Research on the use of different chemical and biological methods for the control of leatherjackets *(Tipulidae)* in sugar beet in the Netherlands and in Belgium 2012

Elma Raaijmakers (IRS) and Barbara Manderyck (KBIVB-IRBAB)

12P07

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 IRS-project 03-01 **KBIVB-IRBAB project no. B12PISXH**

CONTENTS

1. Introduction

Leatherjackets are the larvae of the crane fly (*Tipulidae*). In the Netherlands the insecticide Condor (parathion-methyl) could be used until 2003 and from 2007 till 2009 Talstar (bifenthrin) had a temporary exemption. In Belgium and the Netherlands, the only insecticide that is authorised in sugar beet to treat against attacks of leatherjackets is Poncho Beta (beta-cyfluthrin/clothianidin) as seed treatment. However, the treatment of the seed with Poncho Beta does not allow full control of the leatherjackets when there is a high infestation in a field. Hence, no pest control methods are currently available that gives satisfactory control of leatherjackets in sugar beet in Belgium and the Netherlands.
In 2011 89 hectares had to be redrilled in the Netherlands and 50 hectares in Belgium, due to damage caused by these leatherjackets. The most prevalent species in 2011 was *Nephrotoma appendiculata* (= *N. maculata*). More often the species found to cause damage in Belgium and the Netherlands are *Tipula paludosa* and *Tipula oleracea*.
In order to investigate new pest control methods, research was done with chemical and biological methods to control leatherjackets. These methods were compared with an untreated control.

2. Materials and methods

2.1 Trial sites

The largest problems with leatherjackets can be expected on fields with grass as a pre-crop. For these trials three sites with grass as a pre-crop were selected and checked for leatherjackets in the period November 2011/March 2012. This was done by sampling. A field was divided in four parts and from each part ten subsamples were taken. One subsample consisted of one litre of soil (10 × 10 × 10 cm) and was treated as explained in section 2.7. One field in Belgium (located in Xhendremael) and two fields in the Netherlands (located in Ens and Noordgouwe) were selected. The location in Xhendremael consisted of a silty clay loam soil and the locations in Ens and Noordgouwe of sandy loam and sandy clay, respectively. The field in Xhendremael, Ens and Noordgouwe had 70, 20 and 150 leatherjackets per m^2 respectively. On the fields in Ens and Noordgouwe all of the larvae were *Nephrotoma* spp. In the field in Xhendremael *Tipula* species were found.

2.2 List of products

In table 1 the products used in the trials have been listed. All seed treatments (also the untreated controls) contained the fungicides Proseed (6.5 g thiram) and Tachigaren (14 g hymexazole) to prevent influences of fungi on plant establishment.

Table 1. Products used.

treatmeant	IRS-code	KBIVB-code	substance	applied at the time of sowing/after sowing
1	untreated control	KBIVB1	none	-
2	IRS 657	KBIVB2	chemical	at sowing
3	IRS 657 + IRS 708	KBIVB3	chemical	at sowing + after
4	IRS 657 + barley (70 kg/ha)	KBIVB4	chemical/biological	at sowing
5	IRS 709	KBIVB5	chemical	at sowing
6	IRS 710*	KBIVB6	chemical	at sowing
7	IRS 710 + IRS 711*	KBIVB7	chemical	at sowing
8	IRS 712	KBIVB8	chemical	at sowing
9	IRS 713	KBIVB9	chemical	after
10	IRS 711	*	chemical	at sowing
11	IRS 714	*	biological	after
12	IRS 715	*	biological	at sowing
13	IRS 716 + IRS 717	*	biological	at sowing + after
14	IRS 718	*	chemical	at sowing
15	IRS 719	*	biological	after
16	IRS 720	*	biological	after

* Treatments 6 and 7 were only included in field trials in Belgium and the treatments 10 up to 16 only in the Netherlands. All the other treatments were included in all trials.

2.3 Drilling

In the trials in the Netherlands drilling was done with a standard precision sowing machine (Monozentra). This sowing machine is equipped with a system to change seed batches quickly and to apply in furrow treatments at the time of sowing. The trials were drilled relatively deep (about 4 cm) in order to slow down emergence of plants and to enhance damage due to leatherjackets. The sowing distance in both trials was 18.5 cm and 50 cm between rows. The trials in Noordgouwe and Ens were sown on 17 and 16 April 2012, respectively.
In the trial in Belgium drilling was done with a mechanical precision sowing machine (Gilles Tank-B6 with Gilles microband microgranulate applicator). This sowing machine is equipped with a system to change seed batches quickly and to apply in furrow treatments at the time of sowing. The trial was drilled at a normal sowing depth of 2.5 cm. The sowing distance within the rows was 18.48 cm and 45 cm between the rows. The trial in Xhendremael was drilled on 23 March 2012.

2.4 Spraying and application of treatments after sowing

Spraying was done in Xhendremael on 3 April and in Ens and Noordgouwe on 1 and 2 May 2012, respectively. In Noordgouwe and Ens plants were in cotyledon stage (BBCH 10) at that moment. In Xhendremael the beets were at BBCH 07-09.
In Noordgouwe and Ens spraying was done at around 10 and 11 a.m. on a wet soil and with cloudy conditions. Air temperature was 16 and 18ºC, respectively. Relative humidity was 65 and 60% respectively and wind speed 1.5 and 2 meters per second. For spraying the field trials in Noordgouwe and Ens an AZO apparatus (system Van der Wey) was used with compressed air. The spraying boom of three meters was equipped with six Teejet 6503 E nozzles, with a pressure of 3.6 bar. The spraying volume was 400 liters per hectare for treatment 9 and 600 liters per hectare for treatment 11, 13, 15 and 16. Treatment 9 was applied as a full-field treatment, while the treatments 11, 13, 15 and 16 were applied onto the row. With the row application only 20 cm of the row was sprayed. The same day treatment 3 was applied also as a

full field treatment.
In Xhendremael spraying was done in the morning (8 a.m.) of 3 April 2012 with an air temperature of 8°C, an air humidity of 71%, 0 km per hour wind speed and about 25% cloud cover on a dry soil.
The trial (treatment 9) was sprayed full-field using an EURO-PULVE type ETC sprayer. The spraying boom width is 2.7 meters with six Teejet XR110015 nozzles (150 l/ha), the spraying pressure was 2.8 bar, three passages of 150 liters per hectares at 1 meter per second were done, hence the treatment dose was applied using 450 liters water. The same day treatment 3 was applied also as a full field treatment.

2.5 Statistical design

All trials are designed as randomised blocks in four replications. Gross plot size: six rows and 16.5 meters long in Ens and Noordgouwe. Nett plot size: four rows of 10.0 meters long in Ens and Noordgouwe. Gross plot size: six rows and 16.0 meters long in Xhendremael. Nett plot size: four rows of 10.0 meters long.

2.6 Assessment of efficacy

The effect of the different treatments and formulations on leatherjackets was measured by regular plant counts in the central four rows of each plot. The whole net length was counted. Final plant counts were done at BBCH 16 (six leaves unfolded). It is known that from this stage on, leatherjackets cannot influence plant stand anymore. In Xhendremael the final count was done at BBCH18 (eight leaves unfolded). A total of six counts was performed in Xhendremael, starting during the emergence of the beets. With the last count the crane flies emerged.
At all sites five plant samples were taken in each gross plot. This was done to assess the effect on the number of leatherjackets and to make sure that no other soil pests had influenced the results. Each sample consisted of a plant with soil (10 × 10 × 10 cm). These samples were analysed in the laboratory as described in section 2.7.
In Noordgouwe and Ens, this was done on 14 and 16 May, respectively. Plants were at that time at BBCH 12 to BBCH 14 (2-4 unfolded leaves).
In Xhendremael the first assessment on the untreated control plots was done at BBCH 12. The second assessment was done on 15 May when BBCH 16 was reached. At this time five plants from each plot were sampled.

