IRS

Research on the use of different insecticides for the control of the green peach aphid (Myzus persicae), the vector of beet mild yellowing virus (BMYV) in the Netherlands in 2013

13P02

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 Project: 03-01

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Summary

Virus yellows is an economical important disease in sugar beet. It is caused by Beet Yellowing Virus (BYV) or Beet Mild Yellowing Virus (BMYV). In diagnostic samples sent to the IRS diagnostic service in recent years, only BMYV was detected. This virus is mainly transmitted by the green peach aphid (*Myzus persicae*). It is transmitted in a persistent manner. The spread of the virus in a sugar beet field can be controlled by controlling this aphid species with insecticides. This is common practice for many years now. The insecticides can be applied on the seed pellet or by canopy sprays. In order to investigate new insecticides, research was done on the control of the vector in order to prevent spread of the virus. Different methods were compared with a treatment without insecticide (untreated control). Therefore field trials were conducted in Westmaas and Kortgene. In these trials green peach aphids, infected with BMYV were inoculated twelve and fourteen weeks after sowing (beginning of July).
There was no effect of the insecticides on the number of aphids.
In Kortgene applying IRS 732 resulted in a better control of BMYV than the untreated control, but did not perform better than the other insecticide treatments. There was no significant difference between the way of applying IRS 732. In Westmaas the insecticide treatments did not have a positive effect.
There was no effect of insecticide treatments on sugar yield, sugar content or financial yield due to low expansion of the virus.

Samenvatting

Vergelingsziekte kan veel schade veroorzaken in suikerbieten. Het wordt veroorzaakt door het sterk vergelingsvirus (BYV) en het zwak vergelingsvirus (BMYV), die beide worden overgebracht door de groene perzikbladluis (*Myzus persicae*). De laatste jaren wordt bij de afdeling Diagnostiek van IRS in ingezonden monsters alleen maar BMYV aangetroffen. Dit is een persistent virus. Het virus kan worden aangepakt door de bladluizen te bestrijden en door het gebruik van pillenzaad met insecticiden of met bespuitingen met insecticiden. Doel van dit onderzoek is de effectiviteit bepalen van verschillende soorten insecticiden op pillenzaad of als bespuiting voor de bestrijding van de groene perzikbladluis en daarmee ook het vergelingsvirus.
Daarvoor werden in Westmaas en Kortgene proefvelden aangelegd, waarbij groene perzikbladluizen die vooraf met het virus waren geïnfecteerd, twaalf en ruim veertien weken na zaai (begin juli) werden uitgezet.
Er was geen effect van de insecticiden op het aantal bladluizen.
Alleen op het proefveld in Kortgene had IRS 732 minder planten met BMYV dan het onbehandelde object, maar het was niet beter dan de andere insecticiden. Er was geen verschil in de wijze van toepassing van IRS 732. In Westmaas was er geen effect van behandelingen zichtbaar.
Er was geen effect van de insecticiden op het suikergewicht, het suikergehalte en de financiële opbrengst.

1. Introduction

Virus yellows is an economical important disease in sugar beet. It is caused by the Beet Yellowing Virus (BYV) or Beet Mild Yellowing Virus (BMYV). In diagnostic samples sent to the IRS diagnostic service in recent years, only BMYV was detected. This virus is mainly transmitted by the green peach aphid (*Myzus persicae*) and in a persistent manner. The spread of the virus in a sugar beet field can be controlled by controlling this aphid species with insecticides. This is common practice since many years.
Insecticides in sugar beet can be applied on the seed pellet or by canopy sprays. In order to investigate new insecticides, research was done to control the vector in order to prevent spread of the virus.

2. Materials and methods

2.1 Trial sites

Two locations were selected for these trials: one field in Westmaas and one in Kortgene. These locations are within regions where virus yellows is known to cause damage.

2.2 List of products

In table 1 the products used, type and moment of treatment in the trials are listed. Seeds of all objects (also the untreated controls) were treated with the fungicides Proseed (6.5 g/unit thiram) and Tachigaren (14 g/unit hymexazol) to prevent influences of fungi on plant establishment. With HPLC the dosages of fungicides and insecticides were checked and were in agreement with the target dosages. For insecticides, which are not available in sugar beet in the Netherlands at the moment of writing this report, IRS codes were used.

Table 1. Products used and way of treatment.

treatment	product	target dosage	way of applying
1	untreated control	-	-
2	Poncho Beta	45 g clothianidine + 6 g beta-cyfluthrin	seed treatment
3	Sombrero	60 g imidacloprid	seed treatment
4	IRS 688	60 + 8 g	seed treatment
5	IRS 689	45 + 6 g	seed treatment
6	Calypso before inoculation	0.15 l/ha	spraying
7	IRS 732 before inoculation	0.140 kg/ha	spraying
8	IRS 732 after inoculation	0.140 kg/ha	spraying
9	IRS 732 after inoculation + adjuvant*	0.140 kg/ha	spraying
10	Poncho Beta – not inoculated	45 g clothianidine + 6 g beta-cyfluthrin	seed treatment

*At the field trial in Kortgene 1.0 l/ha adjuvant is applied and in Westmaas 0.5 l/ha.

2.3 Drilling

Drilling was done with a standard precision sowing machine (Monozentra). This sowing machine is equipped with a system to change seed batches quickly. Sowing distance within the rows was 18.5 cm and 50 cm between rows in both trials. The field trials of Westmaas and Kortgene were sown on 8 April and 28 March 2013, respectively. All trials were designed as randomised blocks in four replications. Gross plot size: six rows (3 m) width and 15.5 meters long. Nett plot size: six rows (3 m) × 12 meters.

2.4 Inoculation with aphids

In autumn 2012 sugar beets with confirmed infection of BMYV were taken from an artificial inoculated field in Colijnsplaat, defoliated and stored in a refrigerator at 6^{o}C. In February 2013, these sugar beets were potted in a mixture of sand and potting soil, watered and placed in the laboratory at room temperature to stimulate leaf growth. Meanwhile sugar beets (100 plants) were sown in the climate chambers (23^{o}C for sixteen hours in light (20,000 lux) and 16^{o}C for eight hours in dark). Eleven weeks before inoculation of the plants in the field, green peach aphids were obtained from Koppert Biological Systems in Berkel en Rodenrijs (the Netherlands), where they were cultured on sweet pepper. The aphids were transferred to the naturally infested sugar beet plants from the field in Colijnsplaat for one day. Leaves with aphids were cut off and transferred to young sugar beet plants (8-10th leaf stage). Just before transferring, the plants were taken out of the climate room and transferred to the laboratory at room temperature. The aphids, bearing the virus, were multiplied on those plants.

