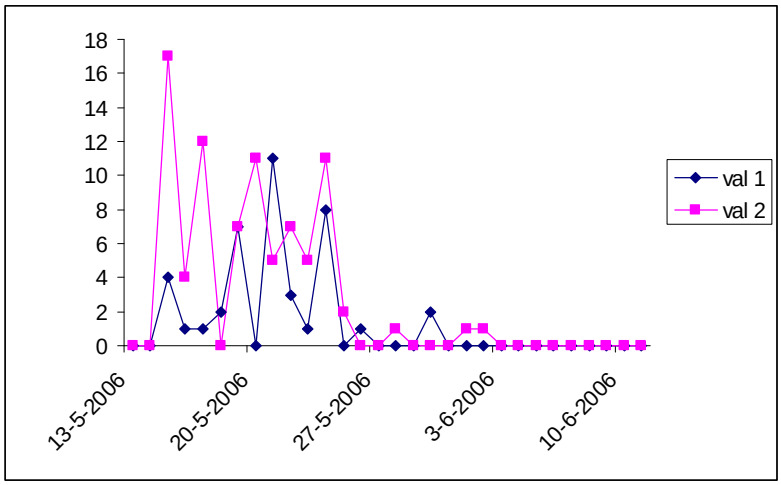

Figure 16. Visualization of M. melolontha *trapped near Kiev*

As can be seen from figure 16 the traps did catch substantial numbers of *M. Melolontha* males. The initial low catch is due to an initial wrong positioning of the traps. After they were relocated the traps started catching insects. Adult *M. melolontha* were already flying at the moment that the first traps were placed so the initial catches are missing. Traps should be placed preferably before the first insects start to fly to be able to see the first insects of the season. Normally a gradual increase in catches is observed which is absent in figure 4. Apparently there exists a large variation in the number of insects that are caught in time and also between the different traps. For example on May 20 one of the traps caught zero beetles whereas a similar trap just 20 meters away caught 11 beetles. The trees surrounding the traps were full with adults and large numbers of adults were flying from the trees towards the fields and vice versa. It is concluded that only a small number of the population is actually trapped in these traps. The potential to develop a mass trapping system for *M. melolontha* with the current pheromone mixture is therefore small. The experience with moth-traps is that substantial numbers of insects are caught even before the first adults were seen or any damage could be detected. In those occasions the pheromone traps could be applied as early warning systems or even be used to trap the moths away (mass trapping). In the case of *M. melolontha* the traps performed insufficiently to be developed it into a mass trapping tool (on a short notice). They can be used to determine the peak in flight activity however more traps should be used to filter out the individual trap catch variability.

Overall it is concluded that the traps that have been tested in the Ukraine are capable of catching *M. melolontha* males but in relative low numbers. The traps can therefore not be used for the development of a mass trap application. They can be used to follow the course of the population and also to determine the population densities and fluctuations from year to year.

4 Comments on trip to Ukraine, 11 to 16 May 2006

A. Ester & L.G. Moraal

12 May:
- Visit to National Agricultural University of Ukraine. Presentation by Associate Professor Tatyana Stefanovska (dept. Integrated Pest Management).

13 May:
- Visit to farm of Lutak Valeriy, Michai Povich, village Proziv Boryspil, region Kierska. Farm 'Eldorado". Farm of 1500 ha, with 15 field. Fields surrounded by trees, mostly birch trees. Crops that are grown: potatoes 200 ha; vegetables 70 ha: onion, cabbage, carrots and beet; oilseed rape 300 ha and wheat 400 ha.
- Visit to 'Companys Farm' Shaslyve in the Boryspyl region. Large-scale growing of lettuce.

14 May:
- Visit to Podillya-Plant, growing only strawberry plants. Damage mostly consists of wilking of planted plants.

15 May:
- Day organized by International Finance Corporation, part of the world bank. Presentation on control possibilities of May beetles. Damage in young fruit trees and rose bushes.
- Visit to Luka Farm (largest fruit farm of Ukraine) in the town Luka. StyleObst is an Austrian company. Fruit that is grown is 100 ha apples, 120 ha strawberries and 100 ha raspberries.
- Visit to Mizhlissya, SVAT "Podillya', growing 40 ha strawberries and 150 ha apples, mainly for industrial purposes. Also arable farming and livestock.

