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CONFIDENTIAL REPORT

Project number [REDACTED]: I-12-6703
Trial numbers included: I-12-6703-1

CONTROL OF *PEPINO MOSAIC VIRUS* IN TOMATO

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Objective: Evaluation of attenuated isolates of *Pepino mosaic virus* for cross-protection.

Period: December 2012 – May 2013

Performer: [REDACTED]
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Date: August 2013

Project coordinator: [REDACTED]

Project manager: [REDACTED]

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Signature:

Date: 22 August 2013

SUMMARY

In 2012-2013 a project was carried out in The Netherlands to test the efficacy of attenuated isolates of *Pepino mosaic virus* for cross-protection in protected tomato. Because of a cross-contamination in trial I-12-6702-2; in treatment VX1/agEU also agCH was detected, an additional trial was carried out. The project consisted of one trial with two treatments:

Treatment	A1	A2
1	untreated	agEU
2	VX1	agEU

Initially four tomato cultivars were used per treatment but due to contamination of one cultivar with VX1 instead of untreated/agEU, this cultivar was removed.

The first foliar spray application with a mild virus isolate of PepMV was carried out when plants were ± 10 cm high. The following application with a virulent PepMV isolate was conducted with a 5-10 weeks interval. Assessments on virus symptoms and phytotoxicity were conducted weekly till harvest. At harvest fruit symptoms were assessed and yield was measured.

PepMV related symptoms on leaves and fruits were strongly reduced in plants infected with an attenuate virus isolate before treating the plants with an aggressive viral isolate. However, no reduction in flowering, setting of trusses and yield was noticed in plants treated with only the aggressive viral isolate agEU when compared with plants that were treated with one of the attenuated isolates first before treating the plants with the aggressive viral isolate agEU.

Stunting of the plants was observed in the virulent virus control agEU.

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1 INTRODUCTION

Pepino mosaic virus (PepMV) is a member of the genus Potexvirus which infects mainly solanaceous plants, including tomato, potato and tobacco. Pepino mosaic virus was first found in Peru in 1974 on pepino. Since then, the virus was first reported on greenhouse tomato (*Lycopersicon esculentum*) in the Netherlands and United Kingdom in 1999. Based on the PepMV genomic RNA analysis, the North American strains (US genotypes), PepMV-US1 and PepMV-US2, are closely related to each other but they differ from the European (EU tomato genotype), Chilean (CH2 genotype) and Peruvian (LP genotype) strains. PepMV is considered as a highly infectious and readily transmittable and it systemically infects tomato.

Symptoms

PepMV can cause various symptoms in tomato. Reports on the disease severity of infected plants vary from minor to severe depending on the type of PepMV strain, age, vigour and cultivar of tomato plant and climatic/growing conditions. Symptoms are often expressed during fall and winter months when temperatures and light levels (daylight) are minimal. Initial symptoms usually appear 2-3 weeks after infection. Early symptoms are noticeable on the growing terminals (heads) of infected plants with light-green, thin or needle-like leaves and stunted growth. On leaves symptoms appear as yellow angular spots and bubbly areas, mild interveinal chlorosis (yellowing) and leaf distortions such as spindly leaves. Streaks of browning may appear on stems and flowering clusters that may affect the development of flowers and fruits. Fruits sometimes show discoloration of yellow-red mosaic patterns, called marbling and may lead to uneven fruit ripening. Severely affected plants become stunted and distorted.

PepMV is transmitted mechanically, particularly by contact. PepMV is readily transmitted by contaminated tools, workers' hands and clothing. Direct plant-to-plant contact and propagation by grafting can also spread the virus.

In 2012-2013 a project was carried out in The Netherlands in protected tomato to examine whether tomato plants can be protected against PepMV (agEU) by a preceding infection with an attenuated isolate of this virus (VX1). The project was conducted conform GEP (Good Experimental Practice) standards.

One efficacy & crop safety trial was carried out, situated in a [REDACTED] (I-12-6703-1). The trial consisted of two objects: one object with agEU and one object with VX1 followed by agEU.

The purposes of the project were:

- evaluation of mild virus isolate VX1 in tomato for the control of a virulent isolate
- determine crop safety

In chapter 2 the materials and methods used in this project will be explained. The results obtained from the trial will be discussed in chapter 3 and the conclusions drawn are given in chapter 4.

Appendix 1 contains the GEP certificate for recognition of efficacy testing and the BBCH growth stage scale is given in appendix 2. Appendix 3 contains the climatic data. Appendix 4 contains the raw data of the assessments.

2 MATERIALS AND METHODS

2.1 Trial site

One trial was laid out in this project, consisting of two treatments (see Table 1). The treatment with only the virulent isolate agEU was used as virulent virus control.

The plots were laid out with 4 replicates. Each replicate consisted of another tomato cultivar; [REDACTED] which are all common commercial cultivars in the Netherlands. Due to contamination of cultivar Souplesse with VX1 in stead of untreated/agEU, this cultivar was removed. Each block of plants was surrounded by sweet pepper plants (non-host for PepMV). The layout of the trials is given in chapter 3.