2.7 Analysing soil samples

After taking soil samples, they were taken into the laboratory. Each soil sample consisted of ten subsamples for finding a location. The soil samples were divided over five buckets (each 12 l). For the assessment of efficacy each soil sample consisted of one subsample. Thereafter, buckets were filled with water and samples were solved. After half an hour, water and soil was flushed over a sieve (mesh width = 1 mm). Leatherjackets were collected from the sieve. If soil had not totally solved, this step was repeated for two times. After that the total number of leatherjackets per sample was counted. The number of leatherjackets was multiplied by ten in case of finding a location and by hundred for assessment of efficacy to express the number of leatherjackets per square meter.

2.8 Analysis of data

Data were analysed by using REML, Linear Mixed Models and by using ANOVA. REML was used for analysing the complete dataset and the field trial in Belgium, because two untreated controls were included. It was also used for analysing the trial in Noordgouwe, because the last

row (plots A11, B6, C14 and D7) was excluded, due to a spraying track. ANOVA was used for analysing the field trial in Ens. Analyses were done with Genstat Software Package 15.0. The overall analysis for all treatments over the three trial sites was performed using a REML. This because some treatments were only included in the trial in Xhendremael or vice versa in Ens and Noordgouwe. The REML procedure compensates for the missing values. This means that for treatments included only in Belgium eight values out of twelve were generated by the method and that for treatments only included in the Netherlands four out of twelve were generated by the REML procedure.

3. Results and discussion

3.1 Germination

There was an interaction between the different field trials and objects (P<0.001). Therefore, results of the germination of the different field trials are shown in separate graphs. In Ens and Noordgouwe trial plants were at BBCH stage 10 or 11. In Xhendremael the first count was performed at the time the beets were at BBCH stage 9 to 10. The plants of objects treatment 4 and treatment 8 germinated significantly slower in comparison with the other objects (figure 1). For treatment 8 it was clearly caused by phytotoxicity of the treatment. For treatment 4 this is due to damage done by birds in the field trial in Xhendremael. Due to practical restrictions the barley was sown at the same time as the beets. The barley was sown between the beet rows (see figure 4). Even before beet plants emerged the barley also germinated. The birds caused damage by eating the freshly germinated barley and also the kernels left in the ground. Their digging for barley caused damage on the sugar beet plants as well (see figure 5). This was not observed at the field trials in Noordgouwe and Ens. Here, the barley was sown about three weeks earlier than the sugar beet seeds. Unfortunately, at these two field trials the barley did not germinate, but were eaten by mice.
At the field trials in Noordgouwe treatment 3 had the highest germination (figure 2). Only treatment 8, 10 and 15 had significantly fewer plants at the first count date compared to treatment 3. In the field trial in Ens treatment 15 had the best germination (figure 3). Only the untreated control and the treatments 4, 8, 10, 13 and 14 had significantly fewer plants than treatment 15. In all three field trials treatment 8 belonged to the objects with the fewest plants.

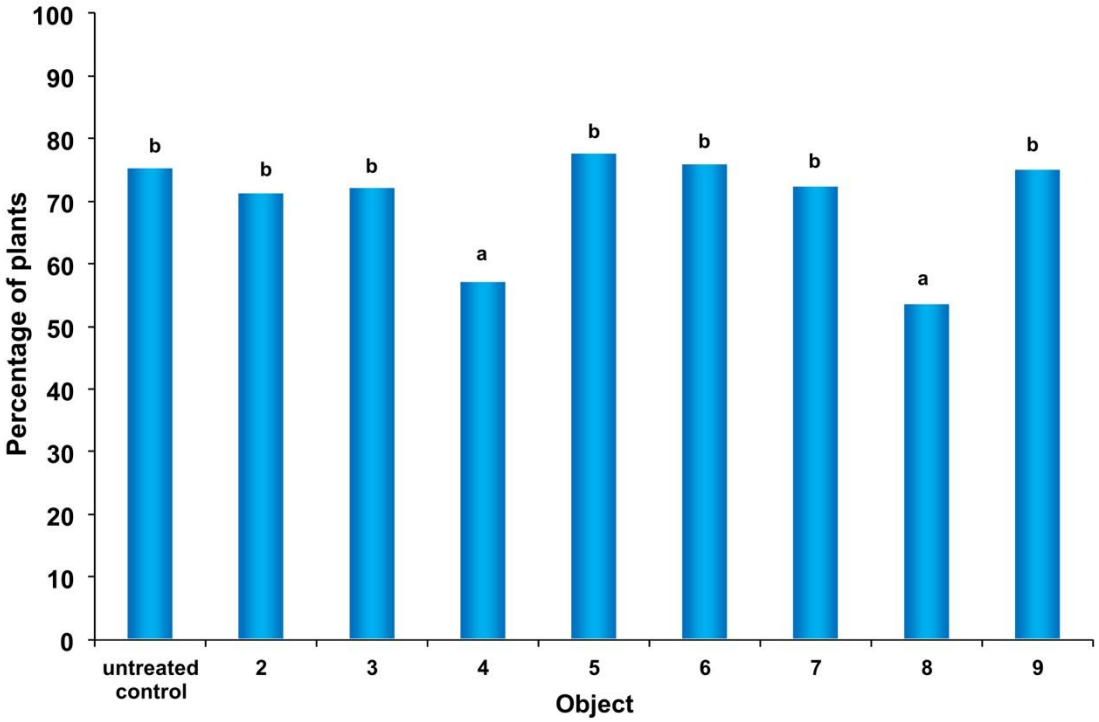

Figure 1. Percentage of plants during germination at the first count (6 April 2012, fourteen days after sowing, BBCH beets 09-10) in Xhendremael (LSD[1] 5% = 7.06; P[2]<0.001).

[1] LSD = least significant difference.
[2] P = probability.

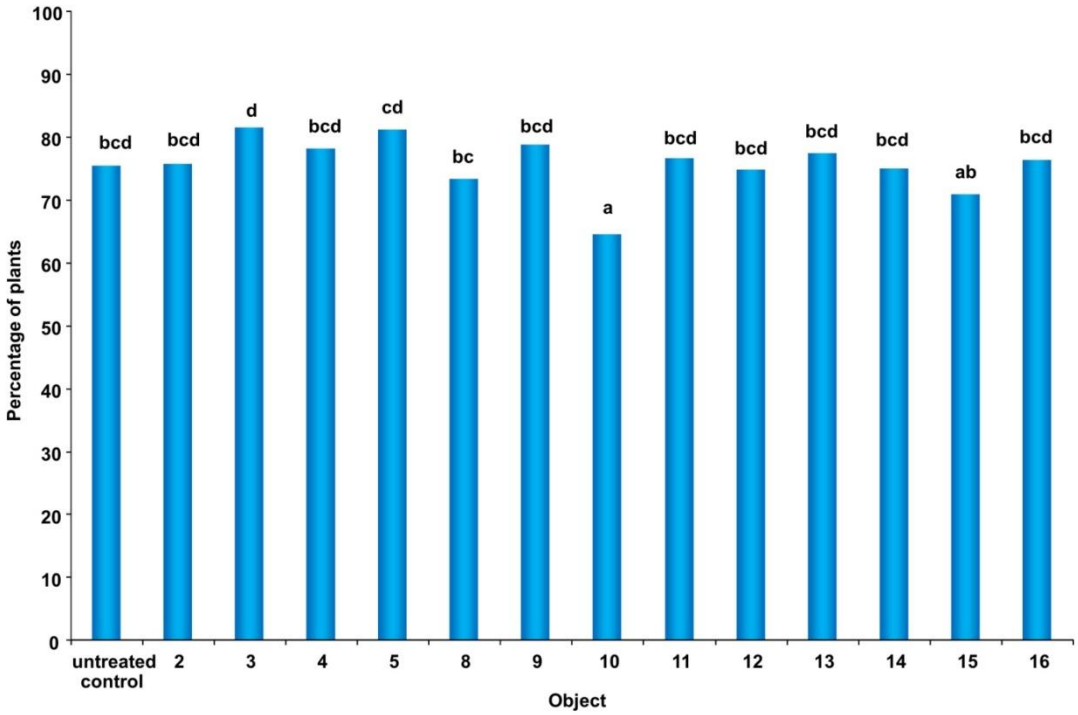

Figure 2. Percentage of plants during germination at the first count (2 May 2012, fifteen days after sowing) in Noordgouwe (LSD[1] 5% = 8.09; P[2]<0.029).