For field inoculation, leaves with aphids from these plants were cut off and carefully transported to the field trials. It was planned to inoculate the plants about seven to eight weeks after sowing. Due to cold and wet weather and slow multiplication of the aphids on the sugar beet plants, the fields were inoculated later than the original plan. The field trial in Westmaas was inoculated on 1 July (twelve weeks after sowing - BBCH 31 [1]) and the field in Kortgene on 4 July (fourteen weeks and two days after sowing - BBCH 31). Every twentieth plant of the four central rows of treatments 1 to 9 was inoculated with two to four aphids per plant. Plants in treatment 10 were not inoculated.

2.5 Spraying

Treatments 6 (Calypso) and 7 (IRS 732) were sprayed six and three days before inoculation in Westmaas and Kortgene, respectively. Treatments 8 (IRS 732) and 9 (IRS 732 with adjuvant) were sprayed three days and one day after inoculation in Westmaas and Kortgene, respectively. Spraying of the treatments was done with Lechler LU (120:03) nozzles and 300 litres water per hectare in Westmaas and with Teejet (110:04) nozzles and 300 litres water per hectare in Kortgene. About one week after inoculation the whole field trial (including the surrounding sugar beet field) was sprayed with Pirimor (pirimicarb, 0.4 kg/ha) to prevent spread of infected aphids.

2.6 Assessment of efficacy

The effect of the different treatments and formulations on controlling green peach aphids was measured by counting the number of aphids on five randomly chosen infected plants per plot. This was done one week after inoculation, just before the field was sprayed with Pirimor. The effect on BMYV was measured by counting the number of plants and the number of plants with yellowing symptoms per plot about seven (20 August) and eleven weeks (20 and 23 September in Kortgene and Westmaas, respectively) after inoculation. This was done in the four central rows. In addition, the field trial in Westmaas was harvested in order to measure the effect on yield. This was done on 27 September. The gross weight of the plot was measured and of each plot one subsample was taken to the tare house of the IRS and there split in two and separately analysed for sugar beet quality.

2.7 Analysis of data

Data were analysed using Analysis of Variance (ANOVA). Analyses were done with Genstat Software Package 15.0.

3. Results and discussion

3.1 Seed treatments

Seeds treated with several insecticides were used and the dosages were checked with HPLC. In table 2 the applied and detected rates are shown. The detected rates of Poncho Beta were a little bit lower than the applied rates. For Sombrero, IRS 688 and IRS 689 the dosages were a little bit higher. All deviations were within the allowed range.

Table 2. Applied and detected rates of insecticides on the different seed treatments.

product	dosage of insecticides	
	target dosage	detected dosage
untreated control	0	0.0
Poncho Beta	45 g clothianidine + 6 g beta-cyfluthrin	43.7 + 5.7
Sombrero	60 g imidacloprid	63.1
IRS 688	60 + 8 g	62.3 + 8.1
IRS 689	45 + 6 g	47.9 + 6.1

3.2 Effect on number of aphids

At both field trials green peach aphids and black bean aphids (*Aphis fabae*) were observed. The black been aphids were not inoculated, but were naturally occurring. There was no interaction between field trial location and treatments on the number of green peach aphids (Probability (P) = 0.921) and on the black bean aphids (P = 0.412). There was also no effect of treatment on both numbers of green peach aphids (P = 0.212) and black bean aphids (P = 0.456). Data are shown in Annexes B and C. No conclusions can be made, since the number of aphids was very low. This was probably due to the weather and also to the plant age at the moment of inoculation.

3.3 Effect on beet mild yellowing virus (BMYV)

The effect on BMYV was measured on 20 August at both locations and on 20 and 23 September in Kortgene and Westmaas, respectively.

In Westmaas, there was a significant difference between the treatments (P<0.001; Figure 1; Annex B). All inoculated treatments (1 to 9) had a significantly higher percentage of plants with virus yellows in comparison with the not inoculated Poncho Beta (treatment 10). There was no significant difference between the treatments that were inoculated with aphids with BMYV.

Also in Kortgene, there was a significant difference between the treatments (P<0.001; Figure 2; Annex C). Only the treatments with IRS 732 and the not-inoculated treatment with Poncho Beta, performed significantly better than the untreated control on 20 September. There was no significant difference between the three different ways of applying IRS 732 and between IRS 732 and Poncho Beta, Sombrero, IRS 689 and Calypso. On 20 August only IRS 732 after inoculation and the not-inoculated Poncho Beta had a significantly lower percentage of plant with virus yellow symptoms than the untreated control (Annex C).

In both field trials the treatment with Poncho Beta that was not inoculated, had the lowest percentage of plants with virus yellow symptoms. This means that none of the treatments that were inoculated with infested aphids, could prohibit virus transmission from the aphids to the plants. This can probably be explained because some aphids might have been sucking from the sugar beet plants before they died from the insecticides.

Overall, the percentage of plants with yellowing symptoms was low in comparison with the results of 2012 [1]. This might be due to leaf stage. Earlier research showed that sugar beet became less suitable for green peach aphids when plants were at the 10 to 12 leaf stage [2]. This is also one of the reason, why the current advice for spraying against these aphids stops at mid July.