Larvae of *Melolontha*-beetles can be very noxious in agricultural fields and nurseries by gnawing the roots of plants. Control of the larvae in the soil is very difficult. Chemical control is not environmental sound and by that not recommended. Biological control with Nematodes has shown to be not very effective. The use of pheromone traps is useful for monitoring but they are probably not effective for mass trapping techniques in order to reduce the population densities of the beetles. It remains unclear how the problems in the Ukraine could be solved but an integrated control seems the best option, in the agricultural fields as well in the natural environment (landscape).

1. Control of the pest in the agriculture
In the breeding of certain crops like strawberries, it is possibly adequate to plough the soil between the plant rows on the right moment (just after egg-depositing) to kill the eggs by desiccation. The use of pheromone traps can be useful to determine the flight of the beetle and the time of egg-deposition. Other possibilities are preventing egg-deposition by the use of nets or plastic foil to cover the soil.

2. Natural environment as source of infestations
In some cases "unused land" is adjacent to the agricultural sites. These (semi) natural sites can be sources of beetle populations because certain weeds can be a suitable food resource for the beetle. An inventory of this (semi) natural sites could possibly lead to an advice to reduce the pest by habitat management.
The inventory should include:
- Digging larvae to determine important beetle populations.
- Use of pheromone traps to determine flight patterns.
- Inventory of the vegetation
- GIS-techniques to use for Pest Risk Analysis.

Suitable ideas can only be developed when the situation on the spot is well defined. Therefore a visit of the locations is necessary for a thorough description of the agricultural techniques and the adjacent (semi) natural sites.

Annex 1. Layout control experiments

Experiment 1 – nematodes and a bacterium

I		II	
1	T2 - L3 - B1	46	T3 - L1 - B3
2	T3 - L3 - B4	47	T1 - L2 - B2
3	T3 - L3 - B1	48	T3 - L1 - B5
4	T1 - L3 - B3	49	T1 - L2 - B4
5	T1 - L3 - B5	50	T1 - L1 - B1
6	T1 - L3 - B4	51	T2 - L1 - B3
7	T2 - L3 - B3	52	T3 - L1 - B2
8	T3 - L2 - B3	53	T2 - L1 - B5
9	T1 - L3 - B2	54	T2 - L2 - B5
10	T2 - L2 - B5	55	T1 - L1 - B2
11	T3 - L3 - B5	56	T3 - L1 - B1
12	T2 - L3 - B4	57	T3 - L1 - B5
13	T2 - L2 - B4	58	T1 - L2 - B3
14	T3 - L2 - B4	59	T1 - L1 - B5
15	T3 - L3 - B4	60	T2 - L1 - B2
16	T3 - L3 - B2	61	T2 - L1 - B4
17	T1 - L2 - B1	62	T1 - L1 - B4
18	T3 - L3 - B1	63	T1 - L2 - B1
19	T1 - L3 - B5	64	T3 - L2 - B3
20	T1 - L2 - B5	65	T2 - L2 - B3
21	T2 - L3 - B4	66	T3 - L1 - B3
22	T1 - L3 - B1	67	T1 - L1 - B3
23	T1 - L2 - B3	68	T2 - L2 - B2
24	T3 - L3 - B3	69	T2 - L1 - B3
25	T1 - L3 - B3	70	T3 - L2 - B4
26	T2 - L3 - B5	71	T1 - L1 - B3
27	T3 - L2 - B2	72	T2 - L1 - B2
28	T2 - L3 - B1	73	T2 - L1 - B4
29	T1 - L2 - B2	74	T1 - L1 - B1
30	T2 - L2 - B1	75	T1 - L2 - B5
31	T2 - L2 - B3	76	T3 - L2 - B5
32	T3 - L3 - B2	77	T2 - L2 - B1
33	T1 - L3 - B4	78	T1 - L1 - B2
34	T1 - L3 - B2	79	T2 - L1 - B5
35	T2 - L3 - B2	80	T2 - L2 - B4
36	T2 - L3 - B3	81	T3 - L2 - B1
37	T3 - L2 - B1	82	T3 - L1 - B2
38	T1 - L2 - B4	83	T2 - L1 - B1
39	T1 - L3 - B1	84	T3 - L2 - B2
40	T3 - L3 - B3	85	T2 - L1 - B1
41	T3 - L2 - B5	86	T3 - L1 - B4
42	T2 - L2 - B2	87	T1 - L1 - B4
43	T3 - L3 - B5	88	T3 - L1 - B4
44	T2 - L3 - B2	89	T3 - L1 - B1
45	T2 - L3 - B5	90	T1 - L1 - B5