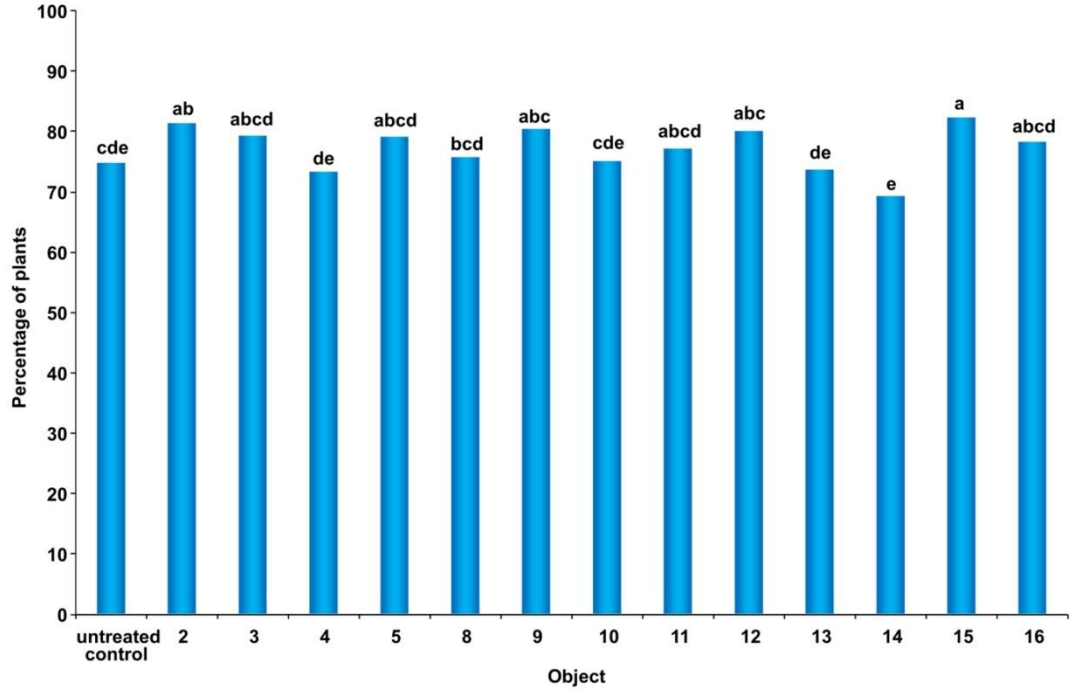

Figure 3. Percentage of plants during germination at the first count (3 May 2012, seventeen days after sowing) in Ens (LSD[1] 5% = 6.18; P[2]<0.005).

[1] LSD = least significant difference.
[2] P = probability.

Figure 4. Sowing of barley seeds in between sown sugar beet rows at Xhendremael on 23 March 2012.

Figure 5. One week after sowing, the barley was germinating and birds had eaten from it and so they damaged the germinating beets at the field trial in Xhendremael. A: plot A of treatment 4 where birds dug up seeds. B: plot A of treatment 6, untouched by birds. C (below): detail of digging done by birds.

3.2 Evolution of plant population at Xhendremael

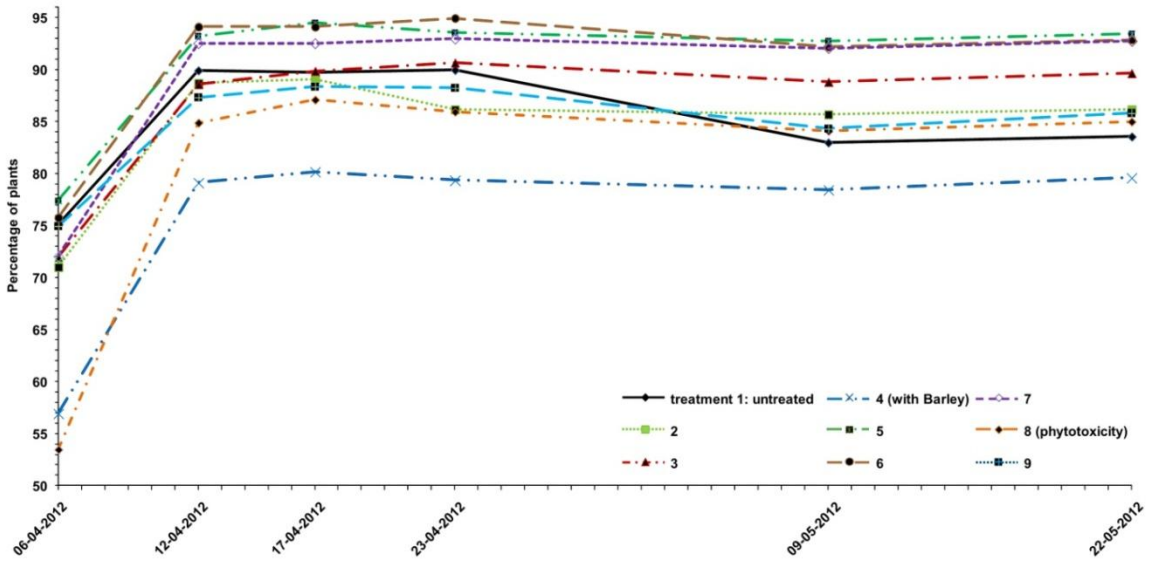

Figure 6. Evolution of plant population at Xhendremael (see table 2 for least significant differences).

In both figure 6 and table 2 it is clear that treatment 8 caused phytotoxicity, which resulted in a slower emergence of the beets. The percentage of plants increased in time subsequently, but it remained comparable to the untreated control. The product may have given some protection against leatherjackets, but the loss of plants due to phytotoxicity was never compensated. Treatment 4 has lower emergence, in following counts it continued to be the worst treatment.

Table 2. Evolution of mean plant population (%) at Xhendremael.

	evolution of plant population at Xhendremael (%)											
	emergence		2nd count		3rd count		4th count		5th count		6th count	
	6-4-2012		12-4-2012		17-4-2012		23-4-2012		9-5-2012		22-5-2012	
1 untreated	75.1	. b	89.9	. . c d .	89.7	. b c .	90.0	. b c .	83.0	a b .	83.6	a b . .
2	71.0	. b	88.7	. b c d .	89.1	. b c .	86.2	. b . .	85.7	. b c	86.2	a b c .
3	72.0	. b	88.6	. b c d .	89.9	. b c d	90.7	. b c d	88.8	. b c	89.6	. b c d
4 (+barley)	56.9	a .	79.1	a	80.2	a . . .	79.4	a . . .	78.4	a . .	79.6	a . . .
5	77.4	. b	93.2	. . . d e	94.5	. . . d	93.6	. . c d	92.8	. . c	93.4	. . . d
6	75.8	. b	94.1	. . . . e	94.1	. . . d	94.9	. . . d	92.2	. . c	92.9	. . c d
7	72.1	. b	92.5	. . . d e	92.5	. . c d	93.0	. . c d	92.1	. . c	92.8	. . c d
8 (phytotoxicity)	53.5	a .	84.9	. b . . .	87.1	. b . .	85.9	. b . .	84.1	a b .	85.0	a b . .
9	75.0	. b	87.3	. b c . .	88.4	. b c .	88.2	. b c .	84.3	a b .	85.8	a b c .
LSD[1] 5%	7.06		4.73		4.89		5.67		7.18		7.16	
P[2]	<0.001		<0.001		<0.001		<0.001		0.002		0.002	

[1] LSD = least significant difference.
[2] P = probability.