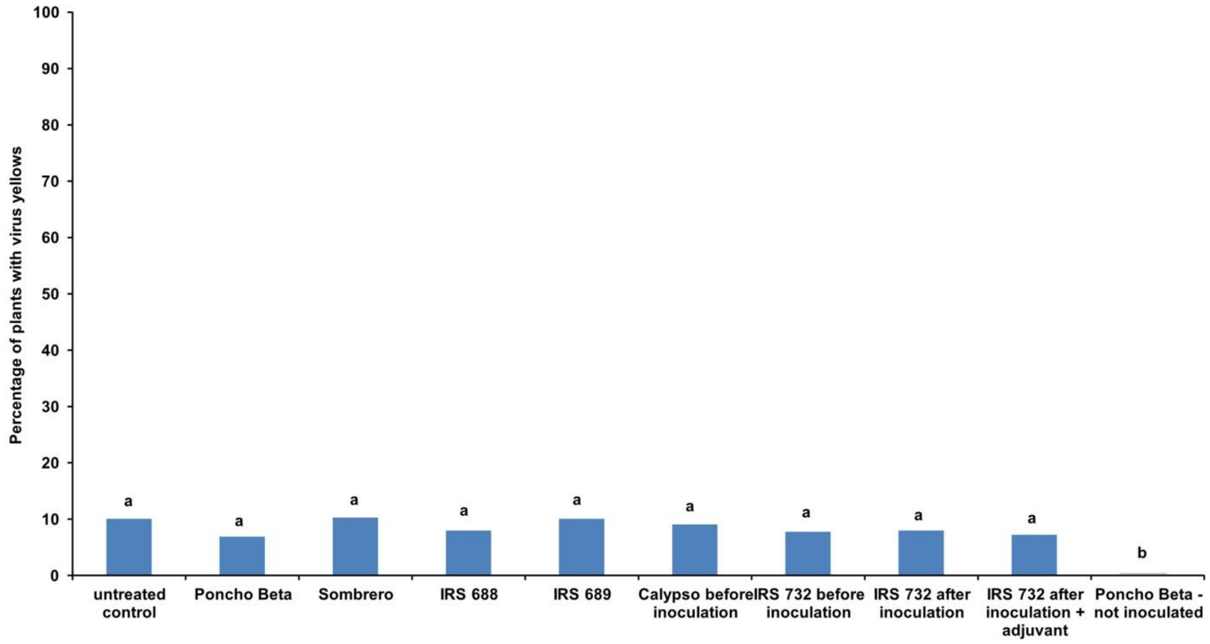


Figure 1. Mean percentage of plants with virus yellows (BMYV) of the field trials in Westmaas (23 September, 2013) (LSD[1] 5% = 3.7; P<0.001).

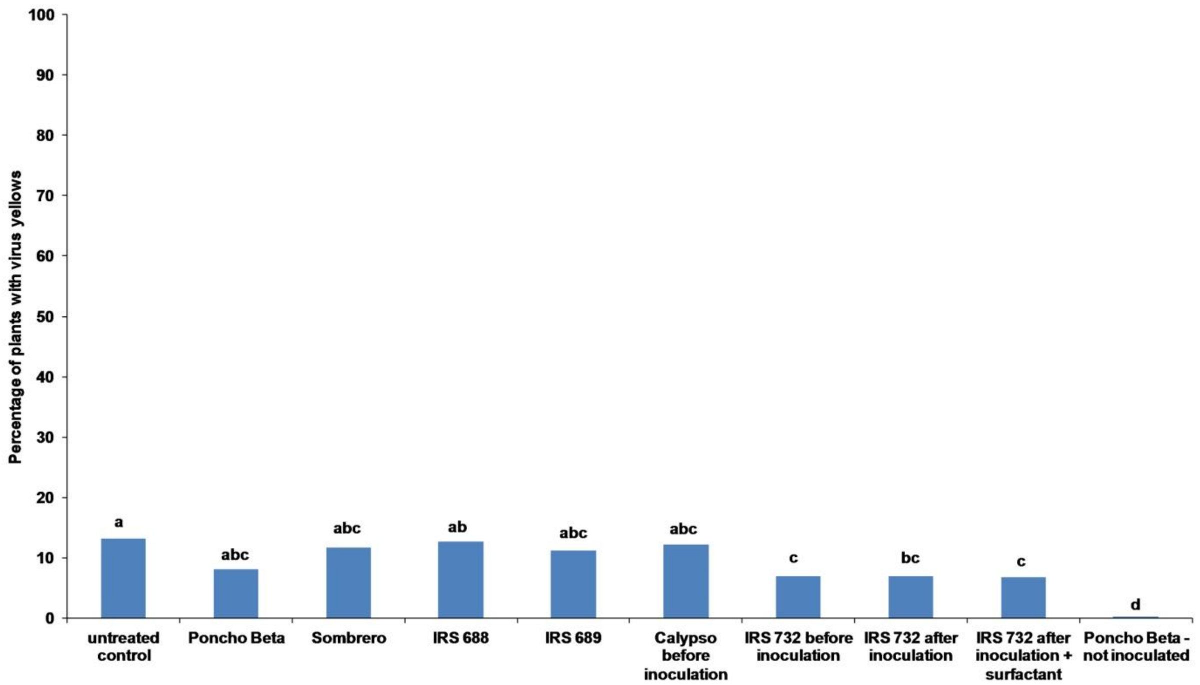


Figure 2. Mean percentage of plants with virus yellows (BMYV) of the field trials in Kortgene (20 September, 2013) (LSD[1] 5% = 5.6; P<0.001).

[1] LSD = least significant difference.

3.4 Effect on yield

There was no effect of treatment on sugar yield (P = 0.546), sugar content (P = 0.44) or financial yield (P = 0.43) at Westmaas (see Annex B). The trial field in Kortgene was not harvested.

4. Conclusions

The aim was to study the efficacy of different insecticides on the control of beet mild yellowing virus (BMYV) and its vector, the green peach aphid (*Myzus persicae*).
There was no effect of the insecticides on the number of aphids.
In Kortgene IRS 732 controlled BMYV better than the untreated control, but not better than the other insecticide treatments. It did not matter how IRS 732 was applied.
There was no effect of insecticide treatments on sugar yield, sugar content or financial yield.

5. Literature

1. Raaijmakers, E.
   Research on the use of different insecticides for the control of the green peach aphid (*Myzus persicae*) (vector of beet mild yellowing virus (BMYV)) in the Netherlands in 2012**.**
   IRS publicatie 12P08, Bergen op Zoom: 28p.

2. Kift, N.B., Dewar, A.M., Dixon, A.F.G.
   Onset of a decline in the quality of sugar beet as a host for the aphid *Myzus persicae*.
   Entomologia Experimentalis et Applicata 1998, 88**:**155-161.