2.2 Treatments

The first application with mild virus was conducted at different locations at different time points when the crop was ± 10 cm high. The following application with the virulent virus was conducted with a 5-10 weeks interval. In Table 1 the different virus isolates are given.

Table 1: Treatments, virus isolates and application timings

Treatment	A1	A2
1	untreated	agEU
2	VX1	agEU

Continued Table 1: Treatments, virus isolates and application timings

Virus isolate	
VX1	mild Peruvian strain
agEU	aggressive European strain

2.3 Application details

The equipment used to carry out the first application with mild virus was a high-pressure spraying arm carrying spraying nozzles of type XRTEEJET 11003VK. The amount of spray liquid used was 0.5 L/m². Carborundum was added to the spray solution (except for cultivar Souplesse) to provide enough abrasion to introduce virus into the plant cells. The second application with the virulent isolate was carried out by dipping fingers (with latex gloves) in the virus suspension (sap from the upper leaves of tomato plants infected with virus) and rubbing two leaves on each plant.

Two-three weeks after the first and second application leaf samples (one sample per plant, 10 plants per cultivar) were taken for ELISA to ensure the absence of virus in the control treatment and to check whether the plants were for 100% infected with the mild and the virulent viruses. To confirm the identity of the mild and virulent viruses in the infected plants real-time PCR was performed on mixtures of leaf samples of each plot of 20 plants.

2.4 Assessment details

Assessments on virus symptoms in the apical leaves and foliage were carried out weekly or with a longer interval. Per plot the percentage nettle head, mosaic, yellow spots, leaf necrosis, stem necrosis, leaf distortion and chlorosis was recorded. Furthermore, flowering and setting of trusses was assessed and fruits were evaluated on viral symptoms. Occurrence of phytotoxic symptoms and crop condition was assessed according to the following scale:

Crop safety (10-1):

- 10 no phytotoxic symptoms compared to the untreated control
- 9 symptoms hardly visible
- 8 a small trace of symptoms
- 7 light, but clearly visible symptoms
- 6 light till moderate symptoms
- 5 moderate symptoms
- 4 severe symptoms
- 3 very severe symptoms
- 2 crop almost dead
- 1 crop dead

Crop condition (10-1)

- 10 excellent crop condition,
- 9 very good crop condition,
- 8 good crop condition,
- 7 reasonable crop condition,
- 6 moderate crop condition,
- 5 unsatisfied crop condition,
- 4 bad crop condition,
- 3 very bad crop condition,
- 2 crop nearly dead,
- 1 crop dead

Tomato fruits (in total three trusses per plant) were harvested nine times from the end of January till mid February. On the day of harvest, fruits were rated for the presence of marbling, fruit discolourations, damage and deformations.

A very strict hygiene protocol was implemented to avoid contaminations between treatments. All personnel followed a specific working order to reduce the consequences of accidental contamination between treatments. The uninfected treatment was always visited prior to the other treatment.

2.5 Guidelines

The project was carried out according to the following EPPO guidelines:

- PP 1/135(3) Phytotoxicity assessment.
- PP 1/152(4) Design and analysis of efficacy evaluation trials.
- PP 1/181(4) Conduct and reporting of efficacy evaluation trials.

2.6 Statistical analysis

Data were analyzed statistically by regression analysis (GENSTAT). Values followed by the same letter did not differ significantly ($p=0.05$).

3 TRIAL SITE DETAILS AND RESULTS

In this chapter the following abbreviations are used:

PESINC Pest incidence
PESSEV Pest severity

For the presentation of the results the following descriptions were discussed:

- treatment 1: virulent virus control
- treatment 2: efficacy VX1

3.1 Trial site details I-12-6703-1

Trial location

Location:		GPS north latitude:	52.015
		GPS east longitude:	4.315
Street:			
Province:			
Country:			

Trial lay-out

section 1: untreated/agEU						section 2: VX1/agEU					
PEPPER	1 P	2 M	3 R	-	PEPPER	PEPPER	1 P	2 M	3 R	4 S	PEPPER

P: Plaisance

M: Merlice

R: Roterno

S: Souplesse

-: Souplesse removed due to contamination

Cultural conditions of the trial site

Soil type:	Rockwool		
Crop:	Tomato	Target temperature:	Day: 20°C Night: 18°C
Cultivars:			
Plot size:		Lit:	No

Data on crop and climatic conditions during the applications

Application date ('12)	2311	2611	3011	2812	010213
Application number	A1	A1	A1	A1	A2
Treatments sprayed	2	2	2	2	1-2
Interval (weeks)	-	-	-	-	5-10
Crop conditions					
Crop stage ¹⁾	13-14	13-14	13-14	13-14	61-62
Crop cultivar	Merlice	Roterno	Plaisance	Souplesse	all cultivars
Wetness of foliage	dry	dry	dry	dry	dry
Wetness of soil surface	moist	moist	moist	moist	moist

¹⁾ BBCH growth stage scale, see appendix 2.

Test site maintenance

Applied chemicals during trial period		
Date	Product	Rate
	none	
Irrigation during trial period		
Date	Method	Amount
daily	trickle irrigation	20-2200 ml (2.8 plants/m ²)








3.2 Results I-12-6703-1

All plants inoculated with PepMV were ELISA positive. A cross-contamination occurred in cultivar Souplesse; in treatment untreated/agEU also VX1 was detected. It was decided to remove this cultivar from the trial.