3.3 Final plant population

Final plant counts were done at BBCH 16 (six leaves unfolded) in Ens and Noordgouwe, at BBCH 16-18 (six till eight leaves unfolded) in Xhendremael. For the overall analysis of the three trial sites the counts at BBCH 16 were taken, for Xhendremael these are the numbers for the fifth count. There was no interaction between the different field trials (P=0.090). Treatments 3, 5, 6 and 7 had significant more plants than the untreated control (figure 7) in the analyses of data of all the three trials. Of course, the results have to be confirmed in another trial year. All the other objects were not significantly different from the untreated control.

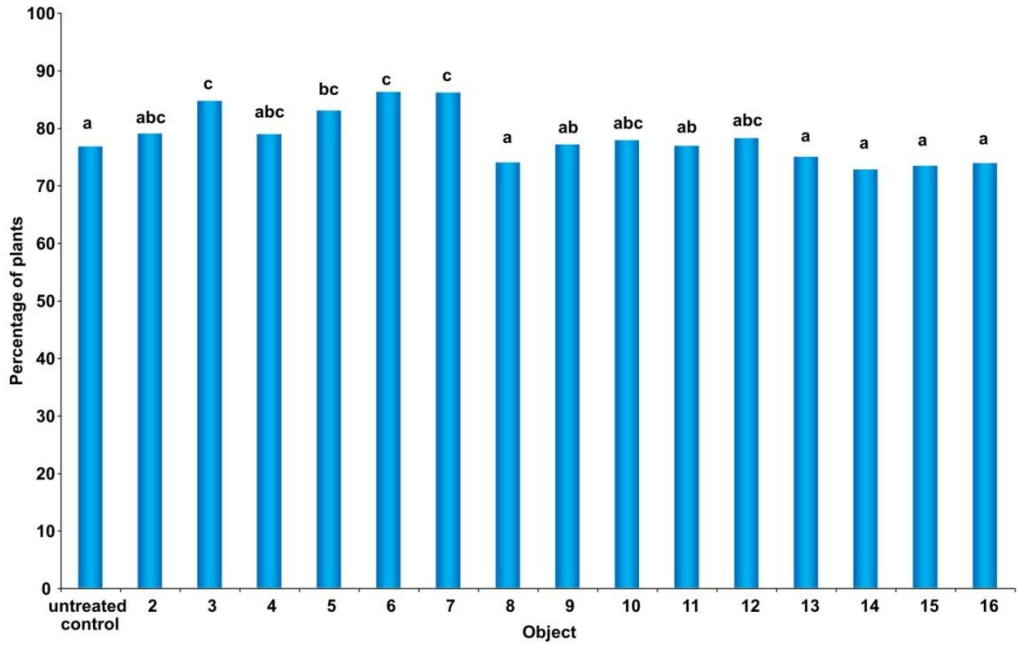

Figure 7. Percentage of plants at BBCH 16-18 from the field trials in Ens, Noordgouwe and Xhendremael (LSD 5% = 6.327; P<0.001). Treatments 6 and 7 were only included in field trials in Belgium and the treatments 10 up to 16 only in the Netherlands. All the other treatments were included in all trials.

In the trial in Xhendremael dead leatherjackets were found on the surface in treatment 3 (see figure 8).

Figure 8. Dead leatherjacket on surface in plot treatment 3 at the field trial in Xhendremael.

3.4 Effect on leatherjackets

The effect of the different control methods on the number of leatherjackets was measured by analysing soil samples. In the untreated control no leatherjackets were found in the soil samples of Noordgouwe and Ens, although many of them were observed on the surface in Ens (figure 9). Therefore, the other samples were not analysed. Moreover, no other soil pests were found either.

Figure 9. One of the leatherjacket that is eating from sugar beet plants in Ens (3 May 2012).

In Xhendremael the presence of the leatherjackets was confirmed on the 23 April at BBCH 10-12. In each of the four plots of the untreated control (treatment 1) five plants and soil were taken as described in section 2.6.

Figure 10. Leatherjackets in plant/soil sample taken from untreated control plot in Xhendremael.

In the 20 plant and soil samples analysed, two leatherjackets were found and no other soil pests. Only non-pathogenic earth-worms were found.
A second sampling was performed on 15 May 2012 when the beets were at BBCH 16. This time all plots were sampled. Some leatherjackets were found in treatments 6 and 1 (untreated), but from the data obtained out of sampling no conclusions could be made concerning efficacy of the treatments.

4. Summary and conclusions

The aim was to study the efficacy of different chemical and biological control methods on the control of leatherjackets in sugar beet.
Treatment 3, 5, 6 and 7 protected the sugar beet plants better against leatherjackets than the untreated control. Of course, the results have to be confirmed in another trial year. All other methods gave a similar degree of protection as the untreated control. Treatment 8 was phytotoxic to the beets. Treatment 4 with barley sown before the sugar beet might be efficient in deterring leatherjackets from eating the young beet plants when sown on full field scale. However, when barley is sown in a small plot in a trial it acts as an attractant for mice and birds.

5. Samenvatting

In België en Nederland werden in 2011 respectievelijk 50 en 89 hectares suikerbietenpercelen opnieuw ingezaaid door schade veroorzaakt door emelten. In beide landen is geen enkel erkend product met een afdoende werking indien de aantasting door emelten aanzienlijk is. De zaadbehandeling met Poncho Beta (beta-cyfluthrin/clothianidine) die erkend is in beide landen, beschermt maar deels tegen aanvallen door emelten. In samenwerking legden het IRS en het KBIVB in 2012 drie proeven aan waarin de effectiviteit van verschillende chemische en biologische middelen werd geëvalueerd. Er werden in proeven (gerandomiseerd, met vier herhalingen) aangelegd te Ens, Noordgouwe en Xhendremael, in totaal vijftien behandelingen vergeleken met een onbehandelde controle. Behandelingen 6 en 7 lagen alleen in de proef in Xhendremael en de behandelingen 10 tot en met 16 alleen in Ens en Noordgouwe. De overige zes behandelingen werden gemeenschappelijk aangelegd. Na een eerste proefjaar werden de volgende conclusies getrokken: behandelingen 3, 5, 6 en 7 beschermden de jonge suikerbieten (significant) beter tegen emelten dan het onbehandelde object. Uiteraard dienen de proeven in de tijd te worden herhaald om deze resultaten te kunnen bevestigen. Alle andere behandelingen hadden geen significant effect op het plantaantal ten opzichte van het onbehandelde object. Behandeling 8 leidde tot fytotoxiteit bij de bietenplanten. Behandeling 4, waarbij gerst was gezaaid, zou efficiënt kunnen zijn als dit volvelds zou worden toegepast, maar in de veldproeven bleek dit aantrekkelijk te zijn voor muizen en vogels, waardoor schade is ontstaan aan de bietenplanten.