Annex A 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 B Field trial Westmaas

Annex B1 Location

GPS location:
51.79124, 4.44524
N 51 47.474, E 4 26.714
N 51 47 28.5, E 4 26 42.9

Annex B2 Trial scheme

Trial field:	Westmaas		
Number of replications:	4		
Nett size (m):	12 × 3	Gross size (m):	15.5 × 3

D				
10	**2**	**5**	**7**	**1**
9	**4**	**3**	**6**	**8**
2	**5**	**7**	**10**	**9**
1	**3**	**6**	**8**	**4**
4	**9**	**10**	**1**	**2**
3	**6**	**8**	**5**	**7**
5	**7**	**1**	**4**	**3**
8	**10**	**9**	**2**	**6**
A				

Annex B3 Treatments

Trial field code: IRS 13-03-01.03
Name of trial field: Beet mild yellowing virus (BMYV), Westmaas
Number of replications: 4
Number of treatments: 10

number	treatment
1	untreated control
2	Poncho Beta
3	Sombrero
4	IRS 688
5	IRS 689
6	Calypso before inoculation
7	IRS 732 before inoculation
8	IRS 732 after inoculation
9	IRS 732 after inoculation + adjuvant
10	Poncho Beta – not inoculated

Annex B4 General data

soil type:	clay soil	
	organic matter	2.2%
	pH-KCl =	7.3
	parts <16 µm =	30%
	K-value =	22
	Pw =	39 mg P_2O_5 per litre of soil
	$CaCO_3$ =	8.5%
preceding crop:	2012 wheat + oil seed radish	
	2011 potatoes	
	2010 wheat	
	2009 sugar beet	
	2008 wheat	
	2007 potatoes	
drilling date:	8 April 2013	
variety:	Bever	
distance in row:	18.5 cm	
distance between rows:	50 cm	

Annex B5 Efficacy

Trial field code: IRS 13-03-01.03
Name of trial field: Beet Mild Yellowing Virus (BMYV), Westmaas

Assessment Number of green peach aphids/plant
Date of assessment 8 July 2013

number	treatment	A	B	C	D	mean
1	untreated control	0.2	0.0	0.0	0.0	0.1
2	Poncho Beta	0.0	0.0	0.0	0.0	0.0
3	Sombrero	0.4	0.0	0.0	0.0	0.1
4	IRS 688	0.0	0.0	0.0	0.0	0.0
5	IRS 689	0.2	0.0	0.0	0.0	0.1
6	Calypso before inoculation	1.0	0.2	0.0	0.0	0.3
7	IRS 732 before inoculation	0.2	0.4	0.0	0.0	0.2
8	IRS 732 after inoculation	0.0	0.0	0.0	0.4	0.1
9	IRS 732 after inoculation + adjuvant	0.0	0.0	0.0	0.0	0.0
10	Poncho Beta - not inoculated	0.0	0.0	0.0	0.0	0.0
	LSD[1] 5%					0.26
	P[2]					0.377
	significance[3]					NS

Assessment Number of black bean aphids/plant
Date of assessment 8 July 2013

number	treatment	A	B	C	D	mean
1	untreated control	0.2	0.4	0.0	0.0	0.2
2	Poncho Beta	0.0	0.2	0.0	0.0	0.1
3	Sombrero	0.0	0.0	0.0	0.0	0.0
4	IRS 688	2.0	0.0	0.0	0.6	0.7
5	IRS 689	1.2	0.0	0.0	0.0	0.3
6	Calypso before inoculation	0.0	0.0	0.0	0.0	0.0
7	IRS 732 before inoculation	0.0	0.0	2.0	0.0	0.5
8	IRS 732 after inoculation	0.0	0.0	0.0	0.0	0.0
9	IRS 732 after inoculation + adjuvant	0.0	0.2	0.0	0.4	0.2
10	Poncho Beta - not inoculated	0.0	0.0	0.0	0.0	0.0
	LSD[1] 5%					0.72
	P[2]					0.549
	significance[3]					NS

[1] LSD = least significant difference.
[2] P = probability.
[3] NS = not significant.

Assessment	Percentage of plants with virus yellows
Date of assessment	20 August 2013

number	treatment	A	B	C	D	mean
1	untreated control	12	8	11	8	9.7
2	Poncho Beta	7	8	8	4	7.0
3	Sombrero	7	7	9	11	8.7
4	IRS 688	9	10	9	4	7.7
5	IRS 689	7	5	11	10	8.2
6	Calypso before inoculation	14	10	3	8	8.7
7	IRS 732 before inoculation	10	9	5	3	6.6
8	IRS 732 after inoculation	11	6	7	5	7.4
9	IRS 732 after inoculation + adjuvant	7	7	8	6	7.1
10	Poncho Beta - not inoculated	2	1	0	0	0.9
	LSD[1] 5%					3.60
	P[2]					0.003
	significance[3]					S

Assessment	Percentage of plants with virus yellows
Date of assessment	23 September 2012

number	treatment	A	B	C	D	mean
1	untreated control	14	10	10	7	10.0
2	Poncho Beta	10	7	6	4	6.8
3	Sombrero	8	10	11	11	10.2
4	IRS 688	9	8	9	6	7.9
5	IRS 689	12	5	13	11	10.0
6	Calypso before inoculation	8	15	4	8	9.0
7	IRS 732 before inoculation	12	10	4	4	7.7
8	IRS 732 after inoculation	11	6	8	7	7.9
9	IRS 732 after inoculation + adjuvant	7	8	7	7	7.1
10	Poncho Beta - not inoculated	0	0	0	0	0.1
	LSD[1] 5%					3.70
	P[2]					<0.001
	significance[3]					VS