PepMV related symptoms on tomato

The figures for the PepMV related symptoms on the apical leaves and foliage per tomato cultivar are given in Table 3. Furthermore, as soon as fruit setting occurred, assessments on PepMV related symptoms on fruits were carried out.








Table 3: Symptom scores on tomato in trial I-12-6703-1 (n= 20 plants).








Description		Nettle head								
Rating Date		140213		220213		010313		080313		
Rating Type		PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC	
Rating Unit		% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS	
Trt	Treatment	cultivar								
(A1)	(A2)									
untr	agEU		10	100	30	100	30	100	25	100
			4.5	85	40	100	50	100	70	100
			5	100	40	100	50	100	60	100
VX1	agEU		0	0	0	0	3.5	35	15.75	85
			0	0	0	0	9.5	45	22	100
			0	0	0	0	3.5	20	14.5	100
			0	0	0	0	2	20	5.5	25








A1 (2311-2812): mild virus, A2 (010213): aggressive virus

untr: untreated

Continued Table 3: Symptom scores on tomato in trial I-12-6703-1 (n= 20 plants).

Description		Nettle head								
Rating Date		250313		050413		150413		220413		
Rating Type		PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC	
Rating Unit		% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS	
Trt (A1)	Treatment (A2)	cultivar								
untr	agEU		30	100	20	100	3	15	10	100
			50	100	30	100	8.5	25	10	100
			50	100	40	100	8.5	80	10	100
VX1	agEU		2	20	1	10	0	0	0	0
			6.5	50	1.5	15	0	0	0	0
			1.5	15	2	20	1.5	15	0	0
			0.75	10	0.5	5	0	0	0	0








Description		Nettle head		
Rating Date		290413		
Rating Type		PESSEV	PESINC	
Rating Unit		% AREA	%PLANTS	
Trt (A1)	Treatment (A2)	cultivar		
untr	agEU		10	100
			10	100
			10	100
VX1	agEU		0	0
			0	0
			0	0
			0	0








Description		Mosaic								
Rating Date		140213		220213		010313		080313		
Rating Type		PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC	
Rating Unit		% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS	
Trt (A1)	Treatment (A2)	cultivar								
untr	agEU		10	100	40	100	30	100	25	100
			10	100	40	100	50	100	70	100
			5	100	40	100	50	100	60	100
VX1	agEU		0	0	0	0	6	45	17	85
			0	0	1	15	10	30	17.5	55
			0	0	0	0	4.5	35	14.5	100
			0	0	0	0	2.5	25	5.5	25








A1 (2311-2812): mild virus, A2 (010213): aggressive virus

untr: untreated

Continued Table 3: Symptom scores on tomato in trial I-12-6703-1 (n= 20 plants).

Description		cultivar	Mosaic							
Rating Date			250313		050413		150413		220413	
Rating Type			PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC
Rating Unit			% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS
Trt (A1)	Treatment (A2)									
untr	agEU		30	100	20	100	7.5	30	25	100
			50	100	40	100	15.5	50	30	100
			50	100	50	100	16	80	25	100
VX1	agEU		4.75	75	8	75	2	20	0.5	5
			6.5	50	4.5	40	0	0	0.5	5
			3.5	55	9	80	0.5	5	0.5	5
			2	30	2.5	25	0	0	0	0








Description		cultivar	Mosaic	
Rating Date			290413	
Rating Type			PESSEV	PESINC
Rating Unit			% AREA	%PLANTS
Trt (A1)	Treatment (A2)			
untr	agEU		25	100
			30	100
			25	100
VX1	agEU		0.5	5
			0	0
			0.5	5
			0	0








Description		cultivar	Necrosis leaf							
Rating Date			140213		220213		010313		080313	
Rating Type			PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC
Rating Unit			% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS
Trt (A1)	Treatment (A2)									
untr	agEU		1	100	10	100	10	100	10	100
			6.5	100	30	100	40	100	40	100
			5	100	20	100	30	100	40	100
VX1	agEU		0	0	0	0	0	0	0	0
			0	0	0	0	0.265	20	0.5	5
			0	0	0	0	0.005	5	0	0
			0	0	0	0	0.015	15	0.05	5








A1 (2311-2812): mild virus, A2 (010213): aggressive virus

untr: untreated

Continued Table 3: Symptom scores on tomato in trial I-12-6703-1 (n= 20 plants).