6. Resumé

En Belgique et les Pays-Bas, on a en 2011 ressemé respectivement 50 et 89 hectares de parcelles de betteraves sucrières par des dégâts causés par des tipules. Dans les deux pays, aucun produit avec une action suffisante n'est agréé si l'infestation par les tipules est significative. Le traitement des semences avec Poncho Beta (beta-cyfluthrine/clothianidine) qui est reconnu dans les deux pays, ne protège que partiellement contre les attaques de tipules. En 2012, l'IRS et l'IRBAB ont conjointement mis en place trois essais dans lesquelles l'efficacité des différents produits chimiques et biologiques a été évaluée. Dans des essais, mis en place (randomisés, en quatre répétitions) à Ens, Noordgouwe et Xhendremael, en total quinze traitements ont été comparé avec un témoin non traité. Six traitements ont été effectués en commun. Après une première année d'essai on a tiré les conclusions suivantes: les traitements 3, 5, 6 et 7 ont mieux (significativement) protégé les jeunes betteraves contre les tipules que dans l'objet non-traité. Evidemment les essais devraient être répétées dans le temps pour confirmer les résultats. Tous les autres traitements n'avaient aucun effet significatif sur le nombre de plantes par rapport à l'objet non-traité. Le traitement 8 a mené à une phytotoxicité chez les plantes de betteraves. Le traitement 4, ou a été semé de l'orge, pourrait être efficace

s’il était appliqué en généralisé, mais les essais sur le terrain se sont avérés attrayant pour les souris et les oiseaux, ce qui a endommagé les plantes de betteraves.

Annex A GEP CERTIFICATE IRBAB

federale overheidsdienst
VOLKSGEZONDHEID,
VEILIGHEID VAN DE VOEDSELKETEN
EN LEEFMILIEU

Directoraat Generaal
Dier, Plant en Voeding

Dienst
Pesticiden en Meststoffen

UW BRIEF VAN
UW REF.
ONZE REF. 4LL70/10/ λ65704
DATUM
(BIJLAGE(N)) 28. 10. 10

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K.B.I.V.B.
T.a.v. de heer Misonne
Molenstraat 45
3300 TIENEN

BETREFT **Erkenning van een station of laboratorium dat bepaalde proeven en analyses verricht met betrekking tot bestrijdingsmiddelen voor landbouwkundig gebruik (vernieuwing)**

Geachte heer,

In toepassing van het ministerieel besluit van 7 april 1995 betreffende de erkenning van stations, of laboratoria die bepaalde proeven en analyses verrichten met betrekking tot bestrijdingsmiddelen voor landbouwkundig gebruik, is het

Koninklijk Belgisch Instituut tot Verbetering van de Biet v.z.w.
Molenstraat 45
3300 Tienen

erkend onder nr. **7 SL.**

De erkenning is geldig voor de volgende proeven:

- de ***werkzaamheid*** van bestrijdingsmiddelen voor landbouwkundig gebruik en de effecten van deze middelen op de opbrengst van planten en plantaardige producten zoals in tabel 1 vermeld;
- de effecten van bestrijdingsmiddelen voor landbouwkundig gebruik op de ***kwaliteit*** van planten en plantaardige producten zoals in tabel 1 vermeld;
- het testen van de ***fytotoxiciteit*** bij met bestrijdingsmiddelen voor landbouwkundig gebruik behandelde planten en plantaardige producten, effecten op volggewassen, effecten op andere planten, inclusief gewassen op aangrenzende velden zoals in tabel 1 vermeld;

*Tabel 1: erkenning voor het testen van de **werkzaamheid**, de effecten op de **kwaliteit** en de **fytotoxiciteit** van de experimentele producten*

Teelten	insecticide/acaricide
Bieten	X

De erkenning is geldig voor:

- Bespuiten
- Microgranulaten
- Behandelde zaaizaden

Deze erkenning is geldig van 26/10/10 tot 01/02/2014.

Belangrijke opmerking:

Indien andere types van behandelingen of proeven (toepassingstechniek of type van product of teelt) moeten gebeuren, is het noodzakelijk het volgende in te dienen:

- een bewijs dat het noodzakelijke materiaal beschikbaar is;
- de procedure(s) voor het gebruik van dit materiaal;
- een aanvraag tot uitbreiding van deze erkenning.

Hoogachtend,

ir Olivier GUELTON
Diensthoofd *a.i.*

Annex B GEP CERTIFICATE IRS

Ministerie van Landbouw, Natuur en Voedselkwaliteit

Landbouw, natuur en voedselkwaliteit

This is to declare that, in conformity with the request of March 4, 2008

Stichting IRS

Residing Van Konijnenburgweg 24, Bergen op Zoom, the Netherlands

HAS OFFICIALLY BEEN RECOGNISED AS AN ORGANISATION FOR EFFICACY TESTING

as has been laid down in the 'Regeling gewasbeschermingsmiddelen en biociden' (Regulation Crop Protection Products and Biocides) of September 26, 2007 (Staatscourant 2007, 386)

This recognition will commence on June 19, 2008 and expire on June 19, 2014

Wageningen, June 9, 2008

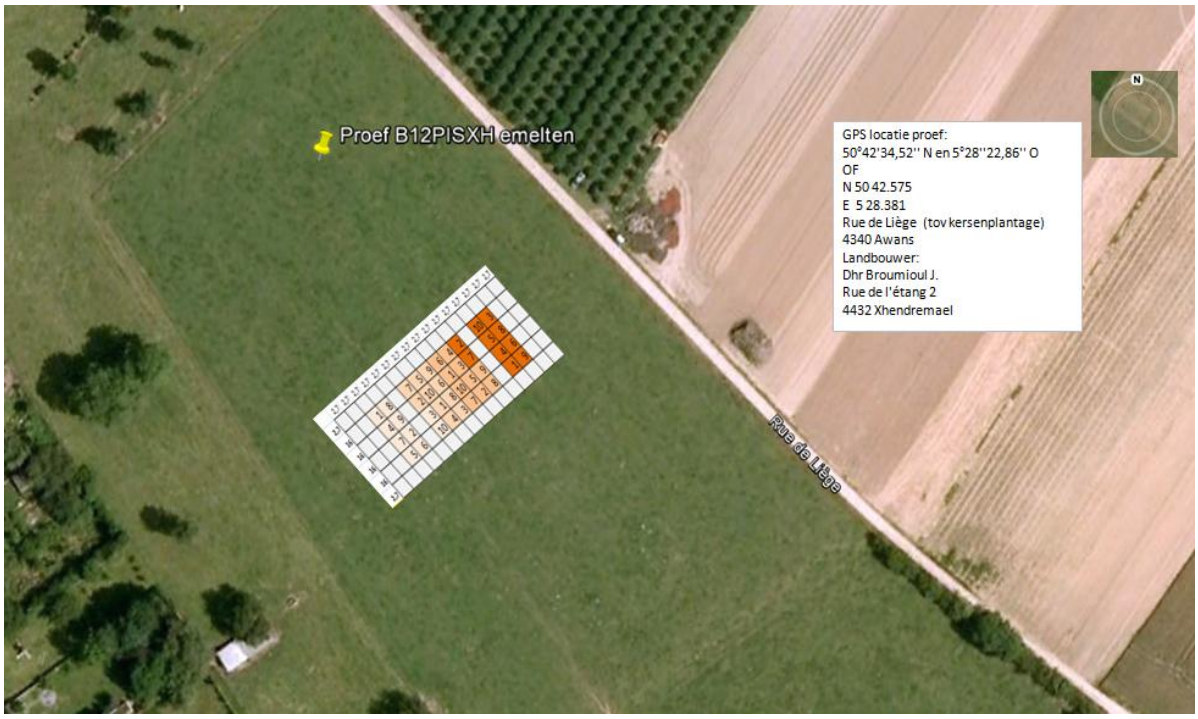
For the Minister of Agriculture, Nature and Food Quality,

Mr. ing. H.A. Harmsma

Acting Director Plant Protection Service

Annexes C Field trial Xhendremael

Annex C1 Location



Annex C2 Trial scheme

Trial field:	B12PISXH	(Xhendremael)	
Number of replications:	4		
Nett size (m):	10*1.8	Gross size (m):	16*2.7