Experiment 2 – nematodes

I			II			III			IV			V			VI		
8	H	L3 - B4	16	A	L1 - B1	24	C	L1 - B3	32	G	L3 - B3	40	F	L3 - B2	48	D	L1 - B4
7	E	L3 - B1	15	F	L3 - B2	23	D	L1 - B4	31	C	L1 - B3	39	C	L1 - B3	47	E	L3 - B1
6	A	L1 - B1	14	B	L1 - B2	22	G	L3 - B3	30	D	L1 - B4	38	H	L3 - B4	46	F	L3 - B2
5	F	L3 - B2	13	H	L3 - B4	21	A	L1 - B1	29	E	L3 - B1	37	G	L3 - B3	45	B	L1 - B2
4	D	L1 - B4	12	E	L3 - B1	20	E	L3 - B1	28	F	L3 - B2	36	D	L1 - B4	44	G	L3 - B3
3	B	L1 - B2	11	D	L1 - B4	19	H	L3 - B4	27	A	L1 - B1	35	E	L3 - B1	43	A	L1 - B1
2	G	L3 - B3	10	G	L3 - B3	18	F	L3 - B2	26	H	L3 - B4	34	B	L1 - B2	42	C	L1 - B3
1	C	L1 - B3	9	C	L1 - B3	17	B	L1 - B2	25	B	L1 - B2	33	A	L1 - B1	41	H	L3 - B4

KAS585 – first screening

I			II			III			IV		
23	K	46	AA	69	O	92	T	115	Q	138	W	161	JJ	184	R
22	MM	45	A	68	Y	91	OO	114	U	137	C	160	AA	183	CC
21	F	44	I	67	H	90	L	113	I	136	DD	159	FF	182	D
20	M	43	J	66	N	89	DD	112	L	135	X	158	L	181	LL
19	JJ	42	W	65	F	88	EE	111	SS	134	E	157	NN	180	TT
18	Z	41	Y	64	QQ	87	LL	110	JJ	133	TT	156	OO	179	SS
17	NN	40	V	63	CC	86	GG	109	BB	132	R	155	I	178	BB
16	B	39	U	62	B	85	J	108	EE	131	H	154	GG	177	B
15	FF	38	RR	61	C	84	W	107	FF	130	J	153	Y	176	N
14	PP	37	C	60	BB	83	Z	106	N	129	NN	152	A	175	DD
13	H	36	L	59	R	82	K	105	B	128	QQ	151	V	174	O
12	Q	35	X	58	A	81	U	104	RR	127	Z	150	M	173	RR
11	P	34	II	57	X	80	FF	103	Y	126	HH	149	S	172	E
10	GG	33	EE	56	NN	79	RR	102	V	125	F	148	W	171	U
9	SS	32	G	55	II	78	JJ	101	KK	124	D	147	Z	170	P
8	O	31	N	54	D	77	M	100	PP	123	T	146	H	169	F
7	E	30	OO	53	E	76	HH	99	AA	122	MM	145	II	168	Q
6	CC	29	QQ	52	KK	75	AA	98	CC	121	OO	144	KK	167	MM
5	R	28	TT	51	MM	74	TT	97	LL	120	K	143	PP	166	G
4	S	27	LL	50	Q	73	P	96	GG	119	O	142	C	165	J
3	BB	26	D	49	SS	72	S	95	A	118	M	141	T	164	EE
2	DD	25	T	48	G	71	V	94	S	117	P	140	HH	163	X
1	KK	24	HH	47	I	70	PP	93	G	116	II	139	QQ	162	K

KAS589 – second screening

I		II		III		IV	
17 Y		50 AA		83 BB		116 J	
16 K	33 BB	49 R	66 U	82 EE	99 F	115 X	132 S
15 DD	32 I	48 B	65 DD	81 FF	98 D	114 N	131 K
14 E	31 S	47 X	64 F	80 N	97 T	113 B	130 C
13 X	30 U	46 I	63 L	79 B	96 H	112 G	129 O
12 D	29 FF	45 EE	62 BB	78 DD	95 W	111 AA	128 BB
11 G	28 Q	44 W	61 FF	77 Y	94 K	110 H	127 GG
10 GG	27 Z	43 CC	60 T	76 V	93 O	109 F	126 A
9 B	26 J	42 S	59 J	75 R	92 M	108 W	125 CC
8 M	25 CC	41 K	58 P	74 E	91 P	107 R	124 U
7 R	24 AA	40 Q	57 C	73 AA	90 Z	106 D	123 DD
6 EE	23 H	39 H	56 GG	72 CC	89 Q	105 T	122 P
5 A	22 T	38 E	55 Z	71 J	88 U	104 Y	121 M
4 V	21 O	37 V	54 O	70 GG	87 I	103 FF	120 Z
3 F	20 L	36 D	53 N	69 A	86 L	102 V	119 I
2 W	19 N	35 M	52 A	68 S	85 C	101 EE	118 Q
1 C	18 P	34 Y	51 G	67 G	84 X	100 L	117 E