Description		Necrosis leaf								
Rating Date		250313		050413		150413		220413		
Rating Type		PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC	
Rating Unit		% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS	
Trt (A1)	Treatment (A2)	cultivar								
untr	agEU		10	100	20	100	25	100	25	100
			40	100	40	100	50	100	50	100
			50	100	60	100	50	100	50	100
VX1	agEU		0	0	0	0	0	0	0	0
			0.25	5	1.2	30	1.3	25	1.25	20
			0	0	0.1	10	0	0	0	0
			0	0	0	0	0.05	5	0	0








Description		Necrosis leaf		
Rating Date			290413	
Rating Type			PESSEV	PESINC
Rating Unit			% AREA	%PLANTS
Trt (A1)	Treatment (A2)	cultivar		
untr	agEU		5	100
			10	100
			25	100
VX1	agEU		0	0
			1	20
			0	0
			0	0

Description		Necrosis stem								
Rating Date		140213		220213		010313		080313		
Rating Type		PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC	
Rating Unit		% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS	
Trt	Treatment	cultivar								
(A1)	(A2)									
untr	agEU		2.75	30	3	55	4.75	50	5	100
			6.75	95	9	85	14	100	25	100
			10.5	90	12	100	13.75	95	25	100
VX1	agEU		0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0








A1 (2311-2812): mild virus, A2 (010213): aggressive virus

untr: untreated

Continued Table 3: Symptom scores on tomato in trial I-12-6703-1 (n= 20 plants).

Description		Necrosis stem								
Rating Date		250313		050413		150413		220413		
Rating Type		PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC	
Rating Unit		% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS	
Trt (A1)	Treatment (A2)	cultivar								
untr	agEU		5	100	5	100	5	100	5	100
			10	100	10	100	10	100	10	100
			5	100	10	100	10	100	10	100
VX1	agEU		0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0








Description		Necrosis stem		
Rating Date		290413		
Rating Type		PESSEV	PESINC	
Rating Unit		% AREA	%PLANTS	
Trt (A1)	Treatment (A2)	cultivar		
untr	agEU	<div><div></div></div>	5	100
		<div><div></div></div>	10	100
		<div><div></div></div>	10	100
VX1	agEU	<div><div></div></div>	0	0
		<div><div></div></div>	0	0
		<div><div></div></div>	0	0
		<div><div></div></div>	0	0

Description		Yellow spot								
Rating Date		140213		220213		010313		080313		
Rating Type		PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC	
Rating Unit		% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS	
Trt	Treatment	cultivar								
(A1)	(A2)									
untr	agEU		0.015	15	0.09	25	0.28	35	0.03	30
			0	0	0.03	30	0.02	20	0.035	15
			0.25	5	0.055	10	0	0	0.005	5
VX1	agEU		0.005	5	0	0	0	0	0	0
			0.01	10	0.015	15	0.02	20	0.01	10
			0	0	0.005	5	0	0	0	0
			0	0	0.005	5	0	0	0	0








A1 (2311-2812): mild virus, A2 (010213): aggressive virus

untr: untreated

Continued Table 3: Symptom scores on tomato in trial I-12-6703-1 (n= 20 plants).

Description		Yellow spots							
Rating Date		250313		050413		150413		220413	
Rating Type		PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC
Rating Unit		% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS
Trt (A1)	Treatment (A2)	cultivar							
untr	agEU		0.03	30	0	0	0	0	100
			0.015	15	0.005	5	0.005	5	100
			0.005	5	0.03	10	0.005	5	100
VX1	agEU		0	0	0.01	10	0.015	15	0
			0.01	10	0.005	5	0.005	5	0
			0.005	5	0	0	0.005	5	0.01
			0.02	20	0.015	15	0	0.015	15








Description		Yellow spots		
Rating Date		290413		
Rating Type		PESSEV	PESINC	
Rating Unit		% AREA	%PLANTS	
Trt (A1)	Treatment (A2)	cultivar		
untr	agEU	<div></div>	10	100
		<div></div>	25	100
		<div></div>	0	0
VX1	agEU	<div></div>	0	0
		<div></div>	0	0
		<div></div>	0.01	10
		<div></div>	0.015	15








Description		Chlorosis							
Rating Date		140213		220213		010313		080313	
Rating Type		PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC
Rating Unit		% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS
Trt (A1)	Treatment (A2)	cultivar							
untr	agEU		0	0	0	0	0	0	0
			0	0	0	0	0	0	0
			0	0	0	0	0	0	0
VX1	agEU		0	0	0	0	0	0	0
			0	0	0	0	0	0	0
			0	0	0	0	0	0	0
			0	0	0	0	0	0	0

A1 (2311-2812): mild virus, A2 (010213): aggressive virus

untr: untreated

Continued Table 3: Symptom scores on tomato in trial I-12-6703-1 (n= 20 plants).

Description		cultivar	Chlorosis							
Rating Date			250313		050413		150413		220413	
Rating Type			PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC	PESSEV	PESINC
Rating Unit			% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS	% AREA	%PLANTS
Trt (A1)	Treatment (A2)									
untr	agEU		0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0
VX1	agEU		0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0
			0	0	0	0	0	0	0	0

Description		cultivar	Chlorosis	
Rating Date			290413	
Rating Type			PESSEV	PESINC
Rating Unit			% AREA	%PLANTS
Trt (A1)	Treatment (A2)			
untr	agEU		0	0
			0	0
			0	0
VX1	agEU		0	0
			0	0
			0	0
			0	0

A1 (2311-2812): mild virus, A2 (010213): aggressive virus

untr: untreated

As shown in Table 3, plants which were only inoculated with the aggressive virus (agEU) showed more severe viral symptoms than plants which were first inoculated with the attenuate virus (VX1).