	2,7	2,7	2,7	2,7	2,7	2,7	2,7	2,7	2,7	2,7	2,7	2,7	2,7	2,7	2,7	2,7
2,7																
16			1	8		7	5	9	6	4	2		10	3		
16			4	9		2	10	6	1	3	7		5	8		
16			7	2		3	1	8	10	5	9		4	6		
16			5	6		10	4	3	7	2	8		1	9		
2,7																

Annex C3 Treatments

Trial field code: B12PISXH
Name of trial field: Xhendremael
Number of replications: 4
Number of treatments: 9

number	treatment
1	untreated control
2	IRS 657
3	IRS 657 + IRS 708
4	IRS 657 + barley
5	IRS 709
6	IRS 710
7	IRS 710 + IRS 711
8	IRS 712
9	IRS 713

Annex C4 General data

soil type:	silty clay-loam soil 4.4% organic matter pH-KCl = 5.3 (acid) parts < 20 µm = 17.4% P = 2 mg/100 g (reference 7-11 mg/100 g) K = 14 mg/100 g (reference 14-21 mg/100 g) Mg = 14 mg/100 g (reference 7-10 mg/100 g) Na = 3 mg/100 g (= medium) Ca = 187 mg/100 g K/Mg = 1 = good Ca/Mg = 13= good B = 0.56 mg/kg = good
preceding crop:	2.7 hectares permanent grassland (25 years)
drilling date:	23 March 2012
variety:	Bever
distance in row:	18.48 cm
distance between rows:	45 cm

Annex C5 Efficacy

Trial field code: B12PISXH
Name of trial field: Leatherjackets, Xhendremael

Assessment Percentage of plants
Date of assessment 6-4-2012

	treatment	A	B	C	D	average
1	untreated control	68.8	73.5	76.7	75.8	73.7
2	IRS 657	62.8	73.9	71.6	75.8	71.0
3	IRS 657 + IRS 708	68.8	72.5	69.8	76.7	72.0
4	IRS 657 + barley	53.1	58.2	58.2	58.2	56.9
5	IRS 709	78.1	73.5	75.3	82.7	77.4
6	IRS 710	80.9	68.8	73.0	80.4	75.8
7	IRS 710 + IRS 711	67.5	79.9	71.1	69.8	72.1
8	IRS 712	52.2	52.7	52.2	56.8	53.5
9	IRS 713	64.7	76.2	77.6	81.3	75.0
1	untreated control	70.7	80.9	86.9	67.9	76.6
	LSD[1] 5%					7.064
	P[2]					<0.001
	significance[3]					VS

Assessment Percentage of plants
Date of assessment 12-4-2012

	treatment	A	B	C	D	average
1	untreated control	84.1	88.7	88.2	88.2	87.3
2	IRS 657	82.7	89.6	91.5	91.0	88.7
3	IRS 657 + IRS 708	84.5	94.2	90.6	85.0	88.6
4	IRS 657 + barley	79.0	75.3	80.9	81.3	79.1
5	IRS 709	92.4	90.1	97.0	93.3	93.2
6	IRS 710	93.8	92.4	94.7	95.6	94.1
7	IRS 710 + IRS 711	92.4	94.7	90.6	92.4	92.5
8	IRS 712	87.3	87.8	82.2	82.2	84.9
9	IRS 713	82.2	84.5	93.8	88.7	87.3
1	untreated control	91.9	92.4	93.8	91.9	92.5
	LSD[1] 5%					4.733
	P[2]					<0.001
	significance[3]					VS

[1] LSD = least significant difference.
[2] P = probability.
[3] VS = very significant.

Assessment Percentage of plants
Date of assessment 17-4-2012

	treatment	A	B	C	D	average
1	untreated control	80.4	91.0	89.6	88.7	87.4
2	IRS 657	85.5	91.0	91.0	88.7	89.1
3	IRS 657 + IRS 708	87.3	93.8	92.9	85.5	89.9
4	IRS 657 + barley	85.5	73.5	80.9	80.9	80.2
5	IRS 709	94.2	91.9	97.5	94.2	94.5
6	IRS 710	93.3	92.4	95.2	95.6	94.1
7	IRS 710 + IRS 711	92.4	93.8	90.1	93.8	92.5
8	IRS 712	87.3	89.2	85.0	86.9	87.1
9	IRS 713	84.5	85.9	94.2	88.7	88.4
1	untreated control	92.4	91.9	91.9	91.5	91.9
	LSD[1] 5%					4.89
	P[2]					<0.001
	significance[3]					VS

Assessment Percentage of plants
Date of assessment 23-4-2012

	treatment	A	B	C	D	average
1	untreated control	79.0	92.4	91.9	87.8	87.8
2	IRS 657	78.1	88.7	88.2	89.6	86.2
3	IRS 657 + IRS 708	86.4	93.3	94.7	88.2	90.7
4	IRS 657 + barley	84.5	73.5	78.1	81.3	79.3
5	IRS 709	93.3	91.5	94.7	94.7	93.6
6	IRS 710	93.8	93.3	97.9	94.7	94.9
7	IRS 710 + IRS 711	93.3	95.2	89.6	93.8	93.0
8	IRS 712	85.5	90.1	83.2	85.0	85.9
9	IRS 713	84.1	85.0	94.7	89.2	88.2
1	untreated control	91.5	91.5	91.0	94.7	92.2
	LSD[1] 5%					5.674
	P[2]					<0.001
	significance[3]					VS

[1] LSD = least significant difference.
[2] P = probability.
[3] VS = very significant.

Assessment	Percentage of plants
Date of assessment	9-5-2012

	treatment	A	B	C	D	average
1	untreated control	67.5	86.4	85.9	79.5	79.8
2	IRS 657	79.0	89.6	89.2	85.0	85.7
3	IRS 657 + IRS 708	84.5	92.9	91.9	85.9	88.8
4	IRS 657 + barley	84.5	71.1	79.5	78.5	78.4
5	IRS 709	91.0	91.0	94.2	94.7	92.7
6	IRS 710	93.3	85.9	94.7	94.7	92.2
7	IRS 710 + IRS 711	89.6	94.2	91.5	92.9	92.1
8	IRS 712	80.9	87.8	79.0	88.7	84.1
9	IRS 713	78.5	82.7	91.0	85.0	84.3
1	untreated control	89.6	87.3	84.1	83.6	86.2
	LSD[1] 5%					7.184
	P[2]					0.002
	significance[3]					S

Assessment	Percentage of plants
Date of assessment	22-5-2012

	treatment	A	B	C	D	average
1	untreated control	66.1	86.9	85.5	83.2	80.4
2	IRS 657	79.5	90.1	88.7	86.4	86.2
3	IRS 657 + IRS 708	85.5	93.3	93.8	85.9	89.6
4	IRS 657 + barley	85.0	73.0	80.4	79.9	79.6
5	IRS 709	91.9	91.9	94.7	95.2	93.4
6	IRS 710	93.3	87.3	95.6	95.2	92.9
7	IRS 710 + IRS 711	90.6	94.2	92.9	93.3	92.7
8	IRS 712	82.7	87.8	79.9	89.6	85.0
9	IRS 713	79.5	82.7	93.3	87.8	85.8
1	untreated control	89.2	87.8	85.0	85.0	86.7
	LSD[1] 5%					7.164
	P[2]					0.002
	significance[3]					S

[1] LSD = least significant difference.
[2] P = probability.
[3] S = Significant.

Annex C6 Weather conditions

Trial field code: B12PISXH
Name of trial field: Leatherjackets, Xhendremael

Table. Conditions at sowing on 23 March (Air).

date and time	T (°C)	relative humidity (%)
23-03-2012 11:06	15.5	57
23-03-2012 11:11	15.6	57
23-03-2012 11:16	15.7	57
23-03-2012 11:21	15.9	57
23-03-2012 11:26	16.1	58
23-03-2012 11:31	16.2	58
23-03-2012 11:36	16.4	57
23-03-2012 11:41	17.6	55
23-03-2012 11:46	20.4	54
23-03-2012 11:51	21.7	49
23-03-2012 11:56	23.1	40
23-03-2012 12:01	23.2	44
23-03-2012 12:06	23.3	49
23-03-2012 12:11	23.7	53
23-03-2012 12:16	22.8	55
23-03-2012 12:21	22.2	59
23-03-2012 12:26	21.4	62

% soil humidity at time of sowing: 16.7.
Coarse sowing bed of good quality.