KAS582 – third screening, using strawberry plants

I	II	III	IV
36 B	72 B	108 S	144 C
35 V	71 EE	107 U	143 BB
34 J	70 CC	106 CC	142 J
33 EE	69 W	105 B	141 P
32 N	68 A	104 B	140 I
31 B	67 Y	103 O	139 S
30 H	66 B	102 C	138 L
29 DD	65 S	101 R	137 E
28 C	64 I	100 F	136 B
27 AA	63 L	99 J	135 AA
26 X	62 X	98 H	134 T
25 R	61 DD	97 K	133 Y
24 O	60 E	96 DD	132 A
23 B	59 Q	95 L	131 U
22 I	58 U	94 P	130 N
21 CC	57 J	93 D	129 H
20 T	56 C	92 G	128 V
19 G	55 H	91 B	127 F
18 L	54 Z	90 EE	126 R
17 BB	53 K	89 W	125 EE
16 S	52 B	88 Z	124 O
15 M	51 F	87 AA	123 B
14 B	50 R	86 I	122 M
13 U	49 AA	85 B	121 G
12 D	48 T	84 Y	120 Q
11 P	47 D	83 T	119 B
10 W	46 B	82 Q	118 B
9 E	45 N	81 M	117 Z
8 Q	44 M	80 B	116 B
7 Y	43 G	79 V	115 CC
6 K	42 B	78 X	114 D
5 Z	41 P	77 E	113 W
4 B	40 B	76 BB	112 DD
3 A	39 V	75 A	111 K
2 F	38 BB	74 N	110 X
1 B	37 O	73 B	109 B

Annex 2. Protocol field testing *Melolontha melolontha* lures

insect

Melolontha melolontha

PREFERRED NAME
Melolontha melolontha Linnaeus, 1758

TAXONOMIC POSITION
Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Coleoptera
Family: Scarabaeidae

OTHER NAMES USED
Scarabaeus melolonthus Linnaeus, 1758
Melolontha vulgaris Fabricius, 1775
Scarabaeus majalis Moll, 1785

COMMON NAMES
- English:
  grub cockchafer
  cockchafer
  may bug
  common European cockchafer
- Germany:
  Engerling
  Maikaefer, Feld-
  Maikaefer, Gemeiner

delivered test samples

Melolontha melolontha lures.
(The lures have a nasty smell and the content is irritating for your eyes).

Yellow unitraps with barrier crosses

Instruction sheets for unitrap

Supplied during the trip of Frans Griepink, Albert Ester and Leen Moraal to the Ukraine 1-16 May 2006.

operating procedure

The unitraps must be installed in accordance with the supplied information sheet. Please be sure that the lures are opened by cutting the top part with a scissor.

Place the unitraps in a tree at eye side. Individual traps of the same species should be separated by a distance of at least 20 meters. The distance between traps for different species should be at least10 meters.
If no insect are caught please make sure that the traps are relocated to a tree edge with preferable oak

trees. Also please place the traps higher, preferably at 5 meters height.

The traps must be checked every other day and preferably each day. The insects must be killed or at least not released in the vicinity of the trap.

The lures do not have to be replaced. However, if there is a doubt about the level of liquid they may be replaced by new ones.

If no insects are being caught after one week the checking can be done once per week.

duration of the test

Maximum one month, so in this case until halfway June, or until no insects are being caught anymore.

contact:

Frans Griepink

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****
Plant Research International BV
***PHERO*BANK**
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email: frans.griepink@wur.nl
www.pherobank.com
****

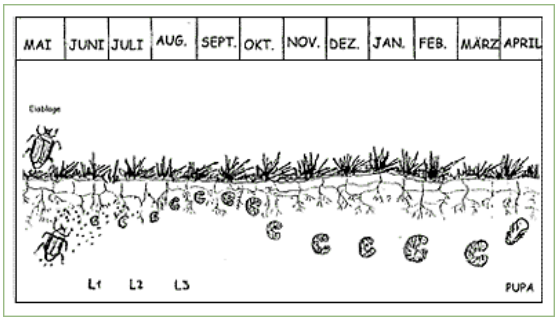
Annex 3. Protocol field testing *Phyllopertha horticola* lures

insect

PREFERRED NAME
Phyllopertha horticola (Linnaeus)

TAXONOMIC POSITION
Kingdom: Animalia
Phylum: Arthropoda
Class: Insecta
Order: Coleoptera
Family: Scarabaeidae

OTHER NAMES USED
Anomala horticola (Linnaeus)



COMMON NAMES
- English:
  bracken clock
  chafer, garden-
- Germany:
  Laubkaefer, Garten-
  Junikaefer
  Rosenkaefer, Kleiner

delivered test samples

Phyllopertha horticola lures
(The lures have a nice smell, however, the content may be irritating for your eyes).