Plants which were inoculated with the attenuate isolate of PepMV (VX1) followed by the aggressive isolate showed symptoms of nettle head and mosaic on the apical leaves from two till ten weeks after the inoculation. Also mild symptoms of necrosis (leaf and stem) and yellow spots were observed on a limited number of plants. Symptoms became less evident as the growing season progressed. More severe PepMV symptoms were visible in plants that were only treated with the aggressive virus when compared with cross-protected plants. Nettle head, mosaic, necrosis (leaf and stem) and yellow spots were visible and remained visible till the end of the trial. Minor differences in PepMV symptoms occurred between the different tomato cultivars. In Table 4 the average symptom scores (expected values from statistic model which come close to calculated values) throughout the trial are given.

Table 4. Average symptom scores on the apical leaves in trial I-12-6703-1

Trt	Symptom		Nettle head	Mosaic	Necrosis leaf	Necrosis stem
	Rating Type		PESSEV area	PESSEV area	PESSEV area	PESSEV area
	Rating Unit		%	%	%	%
	A1	A2				
1	untr	agEU	24.2 a	29.57 a	24.27 a	9.238 a
2	VX1	agEU	2.83 b	3.75 b	0.19 b	0 a

Trt	Symptom		Yellow spots	Chlorosis
	Rating Type		PESSEV area	PESSEV area
	Rating Unit		%	%
	A1	A2		
1	untr	agEU	0.03591 a	0 a
2	VX1	agEU	0.00556 b	0 a

A1 (2311-2812): mild virus, A2 (010213): aggressive virus

untr: untreated (average of 3 cultivars), VX1 (average of 4 cultivars)

As shown in Table 4, PepMV related symptoms (nettle head, mosaic, necrosis on leaf and stem and yellow spots) were significantly reduced in treatment 2. Cross-protection with mild viruses reduces the leaf symptoms caused by more aggressive isolates.

Assessments on setting and flowering of trusses were carried out twice during the trial. In Table 5a the number of trusses is presented per treatment and Table 5b per tomato cultivar. Furthermore, the increase in flowering and setting of trusses is given (comparison between the number of flowers and setted trusses at two assessment dates). Flowering and setting are expressed in decimal figures. The number represents the trusses that flower or have set. Decimals indicate the part of the youngest truss that flowers or has set.

Table 5a. Flowering and setting of trusses in trial I-12-6703-1

Trt	Date		220213	080313	
	Rating Type		flowering	flowering	increase in flowering
	Rating Unit		NUMBER	NUMBER	NUMBER
	A1	A2			
1	untr	agEU	4.2 a	5.16 a	1.36 a
2	VX1	agEU	4.1 a	5.56 a	1.53 a

Trt	Date		220213	080313	
	Rating Type		setting	setting	increase in setting
	Rating Unit		NUMBER	NUMBER	NUMBER
	A1	A2			
1	untr	agEU	3.5 a	4.9 a	1.51 a
2	VX1	agEU	3.5 a	5.0 a	1.48 a

A1 (2311-2812): mild virus, A2 (010213): aggressive virus

untr: untreated (average of 3 cultivars), VX1 (average of 4 cultivars)

Table 5b. Flowering and setting of trusses per tomato cultivar in trial I-12-6703-1

Date	220213		080313		
Rating Type	flowering		flowering		increase in flowering
Cultivar	NUMBER		NUMBER		NUMBER
██████	4.0	b	5.53	ab	1.56 a
██████	4.2	ab	5.36	b	1.16 b
██████	4.4	a	5.90	a	1.49 a
██████	3.7	b	5.49	ab	1.77 a
	220213		080313		
	setting		setting		increase in setting
Cultivar	NUMBER		NUMBER		NUMBER
██████	3.35	a	4.9	a	2 a
██████	3.66	a	5.0	a	1 a
██████	3.73	a	5.1	a	1 a
██████	3.18	a	4.6	a	2 a

As shown in Table 5a, the increase in flowering and setted trusses in treatment 1 (-/agEU) was not significantly different from treatment 2 (VX1/agEU). The increase in flowering was in cultivar Plaisance the lowest (significant) and in cultivar Souplesse the highest (see table 5b). No differences in setting of trusses was noticed.

Fruit symptoms

As soon as fruits had developed, assessments were carried out on PepMV related symptoms. Figures for the average percentage of damaged fruits are presented in Table 6a. Fruit damage per tomato cultivar is presented in Table 6b.

Table 6a. PepMV related symptoms on fruits in trial I-12-6703-1

Trt	Symptoms		blotchy		marble		damaged fruits		deformed	
	Rating Type	Rating Unit	fruits	%	fruits	%	fruits	%	fruits	%
1	untr	agEU	20	a	1.4	a	23.9	a	19.8	a
2	VX1	agEU	2	b	0.0	b	3.9	b	2.5	b

Trt	Symptoms		open		damage calyx		cancker	
	Rating Type	Rating Unit	fruits	%	(brown)	%	fruits	%
1	untr	agEU	0.3	a	67	a	0.0	a
2	VX1	agEU	0.2	a	0	b	0.0	a

A1 (2311-2812): mild virus, A2 (010213): aggressive virus

untr: untreated (average of 3 cultivars), VX1 (average of 4 cultivars)

As shown in Table 6a, the percentage of blotchy fruits, the number of fruits with a damaged calyx and damaged and deformed fruits was strongly reduced in treatment 2 when compared with treatment 1. None of the fruits was marbled in treatment 2 and a limited number was open in treatment 2 as well as in treatment 1.