Table. Soil temperature at sowing.

date and time	T (°C)
23-03-2012 11:14	7.8
23-03-2012 11:19	7.9
23-03-2012 11:24	7.9
23-03-2012 11:29	8.0
23-03-2012 11:34	8.0
23-03-2012 11:39	8.1
23-03-2012 11:44	8.2
23-03-2012 11:49	8.2
23-03-2012 11:54	8.3
23-03-2012 11:59	8.3
23-03-2012 12:04	8.4
23-03-2012 12:09	8.4
23-03-2012 12:14	8.5
23-03-2012 12:19	8.5
23-03-2012 12:24	8.6

Table. Spray conditions (also specified in section 2.4).

date and time	T (°C)	relative humidity (%)
03-04-2012 07:56	8.8	82
03-04-2012 08:01	9.1	78
03-04-2012 08:06	9.3	75
03-04-2012 08:11	9.5	78
03-04-2012 08:16	10.4	89
03-04-2012 08:21	11.6	89
03-04-2012 08:26	12.6	86

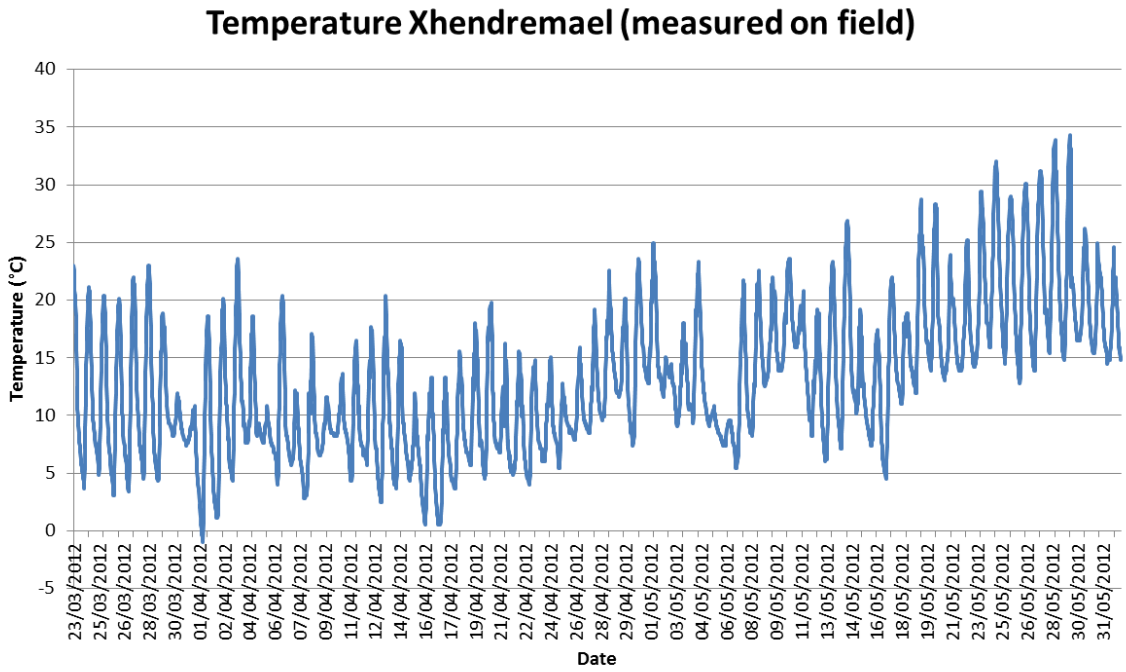


Figure. Temperature measured on field during trial period.

Table. Pluviometry (mm) measured on field during trial period.

Date	pluviometer (mm)
23-03-2012	0
03-04-2012	0
06-04-2012	0
12-04-2012	20
17-04-2012	8
23-04-2012	10
09-05-2012	38
15-05-2012	4
22-05-2012	14

Annexes D Field trial Noordgouwe

Annex D1 Location

GPS location:
51.67399, 3.96184
N 51 40.439, E 3 57.71
N 51 40 26.4, E 3 57 42.6

Annex D2 Trial scheme

Trial field:	Noordgouwe		
Number of replications:	4		
Nett size (m):	10×2	Gross size (m):	16.5×3

A	B	C	D
11	6	14	7
10	2	1	3
8	13	6	5
2	10	12	4
9	4	11	1
6	7	9	2
3	8	5	13
4	11	10	14
7	3	4	11
5	14	13	8
1	12	3	6
14	9	2	12
13	5	8	10
12	1	7	9
A	B	C	D

Annex D3 Treatments

Trial field code: IRS 12-03-01.02
Name of trial field: Leatherjackets, Noordgouwe
Number of replications: 4
Number of treatments: 14

number	treatment
1	untreated control
2	IRS 657
3	IRS 657 + IRS 708
4	IRS 657 + barley
5	IRS 709
8	IRS 712
9	IRS 713
10	IRS 711
11	IRS 714
12	IRS 715
13	IRS 716 + IRS 717
14	IRS 718
15	IRS 719
16	IRS 720

Annex D4 General data

soil type:	clay soil 3.8% organic matter pH-KCl = 7.3 parts <16 µm = 32% K-value = 33 Pw = 43 mg P_2O_5 per liter of soil $CaCO_3$ = 4.1%
preceding crop:	2011 grass 2010 grass 2009 grass 2008 grass 2007 maize 2006 sugar beet
drilling date:	17 April 2012
variety:	Bever
distance in row:	18.5 cm
distance between rows:	50 cm

Annex D5 Efficacy

Trial field code: IRS 12-03-01.02
Name of trial field: Leatherjackets, Noordgouwe

Assessment Percentage of plants
Date of assessment 2-5-2012

	treatment	A	B	C	D	average
1	untreated control	64.8	78.2	85.1	73.1	75.3
2	IRS 657	79.6	68.5	82.8	71.7	75.6
3	IRS 657 + IRS 708	84.2	81.4	82.8	77.2	81.4
4	IRS 657 + barley	74.0	87.4	77.2	78.2	79.2
5	IRS 709	83.7	*	86.5	74.5	81.6
8	IRS 712	77.2	72.6	74.9	67.5	73.1
9	IRS 713	74.0	75.4	*	79.1	76.2
10	IRS 711	69.8	60.6	62.9	*	64.4
11	IRS 714	80.0	78.2	66.1	83.3	76.9
12	IRS 715	77.2	76.3	77.2	68.5	74.8
13	IRS 716 + IRS 717	80.5	81.9	72.2	74.9	77.4
14	IRS 718	*	72.2	69.8	79.1	73.7
15	IRS 719	72.6	62.4	77.7	65.2	69.5
16	IRS 720	73.1	77.7	78.6	76.3	76.4
	LSD[1] 5%					8.093
	P[2]					0.029
	significance[3]					S

[1] LSD = least significant difference.
[2] P = probability.
[3] S = Significant.
* = not counted