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

Yield
13-03-01.03: Westmaas

Assessment Yield
Date of assessment 27 September 2013

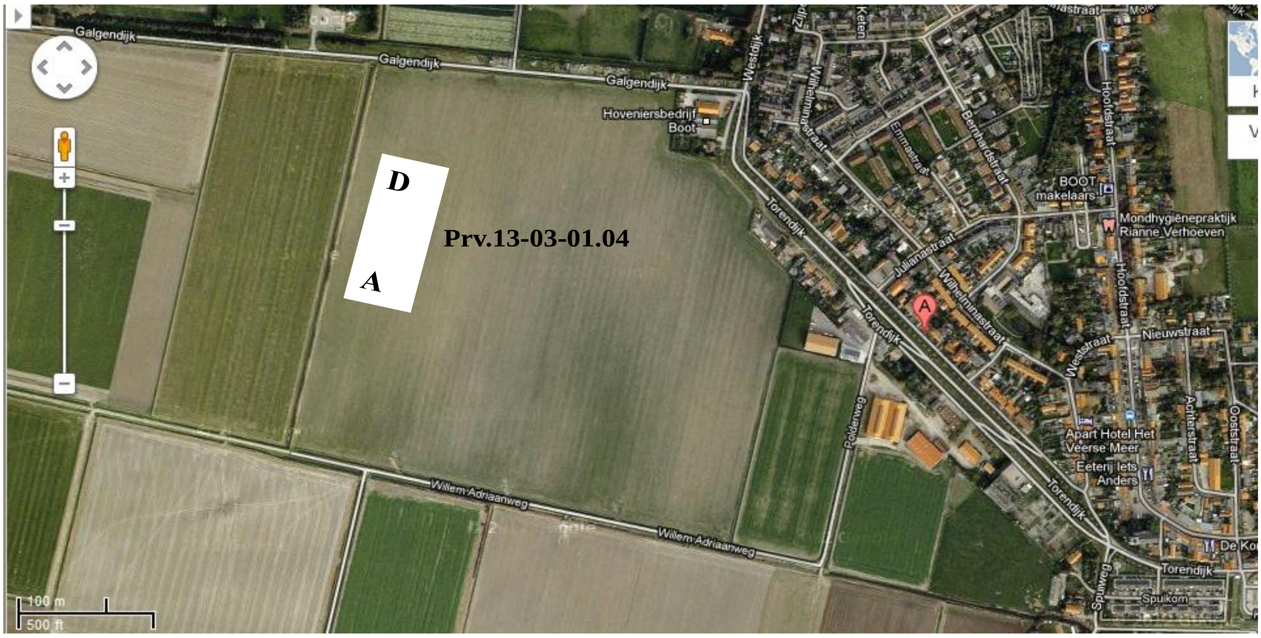
number	treatment	root yield (t/ha)	sugar content (%)	sugar yield (t/ha)	soil tare (%)	financial yield (€/ha)
1	untreated control	96.7	17.2	16.6	7.0	3,894
2	Poncho Beta	96.1	17.2	16.6	6.8	3,893
3	Sombrero	94.5	17.0	16.1	7.2	3,730
4	IRS 688	96.8	17.2	16.6	6.0	3,892
5	IRS 689	96.7	17.4	16.8	7.3	3,948
6	Calypso before inoculation	95.6	16.9	16.1	7.0	3,730
7	IRS 732 before inoculation	96.3	17.2	16.6	6.9	3,890
8	IRS 732 after inoculation	97.2	17.3	16.8	7.0	3,944
9	IRS 732 after inoculation + adjuvant	96.7	17.4	16.8	6.4	3,969
10	Poncho Beta - not inoculated	96.6	17.1	16.5	8.2	3,830
	LSD[1] 5%	3.58	0.47	0.8	2.1	245
	P[2]	0.91	0.44	0.54	0.74	0.43
	significance[3]	NS	NS	NS	NS	NS

1 LSD = least significant difference.
2 P = probability.
3 NS = not significant.

Annexes C Field trial Kortgene

Annex C1 Location

GPS location:
51.55804, 3.89706
N 51 33.482, E 3 47.344
N 51 33 28.9, E 3 47 20.6



Annex C2 Trial scheme

Trial field:	Kortgene		
Number of replications:	4		
Nett size (m):	12×3	Gross size (m):	15.5×3

D				
4	**7**	**3**	**6**	**9**
1	**10**	**5**	**8**	**2**
9	**6**	**4**	**7**	**1**
8	**2**	**10**	**3**	**5**
5	**3**	**8**	**9**	**7**
2	**1**	**6**	**4**	**10**
3	**9**	**7**	**2**	**6**
10	**8**	**1**	**5**	**4**
A				

Annex C3 Treatments

Trial field code: IRS 13-03-01.04
Name of trial field: Beet Mild Yellowing Virus (BMYV), Kortgene
Number of replications: 4
Number of treatments: 7

number	treatment
1	untreated control
2	Poncho Beta
3	Sombrero
4	IRS 688
5	IRS 689
6	Calypso before inoculation
7	IRS 732 before inoculation
8	IRS 732 after inoculation
9	IRS 732 + adjuvant after inoculation
10	Poncho Beta – not inoculated

Annex C4 General data

soil type:	clay soil	
	organic matter	1.4%
	parts <16 µm =	34%
	Pw =	34 mg P_2O_5 per litre of soil
	$CaCO_3$ =	4.1%
preceding crop:	2012 potatoes	
	2011 onions	
	2010 wheat	
	2009 potatoes	
	2008 sugar beet	
	2007 wheat	
drilling date:	28 March 2013	
variety:	Bever	
distance in row:	18.5 cm	
distance between rows:	50 cm	

Annex C5 Efficacy

Trial field code: IRS 13-03-01.04
Name of trial field: Beet mild yellowing virus (BMYV), Kortgene

Assessment: Number of green peach aphids/plant
Date of assessment: 11 July 2013

number	treatment	A	B	C	D	mean
1	untreated control	0.0	0.0	0.0	0.6	0.2
2	Poncho Beta	0.0	0.0	0.0	0.0	0.0
3	Sombrero	0.0	0.0	0.0	0.4	0.1
4	IRS 688	0.0	0.0	0.0	0.0	0.0
5	IRS 689	0.0	0.0	0.0	0.0	0.0
6	Calypso before inoculation	0.0	0.0	0.6	0.0	0.2
7	IRS 732 before inoculation	0.0	0.0	0.0	0.0	0.0
8	IRS 732 after inoculation	0.0	0.0	0.0	0.0	0.0
9	IRS 732 after inoculation + adjuvant	0.0	0.0	0.0	0.0	0.0
10	Poncho Beta - not inoculated	0.0	0.0	0.0	0.0	0.0
	LSD[1] 5%					0.21
	P[2]					0.623
	significance[3]					NS

Assessment: Number of black bean aphids per plant
Date of assessment: 11 July 2013

number	treatment	A	B	C	D	mean
1	untreated control	0.0	0.0	21.0	0.0	5.3
2	Poncho Beta	36.4	0.0	8.8	0.0	11.3
3	Sombrero	11.4	0.0	0.0	0.0	2.9
4	IRS 688	0.0	0.6	0.0	0.0	0.2
5	IRS 689	0.0	17.2	0.0	0.2	4.4
6	Calypso before inoculation	0.0	0.0	0.4	0.0	0.1
7	IRS 732 before inoculation	0.4	0.0	0.0	0.0	0.1
8	IRS 732 after inoculation	0.2	0.0	3.8	0.0	1.0
9	IRS 732 after inoculation + adjuvant	0.0	0.4	0.0	0.2	0.2
10	Poncho Beta - not inoculated	0.0	0.0	0.0	0.0	0.0
	LSD[1] 5%					10.53
	P[2]					0.457
	significance[3]					NS

[1] LSD = least significant difference.
[2] P = probability.
[3] NS = not significant.