Table 6b. PepMV related symptoms on fruits per tomato cultivar in trial I-12-6703-1

Symptoms Rating Type Cultivar	blotchy % fruits		marble % fruits		damaged fruits fruits %		deformed % fruits	
██████	10	a	0.49	c	11.3	a	8.9	a
██████	12	a	0.27	d	13.8	a	13.7	a
██████	8	a	1.08	a	11.8	a	7.4	a
██████	8	a	0.54	b	15.8	a	5.4	a

Symptoms Rating Type Cultivar	open % fruits		damage calyx % fruits		canker % fruits	
██████	0.1	a	29.1	a	0.0	a
██████	0.5	a	42.3	a	0.0	a
██████	0.1	a	31.4	a	0.0	a
██████	0.0	a	0.0	a	0.0	a

Fruit symptoms hardly differ between the different tomato cultivars. The percentage of marbled fruits was in cultivar Roterno the highest, followed by Souplesse. Merlice and Plaisance had the lowest percentage of marbled fruits.

Yield

Assessments on yield were carried out by counting and weighing the number of harvested fruits. Figures are presented in Table 7a. In Table 7b, yield per tomato cultivar is presented.

Table 7a. Total number and weight of fruits (n= 20 plants) in trial I-12-6703-1

Trt	Rating Type Rating Unit		YIELD NUMBER total		WEIGHT kg		AVG fruit weight g	
	A1	A2						
1	untr	agEU	337	a	30	a	89.8	a
2	VX1	agEU	321	a	27	a	85.0	a

A1 (2311-2812): mild virus, A2 (010213): aggressive virus

untr: untreated (average of 3 cultivars), VX1 (average of 4 cultivars)

The number of fruits as well as the weight of the harvested fruits in plants treated with the attenuate virus (treatment 2) and the viral control (treatment 1) was comparable.

Table 7b. Total number and weight of fruits per tomato cultivar (n= 20 plants) in trial I-12-6703-1

Rating Type Rating Unit	YIELD NUMBER total		WEIGHT kg		AVG fruit weight g	
██████	341	a	32	a	93	a
██████	310	a	29	a	95	a
██████	337	a	24	a	71	a
██████	323	a	30	a	92	a

Numbers are based on two treatments, except for Souplesse, where only treatment 2 was included.

The yield and weight of all tomato cultivars was comparable.

Crop safety

Some stunting was observed during the trial. Figures are presented in Table 8.

Table 8. Average phytotoxicity symptoms (%) in trial I-12-6703-1

Trt	Symptom Rating Type		Stunting %
	A1	A2	
1	untr	agEU	41.5 a
2	VX1	agEU	0.8 b

A1 (2311-2812): mild virus, A2 (010213): aggressive virus

untr: untreated (average of 3 cultivars), VX1 (average of 4 cultivars)

Significantly more stunting occurred in treatment 1 (-/agEU) when compared with treatment 2 (VX1/agEU).

Crop condition

Figures on crop condition are presented in Table 9.

Table 9. Crop condition (10-1) in trial I-12-6703-1

Trt	Description Rating Date		Vigor						
	A1	A2	140213	220213	010313	080313	250313	050413	150413
1	untr	agEU	5.0	5.0	5.0	5.0	4.3	5.0	5.0
2	VX1	agEU	9.0	9.0	7.5	7.4	7.9	7.5	8.0

Trt	Description Rating Date		Vigor	
	A1	A2	220413	290413
1	untr	agEU	4.7	5.2
2	VX1	agEU	8.0	8.0

A1 (2311-2812): mild virus, A2 (010213): aggressive virus

untr: untreated (average of 3 cultivars), VX1 (average of 4 cultivars)

Crop condition in treatment 1 with a single infection of agEU + agCH became quite worse due to severe symptoms of necrosis in the apical leaves and nettle head. Crop condition in all other treatments was moderate to reasonable but recovered and was finally good (score 8).

Presence of PepMV

By means of real-time PCR the presence of the correct viral strains after the second application was determined. The presence of the mild variants and the presence of the challenge isolates in the 'cross-protection' treatments was confirmed. An accidental contamination with VX1 occurred in cultivar Souplesse in the treatment where only agEU was introduced.

4 CONCLUSIONS

In 2012-2013 a project was carried out in The Netherlands to test the efficacy of attenuated isolates of *Pepino mosaic virus* for cross-protection in protected tomato. Because of a cross-contamination in trial I-12-6702-2; in treatment VX1/agEU also agCH was detected, an additional trial was carried out. The project consisted of one trial with two treatments: untreated/agEU and VX1/agEU.

PepMV related symptoms on leaves and fruits were strongly reduced in plants infected with an attenuate virus isolate before treating the plants with an aggressive viral isolate. However, no reduction in flowering, setting of trusses and yield was noticed in plants treated with only the aggressive viral isolate agEU when compared with plants that were treated with the attenuated isolate first before treating the plants with the aggressive viral isolate agEU.

Stunting of the plants was observed in the virulent virus control agEU.