Assessment Percentage of plants
Date of assessment 14-5-2012

	treatment	A	B	C	D	average
1	untreated control	60.6	72.2	83.7	74.9	72.8
2	IRS 657	64.8	59.7	81.4	77.2	70.8
3	IRS 657 + IRS 708	78.2	81.4	83.3	74.5	79.3
4	IRS 657 + barley	74.0	77.7	77.2	78.2	76.8
5	IRS 709	79.1	*	87.4	60.1	75.5
8	IRS 712	72.6	61.5	71.2	60.6	66.5
9	IRS 713	60.1	70.8	*	68.9	66.6
10	IRS 711	77.2	71.2	73.5	*	74.0
11	IRS 714	72.2	73.5	65.2	75.9	71.7
12	IRS 715	70.8	72.6	78.6	67.1	72.3
13	IRS 716 + IRS 717	69.4	76.3	67.5	64.3	69.4
14	IRS 718	*	67.1	68.0	64.3	66.4
15	IRS 719	63.4	58.3	78.2	58.7	64.6
16	IRS 720	65.2	69.8	70.8	75.4	70.3
	LSD[1] 5%					7.785
	P[2]					0.009
	significance[3]					S

[1] LSD = least significant difference.
[2] P = probability.
[3] S = Significant.
* = not counted

Assessment Percentage of plants
Date of assessment 30-5-2012

	treatment	A	B	C	D	average
1	untreated control	61.5	71.7	86.5	83.7	75.9
2	IRS 657	67.1	57.8	79.1	80.5	71.1
3	IRS 657 + IRS 708	87.9	86.0	84.2	77.7	83.9
4	IRS 657 + barley	78.2	91.6	83.3	78.6	82.9
5	IRS 709	81.9	*	91.1	60.1	77.7
8	IRS 712	78.6	61.5	69.8	68.5	69.6
9	IRS 713	61.1	72.2	*	72.6	68.6
10	IRS 711	75.9	78.6	69.8	*	74.8
11	IRS 714	78.6	74.9	65.2	81.9	75.2
12	IRS 715	75.4	75.4	78.2	72.2	75.3
13	IRS 716 + IRS 717	79.1	77.2	71.2	67.5	73.8
14	IRS 718	*	74.5	69.8	71.2	71.8
15	IRS 719	69.8	59.2	76.8	62.4	67.1
16	IRS 720	60.6	76.3	70.8	77.7	71.3
	LSD[1] 5%					9.478
	P[2]					0.015
	significance[3]					S

[1] LSD = least significant difference.
[2] P = probability.
[3] S = Significant.
* = not counted

Annexes E Field trial Ens

Annex E1 Location

GPS location:
52.64700, 5.85704
N 52 38.82, E 5 51.422
N 52 38 49.2, E 5 51 25.3

Annex E2 Trial scheme

Trial field:	Ens		
Number of replications:	4		
Nett size (m):	10×2	Gross size (m):	16.5×3

A	B	C	D
7	**1**	**14**	**8**
2	**3**	**6**	**13**
10	**7**	**5**	**9**
8	**6**	**1**	**11**
12	**5**	**3**	**14**
4	**13**	**11**	**10**
5	**2**	**7**	**4**
3	**14**	**9**	**12**
9	**8**	**10**	**3**
6	**11**	**13**	**1**
14	**4**	**12**	**7**
1	**10**	**8**	**2**
13	**9**	**4**	**5**
11	**12**	**2**	**6**
A	**B**	**C**	**D**

Annex E3 Treatments

Trial field code: IRS 12-03-01.01
Name of trial field: Leatherjackets, Ens
Number of replications: 4
Number of treatments: 14

number	treatment
1	untreated control
2	IRS 657
3	IRS 657 + IRS 708
4	IRS 657 + barley
5	IRS 709
8	IRS 712
9	IRS 713
10	IRS 711
11	IRS 714
12	IRS 715
13	IRS 716 + IRS 717
14	IRS 718
15	IRS 719
16	IRS 720

Annex E4 General data

soil type:	clay soil 1.5% organic matter parts < 16 µm = 25%
preceding crop:	2011 grass 2010 grass 2009 seed potatoes 2008 onions 2007 grass 2006 grass 2005 sugar beet
drilling date:	16 April 2012
variety:	Bever
distance in row:	18.5 cm
distance between rows:	50 cm

Annex E5 Efficacy

Trial field code: IRS 12-03-01.01
Name of trial field: Leatherjackets, Ens

Assessment Percentage of plants
Date of assessment 1-5-2012

	treatment	A	B	C	D	average
1	untreated control	76.3	72.2	77.2	73.1	74.7
2	IRS 657	74.9	83.3	82.8	84.2	81.3
3	IRS 657 + IRS 708	74.0	77.7	81.4	83.7	79.2
4	IRS 657 + barley	69.8	68.9	79.6	74.5	73.2
5	IRS 709	77.2	74.9	76.3	87.9	79.1
8	IRS 712	72.6	76.8	80.5	72.6	75.6
9	IRS 713	78.6	82.8	79.1	80.5	80.2
10	IRS 711	71.2	72.2	74.0	82.8	75.0
11	IRS 714	74.5	73.5	78.6	81.9	77.1
12	IRS 715	77.2	83.3	84.6	74.9	80.0
13	IRS 716 + IRS 717	73.5	80.0	70.8	69.8	73.5
14	IRS 718	75.4	68.5	72.6	60.1	69.1
15	IRS 719	83.7	85.1	80.9	79.6	82.3
16	IRS 720	74.5	80.5	82.3	75.9	78.3
	LSD[1] 5%					6.184
	P[2]					0.005
	significance[3]					S

[1] LSD = least significant difference.
[2] P = probability.
[3] S = Significant.

Assessment	Percentage of plants
Date of assessment	16-5-2012

	treatment	A	B	C	D	average
1	untreated control	65.7	59.7	71.7	70.8	66.9
2	IRS 657	75.9	78.2	77.2	80.5	77.9
3	IRS 657 + IRS 708	77.2	80.5	81.4	82.3	80.4
4	IRS 657 + barley	64.8	71.2	78.2	68.9	70.8
5	IRS 709	77.7	67.1	74.0	91.1	77.5
8	IRS 712	64.8	67.5	73.5	63.4	67.3
9	IRS 713	73.5	74.9	73.1	74.9	74.1
10	IRS 711	63.8	65.2	74.0	77.7	70.2
11	IRS 714	67.5	65.2	73.1	78.2	71.0
12	IRS 715	63.8	73.5	69.4	71.7	69.6
13	IRS 716 + IRS 717	63.4	66.1	64.8	62.9	64.3
14	IRS 718	72.2	64.8	64.8	58.7	65.1
15	IRS 719	70.8	68.0	73.1	69.4	70.3
16	IRS 720	62.4	74.9	69.8	66.1	68.3
	LSD[1] 5%					6.886
	P[2]					<0.001
	significance[3]					VS

[1] LSD = least significant difference.
[2] P = probability.
[3] VS = very significant.

Assessment Percentage of plants
Date of assessment 1-6-2012

	treatment	A	B	C	D	average
1	untreated control	73.5	63.4	75.9	73.1	71.5
2	IRS 657	76.3	83.3	78.6	82.3	80.1
3	IRS 657 + IRS 708	80.0	79.1	84.2	82.8	81.5
4	IRS 657 + barley	70.8	75.4	80.9	74.5	75.4
5	IRS 709	76.3	72.2	75.4	87.9	77.9
8	IRS 712	68.5	67.5	71.7	65.7	68.3
9	IRS 713	74.0	80.9	75.9	77.2	77.0
10	IRS 711	69.4	70.3	76.8	83.7	75.0
11	IRS 714	71.2	65.7	75.4	78.2	72.6
12	IRS 715	68.5	80.5	79.6	72.6	75.3
13	IRS 716 + IRS 717	70.8	68.5	76.8	64.8	70.2
14	IRS 718	72.6	68.5	69.4	62.9	68.3
15	IRS 719	76.3	69.4	77.2	72.6	73.9
16	IRS 720	66.1	75.4	73.5	67.1	70.5
	LSD[1] 5%					6.404
	P[2]					0.001
	significance[3]					S

[1] LSD = least significant difference.
[2] P = probability.
[3] S = significant.