Assessment: Percentage of plants with virus yellows
Date of assessment: 20 August 2013

number	treatment	A	B	C	D	mean
1	untreated control	7	6	4	6	5.8
2	Poncho Beta	5	6	4	2	4.1
3	Sombrero	9	6	6	8	7.1
4	IRS 688	6	8	8	7	7.6
5	IRS 689	5	4	7	5	5.1
6	Calypso before inoculation	8	10	7	4	7.2
7	IRS 732 before inoculation	6	4	4	4	4.7
8	IRS 732 after inoculation	5	3	2	5	3.8
9	IRS 732 after inoculation + adjuvant	6	5	3	3	4.1
10	Poncho Beta - not inoculated	1	0	0	0	0.3
	LSD[1] 5%					1.93
	P[2]					<0.001
	significance[3]					VS

Assessment: Percentage of plants with virus yellows
Date of assessment: 20 September 2013

number	treatment	A	B	C	D	mean
1	untreated control	14	10	8	21	13.2
2	Poncho Beta	7	7	10	8	8.1
3	Sombrero	12	13	8	14	11.8
4	IRS 688	12	13	14	11	12.6
5	IRS 689	13	8	11	12	11.2
6	Calypso before inoculation	18	13	13	6	12.3
7	IRS 732 before inoculation	8	7	4	8	6.9
8	IRS 732 after inoculation	8	8	6	7	7.0
9	IRS 732 after inoculation + adjuvant	11	7	4	6	6.9
10	Poncho Beta - not inoculated	0	0	1	0	0.3
	LSD[1] 5%					5.630
	P[2]					<0.001
	significance[3]					VS

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

Annex 4 Weather data

Weather station Rotterdam (about 20 km away from Westmaas)

date	temperature	radiation	precipitation (mm)	evaporation (mm)	relative humidity (%)
01-3-2013	3.6	316	0.1	0.4	84
02-3-2013	3	410	0	0.5	78
03-3-2013	4.3	294	0.1	0.4	84
04-3-2013	5	1262	0	1.6	70
05-3-2013	9.2	1192	0	1.7	56
06-3-2013	9.5	640	0	0.9	70
07-3-2013	9	619	0.7	0.9	76
08-3-2013	9.8	821	3.4	1.2	86
09-3-2013	5.1	164	26.1	0.2	96
10-3-2013	0.4	206	2.4	0.2	80
11-3-2013	-1.7	505	0.1	0.5	70
12-3-2013	-2.2	1053	0	1	67
13-3-2013	0	1192	0.3	1.3	78
14-3-2013	-0.1	1037	0.5	1.1	76
15-3-2013	1.5	442	2.6	0.5	84
16-3-2013	5.4	625	1.1	0.8	64
17-3-2013	6.5	687	1	0.9	78
18-3-2013	5.3	996	1	1.3	84
19-3-2013	3.4	497	1	0.6	88
20-3-2013	1.5	676	0.1	0.8	82
21-3-2013	1.2	697	0.1	0.8	73
22-3-2013	1	1241	0	1.4	76
23-3-2013	0.2	797	0	0.9	56
24-3-2013	-0.4	546	0	0.6	36
25-3-2013	0.5	1610	0	1.7	44
26-3-2013	0.7	1822	0	2	46
27-3-2013	0.6	1831	0	2	54
28-3-2013	0.1	1166	0.1	1.2	68
29-3-2013	-0.7	923	0.1	1	80
30-3-2013	0	784	0.1	0.8	79
31-3-2013	1	904	0.1	1	76
01-4-2013	2.3	1852	0	2.1	64
02-4-2013	3.2	2013	0	2.4	53
03-4-2013	3.2	1248	0	1.5	53
04-4-2013	2.8	663	0.1	0.8	68
05-4-2013	4.1	994	0	1.2	63
06-4-2013	3.7	1508	0	1.8	71
07-4-2013	3	1573	0	1.9	73
08-4-2013	6	1573	0	2.1	56
09-4-2013	6.6	896	0.7	1.2	82

date	temperature	radiation	precipitation (mm)	evaporation (mm)	relative humidity (%)
10-4-2013	5.4	662	0.1	0.9	90
11-4-2013	6.6	435	4.9	0.6	94
12-4-2013	8.1	836	6.3	1.2	87
13-4-2013	9.8	1453	2.5	2.1	76
14-4-2013	16.5	1513	0.3	2.6	72
15-4-2013	11.8	1402	1	2.2	80
16-4-2013	11.6	1058	0	1.6	82
17-4-2013	15.3	1202	0	2	74
18-4-2013	11.2	2308	0	3.5	68
19-4-2013	7.4	1461	0.1	2	84
20-4-2013	6.4	2384	0	3.2	75
21-4-2013	6.4	2154	0	2.9	74
22-4-2013	9	1992	0.1	2.8	76
23-4-2013	10.6	1125	0.8	1.7	82
24-4-2013	12.1	2014	0	3.1	81
25-4-2013	14.5	1586	0.1	2.6	77
26-4-2013	7.1	309	6.6	0.4	90
27-4-2013	5.9	2188	0	2.9	80
28-4-2013	6.5	1901	0	2.5	76
29-4-2013	8.2	1937	1.3	2.7	74
30-4-2013	8.6	1844	0	2.6	77
01-5-2013	10.3	2487	0	3.7	68
02-5-2013	11.1	968	0.1	1.5	67
03-5-2013	11.4	2325	0	3.5	76
04-5-2013	11.6	2556	0	3.9	73
05-5-2013	12.3	2451	0	3.8	76
06-5-2013	13	2461	0	3.9	72
07-5-2013	15.8	1853	0.3	3.1	69
08-5-2013	15.4	1421	7.9	2.4	78
09-5-2013	12.4	2108	0.1	3.3	71
10-5-2013	12.2	1403	0.6	2.2	77
11-5-2013	10.4	1002	5.5	1.5	78
12-5-2013	9.3	1379	3.3	2	82
13-5-2013	11.2	1258	4.9	1.9	78
14-5-2013	9.5	1020	3.7	1.5	80
15-5-2013	11	1826	0.9	2.8	75
16-5-2013	8.5	433	15.1	0.6	90
17-5-2013	8.7	391	15	0.6	94
18-5-2013	9.4	594	0.1	0.9	84
19-5-2013	10.8	2227	0.1	3.3	85
20-5-2013	10.8	340	22.5	0.5	96
21-5-2013	9.5	347	4	0.5	93
22-5-2013	9.2	1443	0.1	2.1	85
23-5-2013	7	1738	1.7	2.3	78