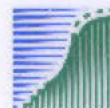
5 TEST FACILITY [REDACTED]

[REDACTED] is officially recognised as an organisation for efficacy testing (see appendix 3) as has been laid down in the “Regulation Crop Protection Products and Biocides” of September 26, 2007.

All raw data of this project will be stored at [REDACTED]. The archived data and documents will be retained for a period of 10 years. Thereafter the sponsor will be contacted. No archived material will be disposed of without the permission of the sponsor.

APPENDIX 1 GEP CERTIFICATE

Ministerie van
Landbouw, Natuur en Voedselkwaliteit



landbouw, natuur en
voedselkwaliteit

This is to declare that, in conformity with the request of November 22, 2007



HAS OFFICIALLY BEEN RECOGNISED AS AN ORGANISATION FOR EFFICACY TESTING
on January 17, 2008

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 January 22, 2008 and expire on January 22, 2014

Wageningen, January 17, 2008

For the Minister of Agriculture,
Nature and Food Quality,



APPENDIX 2 BBCH GROWTH STAGE SCALE

Code	Description
0	Germination, sprouting, bud development
00	Dry seed
01	Beginning of seed imbibition
03	Seed imbibition complete
05	Radicle (root) emerged from seed
06	Elongation of radicle, form. of root hairs
07	Hypocotyl breaking through seed coat
08	Hypocotyl growing towards soil surface
09	Emergence: cotyl. break through soil surf.
1	Leaf development (main shoot)
10	Cotyledons completely unfolded
11	1 true leaf, leaf pair or whorl unfolded
12	2 true leaves, leaf pairs or whorls unfolded
13	3 true leaves, leaf pairs or whorls unfolded
14	4 true leaves, leaf pairs or whorls unfolded
15	5 true leaves, leaf pairs or whorls unfolded
16	6 true leaves, leaf pairs or whorls unfolded
17	7 true leaves, leaf pairs or whorls unfolded
18	8 true leaves, leaf pairs or whorls unfolded
19	9 or m. true leaves, l. pairs, whorls unfolded
2	Formation of side shoots
21	First side shoot visible
22	2 side shoots visible
23	3 side shoots visible
24	4 side shoots visible
25	5 side shoots visible
26	6 side shoots visible
27	7 side shoots visible
28	8 side shoots visible
29	9 or more side shoots visible
3	Stem elong. or rosette growth, shoot dev.
31	Stem (rosette) 10% of final length (diam.)
32	Stem (rosette) 20% of final length (diam.)
33	Stem (rosette) 30% of final length (diam.)
34	Stem (rosette) 40% of final length (diam.)
35	Stem (rosette) 50% of final length (diam.)
36	Stem (rosette) 60% of final length (diam.)
37	Stem (rosette) 70% of final length (diam.)
38	Stem (rosette) 80% of final length (diam.)
39	Max. stem length or rosette diam. reached

Code	Description
4	Devel. of harvestable vegetat. plant parts
41	Harvest. veg. plant parts begin to develop
43	Harvest. veg. plant parts 30% of final size
45	Harvest. veg. plant parts 50% of final size
47	Harvest. veg. plant parts 70% of final size
49	Harvest. veg. plant parts have final size
5	Inflorescence emergence
51	Inflorescence or flower buds visible
55	1. individual flowers vis. (still closed)
59	1. flower pedals vis. (in petalled forms)
6	Flowering (main shoot)
60	First flowers open
61	10% of flow. open or 10% of plants in bloom
63	30% of flow. open or 30% of plants in bloom
65	Full flow.: 50% flow. open/50% pl. in bloom
67	Flower. finishing: majority of petals fallen
69	End of flowering: fruit set visible
7	Development of fruit
71	10% fruits h. final size/fr. 10% of final size
73	30% fruits h. final size/fr. 30% of final size
75	50% fruits h. final size/fr. 50% of final size
77	70% fruits h. final size/fr. 70% of final size
79	Nearly all fruits have reached final size
8	Ripening or maturity of fruit and seed
81	Beginning of ripening or fruit colouration
85	Advanced ripening or fruit colouration
89	Fully ripe
9	Senescence, beginning of dormancy
93	Leaves begin to change colour or fall
95	50% of leaves discoloured or fallen
97	End of leaf fall: plants dead or dormant
99	Harvested product

APPENDIX 3 CLIMATIC DATA

Climatic data registered by means of a datalogger.