Yellow unitraps with barrier crosses

Instruction sheets for unitrap

Supplied during the trip of Frans Griepink, Albert Ester and Leen Moraal to the Ukraine 1-16 May 2006.

operating procedure

The unitraps must be installed in accordance with the supplied information sheet. Please be sure that the lures are opened by cutting the top part with a scissor. The lures have a nice smell.

Place the unitraps in a tree at eye side. Individual traps of the same species should be separated by a distance of at least 20 meters. The distance between traps for different species should be at least10 meters.

The traps must be checked every other day and preferably each day. The insects must be killed or at least not released in the vicinity of the trap.

The lures do not have to be replaced. However, if there is a doubt about the level of liquid they may be replaced by new ones.

If no insects are being caught after one week the checking can be done once per week.

duration of the test

Maximum one month, so in this case until halfway June, or until no insects are being caught anymore.

contact:

Frans Griepink

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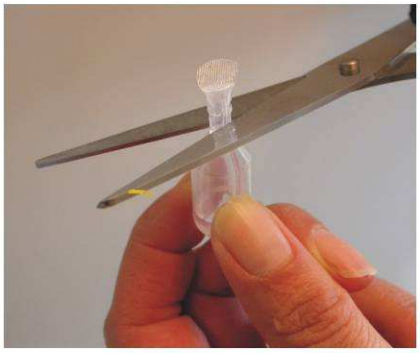
Annex 4. Protocol for installing lures

PHEROBANK

Melolontha dispenser handling



1. Cut off the dispenser tube (preferably) as close to the reservoir as possible.

2. Place the dispenser with the open end upwards in the green holder. Close the holder with the white cap.

3. Place the holder with dispenser in the trap.

Pheromone lures are best kept in th refrigerator or freezer in the tightly close original packaging. Before use allow the pheromone dispensers to acclimatize to room temperature in the original packaging before opening to prevent condensation.

Produced by:
Plant Research International BV, ***PHERO*BANK**
P.O. Box 16, 6700 AA Wageningen
phone. +31 317 475937

www.pherobank.com

Annex 5. Data sheet for field trapping experiments

Insect species: Melolontha melolontha

trap type: UNITRAP, yellow plus barrier crosses

location: Protzev, Borispilskiy rayon, Kyivska region

start date: 13.04.2006 11-06-2006

	trap No. 1	trap No. 2	trap No.3	trap No.4	trap No.5	trap No.6	remarks
Date							
13-04-2006							
14-05-2006	0	0	0	0	0	0	Traps were replaced to new place
15-05-2006	4	17	1	1	2	0	
16-05-2006	1	4	0	0	0	0	
17-05-2006	1	12	0	0	0	0	
18-05-2006	2	0	0	0	0	0	
19-05-2006	7	7	0	0	0	0	
20-05-2006	0	11	0	0	3	1	
21-05-2006	11	5	0	0	0	0	
22-05-2006	3	7	0	0	0	0	
23-05-2006	1	5	0	0	0	0	
24-05-2006	8	11	1	0	0	0	
25-05-2006	0	2	0	0	0	0	
26-05-2006	1	0	0	0	0	0	
27-05-2006	0	2	0	0	0	0	
28-05-2006	1	1	0	0	0	0	
29-05-2006	0	0	0	0	0	0	
30-05-2006	0	0	0	0	0	0	
31-05-2006	0	0	0	0	0	0	
1-06-2006	2	1	0	0	0	0	
2-06-2006	0	1	0	0	0	0	
3-06-2006	0	0	0	0	0	0	
4-06-2006	0	0	0	0	0	0	
5-06-2006	0	0	0	0	0	0	
6-06-2006	0	0	0	0	0	0	
7-06-2006	0	0	0	0	0	0	
8-06-2006	0	0	0	0	0	0	
9-06-2006	0	0	0	0	0	0	
10-06-2006	0	0	0	0	0	0	
11-06- 2006	0	0	0	0	0	0	
total	42	86	2	2	5	1	