date	temperature	radiation	precipitation (mm)	evaporation (mm)	relative humidity (%)
24-5-2013	7.3	1384	4.4	1.9	81
25-5-2013	8.4	1935	4.4	2.7	85
26-5-2013	9.4	1650	3.1	2.4	82
27-5-2013	12.5	2934	0	4.6	66
28-5-2013	15.2	2593	0	4.3	67
29-5-2013	10.8	508	0.7	0.8	94
30-5-2013	12.9	1283	2	2	82
31-5-2013	12.9	2463	0	3.9	84
01-6-2013	9.8	1131	0	1.7	88
02-6-2013	11	2991	0	4.5	77
03-6-2013	10.3	1624	0	2.4	82
04-6-2013	13.5	2719	0	4.3	76
05-6-2013	15.5	2848	0	4.8	74
06-6-2013	17.4	2810	0	4.9	68
07-6-2013	17.6	2845	0	5	66
08-6-2013	14.4	2905	0	4.7	74
09-6-2013	12.6	1754	0	2.7	71
10-6-2013	12.1	1480	0	2.3	78
11-6-2013	15.3	1881	0	3.1	72
12-6-2013	17.8	1040	1.9	1.8	80
13-6-2013	15.5	1196	2.2	2	82
14-6-2013	15.6	2107	0	3.5	66
15-6-2013	14.9	2451	0.9	4	66
16-6-2013	14.2	2061	0.1	3.3	74
17-6-2013	18	2031	0.1	3.6	72
18-6-2013	21.5	2237	0	4.2	68
19-6-2013	20.1	1281	0.1	2.3	82
20-6-2013	20.1	1112	0.3	2	84
21-6-2013	15.8	506	8.5	0.9	90
22-6-2013	15.6	1023	2.8	1.7	82
23-6-2013	14.7	1389	11.9	2.3	84
24-6-2013	12.7	902	2	1.4	86
25-6-2013	13.2	2672	0	4.2	74
26-6-2013	13	1813	9.8	2.9	76
27-6-2013	13.1	1599	0.7	2.5	78
28-6-2013	14.3	900	10.2	1.5	84
29-6-2013	13.9	1989	0.4	3.2	77
30-6-2013	17.2	2375	0	4.1	76
01-7-2013	15.5	1764	0	2.9	78
02-7-2013	17.2	2024	5.7	3.5	73
03-7-2013	16.2	623	3.8	1.1	90
04-7-2013	17.5	1478	0	2.6	83
05-7-2013	17.5	2388	0	4.2	76
06-7-2013	19.4	2628	0	4.7	76

date	temperature	radiation	precipitation (mm)	evaporation (mm)	relative humidity (%)
07-7-2013	19.9	2889	0	5.2	68
08-7-2013	19.3	2875	0	5.2	72
09-7-2013	18.2	2877	0	5.1	66
10-7-2013	16.3	1973	0	3.3	81
11-7-2013	14.8	1638	0.1	2.7	74
12-7-2013	14	1408	0.1	2.3	86
13-7-2013	16.1	2483	0	4.2	80
14-7-2013	16.9	1911	0	3.3	80
15-7-2013	19.3	2724	0	4.9	72
16-7-2013	19	1910	0	3.4	74
17-7-2013	20.4	2028	0	3.7	73
18-7-2013	21.2	2701	0	5	72
19-7-2013	20.7	2776	0	5.1	70
20-7-2013	18.5	1143	0.1	2	80
21-7-2013	22.7	2658	0	5	70
22-7-2013	24.1	2348	0	4.5	68
23-7-2013	23.4	2555	0	4.9	68
24-7-2013	20.2	1948	0.1	3.6	75
25-7-2013	21.4	2310	0.1	4.3	75
26-7-2013	20.9	1244	5.4	2.3	84
27-7-2013	20.9	1207	24.3	2.2	84
28-7-2013	20.2	1962	0	3.6	75
29-7-2013	20.3	1928	0.3	3.5	72
30-7-2013	18.2	960	4	1.7	80
31-7-2013	20.1	1583	0.1	2.9	79

Weather station Vlissingen (about 15 km away from Kortgene)

date	temperature	radiation	precipitation (mm)	evaporation (mm)	relative humidity (%)
01-3-2013	3.3	391	0.1	0.5	80
02-3-2013	2.9	352	0	0.4	74
03-3-2013	3.9	440	0	0.5	80
04-3-2013	4.9	1296	0	1.6	73
05-3-2013	7.8	1179	0	1.6	64
06-3-2013	10.1	790	0.1	1.2	59
07-3-2013	8.9	508	2.9	0.7	82
08-3-2013	10	636	9.2	0.9	90
09-3-2013	5.1	313	9.1	0.4	92
10-3-2013	1.4	208	2.4	0.2	80
11-3-2013	-1.3	305	0.3	0.3	76
12-3-2013	-1.4	1207	0.1	1.2	63
13-3-2013	1.1	1008	0.2	1.1	74
14-3-2013	1.5	1243	0.1	1.4	70
15-3-2013	2.2	507	1	0.6	81
16-3-2013	5.2	888	0.6	1.1	67
17-3-2013	5.5	835	0.7	1.1	82
18-3-2013	4.6	635	2.1	0.8	84
19-3-2013	3.8	297	0.1	0.4	90
20-3-2013	1.9	382	2.4	0.4	84
21-3-2013	2.6	657	0	0.8	69
22-3-2013	2.3	1065	0	1.2	70
23-3-2013	0.3	344	1.6	0.4	74
24-3-2013	-1.1	457	7.5	0.5	64
25-3-2013	0.5	1171	0	1.3	51
26-3-2013	0.8	1780	0	2	52
27-3-2013	1.3	1760	0	2	56
28-3-2013	0.9	1218	0.1	1.3	63
29-3-2013	0.9	1249	0.1	1.4	71
30-3-2013	1.2	1106	0	1.2	74
31-3-2013	1.8	929	0.1	1.1	70
01-4-2013	3	1828	0	2.2	60
02-4-2013	3.3	1975	0	2.4	52
03-4-2013	3	1211	0	1.4	58
04-4-2013	3.1	1262	0.1	1.5	61
05-4-2013	3.6	590	0	0.7	68
06-4-2013	4.2	1521	0	1.9	66
07-4-2013	4.5	1977	0	2.5	70
08-4-2013	5.9	1584	0	2.1	62
09-4-2013	5.7	753	1.2	1	82

date	temperature	radiation	precipitation (mm)	evaporation (mm)	relative humidity (%)
10-4-2013	5.4	1008	2.3	1.3	88
11-4-2013	6.5	545	5.7	0.7	93
12-4-2013	7.7	1065	3.4	1.5	84
13-4-2013	9	1319	1.9	1.9	76
14-4-2013	12.4	1505	0.2	2.3	74
15-4-2013	10	1294	0.9	1.9	81
16-4-2013	10.1	809	0.1	1.2	84
17-4-2013	11.5	1431	0.1	2.2	76
18-4-2013	9.7	1645	0	2.4	74
19-4-2013	7.4	1752	0.1	2.4	82
20-4-2013	7	2181	0	2.9	72
21-4-2013	7.3	2360	0	3.2	66
22-4-2013	8.1	1786	0	2.5	82
23-4-2013	9.9	1748	1	2.6	86
24-4-2013	9.8	2034	0	3	86
25-4-2013	13.4	1979	0	3.2	75
26-4-2013	7.1	325	3.2	0.4	90
27-4-2013	7	2155	0	2.9	72
28-4-2013	7.3	2157	0	2.9	72
29-4-2013	8.5	1951	0.1	2.8	72
30-4-2013	9.2	1999	0	2.9	70
01-5-2013	10.4	2406	0	3.6	65
02-5-2013	10.7	872	2.6	1.3	74
03-5-2013	11.6	2387	0	3.6	70
04-5-2013	10.6	2231	0.4	3.3	79
05-5-2013	11.5	2585	0	3.9	75
06-5-2013	12.8	2437	0	3.8	69
07-5-2013	14.4	1979	0.1	3.2	70
08-5-2013	12.8	1209	9.1	1.9	84
09-5-2013	11.9	2105	0.1	3.2	69
10-5-2013	11.6	1391	0.1	2.1	80
11-5-2013	10.6	1241	2.5	1.9	80
12-5-2013	9.7	1561	4.2	2.3	82
13-5-2013	11	1483	2.9	2.2	82
14-5-2013	9.9	938	4.5	1.4	82
15-5-2013	11.2	1915	1.2	2.9	72
16-5-2013	9.2	493	5.4	0.7	83
17-5-2013	8.7	461	4.4	0.7	91
18-5-2013	10.6	1254	0	1.9	80
19-5-2013	11.6	2482	0	3.8	82
20-5-2013	10.1	320	13.8	0.5	92
21-5-2013	9.1	396	1.9	0.6	92
22-5-2013	9.6	1417	12.2	2.1	82

date	temperature	radiation	precipitation (mm)	evaporation (mm)	relative humidity (%)
23-5-2013	7.4	1623	4.9	2.2	79
24-5-2013	6.8	789	15.3	1.1	86
25-5-2013	8.8	1818	2.2	2.6	82
26-5-2013	10.7	1820	4.4	2.7	80
27-5-2013	12.5	2906	0	4.5	60
28-5-2013	13.3	1896	7.3	3	73
29-5-2013	10.1	316	5.6	0.5	92
30-5-2013	11.3	979	0.1	1.5	92
31-5-2013	11.5	2465	0	3.8	86
01-6-2013	10	1061	0	1.6	88
02-6-2013	11.5	2922	0	4.5	73
03-6-2013	11	2086	0	3.1	77
04-6-2013	12	2290	0	3.5	82
05-6-2013	14.2	2940	0	4.8	80
06-6-2013	16.2	2820	0	4.8	75
07-6-2013	16.2	2784	0	4.7	77
08-6-2013	13.6	2830	0	4.5	78
09-6-2013	12.2	1789	0	2.8	77
10-6-2013	11.8	1067	0	1.6	74
11-6-2013	15.1	1729	0.1	2.9	74
12-6-2013	16.3	1099	1.5	1.9	86
13-6-2013	14.9	940	0.8	1.6	82
14-6-2013	14.6	2502	0	4.1	77
15-6-2013	14.5	2246	1.5	3.7	72
16-6-2013	14	1775	0.1	2.9	74
17-6-2013	18	1802	0.1	3.2	66
18-6-2013	19.9	2039	0.1	3.7	78
19-6-2013	18	1225	0.7	2.1	80
20-6-2013	19.2	1643	1.3	2.9	84
21-6-2013	15.4	598	10.6	1	91
22-6-2013	15	989	2.7	1.6	82
23-6-2013	14.7	1447	1.2	2.4	84
24-6-2013	12.3	499	3	0.8	84
25-6-2013	13.8	2345	0.4	3.8	74
26-6-2013	14.2	1970	6.1	3.2	72
27-6-2013	13.3	1989	0.1	3.2	77
28-6-2013	14.6	1020	11.6	1.7	88
29-6-2013	14.8	1962	0.3	3.2	74
30-6-2013	16.6	2679	0	4.6	80
01-7-2013	15.4	2048	0	3.4	82
02-7-2013	16.2	1885	9.2	3.2	80
03-7-2013	16	875	7.6	1.5	91
04-7-2013	16.9	1396	0	2.4	84
05-7-2013	16.9	2429	0	4.2	78

date	temperature	radiation	precipitation (mm)	evaporation (mm)	relative humidity (%)
06-7-2013	19.8	2695	0	4.9	76
07-7-2013	20.3	2895	0	5.3	70
08-7-2013	19.4	2814	0	5.1	74
09-7-2013	18.1	2861	0	5	66
10-7-2013	16.6	1946	0.1	3.3	79
11-7-2013	15.1	1774	0	2.9	70
12-7-2013	14.7	913	0	1.5	81
13-7-2013	16.3	2410	0	4.1	78
14-7-2013	18.3	2345	0	4.1	81
15-7-2013	19.1	2838	0	5.1	73
16-7-2013	19.9	2062	0	3.7	72
17-7-2013	20.9	2129	0	3.9	70
18-7-2013	20.2	2706	0	4.9	80
19-7-2013	20.9	2750	0	5.1	68
20-7-2013	18.7	1199	0	2.1	82
21-7-2013	22.5	2737	0	5.2	70
22-7-2013	24.8	2691	0	5.3	60
23-7-2013	23.5	2368	0	4.5	65
24-7-2013	20.4	2142	0.1	3.9	75
25-7-2013	21.5	2443	0.3	4.6	73
26-7-2013	20.9	1546	14.8	2.9	80
27-7-2013	20.8	1200	24.5	2.2	86
28-7-2013	20.6	2171	0	4	74
29-7-2013	19.9	2215	0.1	4	76
30-7-2013	18.1	819	1.2	1.4	83
31-7-2013	19.8	1531	0	2.8	82