Location I-12-6703-1: XXXXXXXXXX

date	mean temp. (°C)	RH (%)	daily light sum (J/cm ²)	date	mean temp. (°C)	RH (%)	daily light sum (J/cm ²)
11-12-2012	20.3	54.59	348	19-01-2013	15.5	59.15	231
12-12-2012	20.3	60.62	175	20-01-2013	15.9	61.70	140
13-12-2012	20.3	58.36	161	21-01-2013	15.8	64.57	92
14-12-2012	20.3	65.07	93	22-01-2013	16.1	63.65	412
15-12-2012	20.3	71.13	193	23-01-2013	16.0	61.71	395
16-12-2012	20.3	70.69	117	24-01-2013	16.3	62.21	479
17-12-2012	20.3	69.84	87	25-01-2013	16.3	61.61	550
18-12-2012	20.0	70.34	176	26-01-2013	15.8	63.78	151
19-12-2012	19.6	72.34	102	27-01-2013	16.0	71.45	140
20-12-2012	19.1	64.57	82	28-01-2013	16.4	73.40	467
21-12-2012	18.7	71.64	171	29-01-2013	16.6	80.16	89
22-12-2012	18.6	74.23	42	30-01-2013	17.2	77.55	304
23-12-2012	18.6	75.15	106	31-01-2013	17.4	73.45	345
24-12-2012	18.6	76.84	92	01-02-2013	17.1	70.92	112
25-12-2012	18.6	75.42	154	02-02-2013	17.5	65.28	538
26-12-2012	18.6	73.79	214	03-02-2013	17.2	69.29	193
27-12-2012	18.6	73.68	101	04-02-2013	17.7	75.22	558
28-12-2012	18.7	73.01	115	05-02-2013	17.2	69.27	357
29-12-2012	18.7	75.08	242	06-02-2013	17.6	67.18	592
30-12-2012	18.6	72.57	211	07-02-2013	17.4	65.13	451
31-12-2012	18.6	75.35	97	08-02-2013	17.8	66.92	616
01-01-2013	18.6	70.81	188	09-02-2013	17.5	68.70	483
02-01-2013	18.7	71.25	274	10-02-2013	17.4	66.06	594
03-01-2013	18.0	79.45	124	11-02-2013	17.2	62.02	237
04-01-2013	16.9	81.91	61	12-02-2013	17.6	63.72	312
05-01-2013	17.0	78.36	41	13-02-2013	18.4	66.61	577
06-01-2013	16.9	78.89	298	14-02-2013	17.3	62.51	181
07-01-2013	17.0	77.00	52	15-02-2013	18.5	74.44	527
08-01-2013	17.0	76.76	74	16-02-2013	18.0	79.83	468
09-01-2013	16.9	74.07	72	17-02-2013	18.9	75.61	889
10-01-2013	16.6	74.24	260	18-02-2013	18.8	74.92	594
11-01-2013	16.0	70.70	339	19-02-2013	18.0	76.55	474
12-01-2013	15.9	67.40	376	20-02-2013	18.5	71.49	543
13-01-2013	16.1	61.10	450	21-02-2013	18.4	69.59	714
14-01-2013	16.0	62.02	314	22-02-2013	18.2	68.50	436
15-01-2013	15.7	61.09	259	23-02-2013	18.0	66.67	265
16-01-2013	16.2	59.54	527	24-02-2013	17.6	68.46	192
17-01-2013	15.9	65.75	157	25-02-2013	18.0	71.81	179
18-01-2013	16.0	64.20	254	26-02-2013	18.1	76.04	323

CONTINUED: Climatic data registered by means of a datalogger.

Location I-12-6703-1: [REDACTED]

date	mean temp. (°C)	RH (%)	daily light sum (W/cm ²)	date	mean temp. (°C)	RH (%)	daily light sum (W/cm ²)
27-02-2013	18.1	73.79	303	08-04-2013	20.1	71.75	1502
28-02-2013	18.4	74.90	548	09-04-2013	19.6	78.97	786
01-03-2013	18.1	76.02	317	10-04-2013	19.1	81.53	540
02-03-2013	18.2	75.90	376	11-04-2013	18.9	80.09	433
03-03-2013	18.1	79.28	690	12-04-2013	19.4	80.48	685
04-03-2013	19.3	74.34	1195	13-04-2013	19.8	76.59	1194
05-03-2013	19.8	71.54	885	14-04-2013	20.9	70.38	1301
06-03-2013	19.4	80.20	623	15-04-2013	19.9	78.55	1334
07-03-2013	19.1	82.41	613	16-04-2013	19.6	80.97	849
08-03-2013	19.3	83.51	401	17-04-2013	20.2	72.38	1416
09-03-2013	18.7	78.32	215	18-04-2013	20.3	70.64	1893
10-03-2013	18.6	71.54	374	19-04-2013	19.8	76.54	1562
11-03-2013	18.6	69.49	626	20-04-2013	20.4	67.65	2217
12-03-2013	19.1	69.62	1045	21-04-2013	20.5	70.13	2046
13-03-2013	19.5	71.23	1082	22-04-2013	20.4	72.57	1419
14-03-2013	19.3	71.84	647	23-04-2013	19.8	80.13	1364
15-03-2013	18.8	73.33	476	24-04-2013	20.4	76.60	1654
16-03-2013	19.0	77.02	632	25-04-2013	20.7	75.50	1198
17-03-2013	19.2	79.75	980	26-04-2013	19.0	77.42	906
18-03-2013	19.4	78.15	615	27-04-2013	20.2	75.10	1552
19-03-2013	18.9	79.72	446	28-04-2013	20.6	72.13	1330
20-03-2013	19.0	74.60	782	29-04-2013	19.1	72.96	1532
21-03-2013	19.1	75.98	989				
22-03-2013	19.5	72.04	811				
23-03-2013	19.2	71.32	681				
24-03-2013	18.9	68.78	1183				
25-03-2013	19.6	69.52	1725				
26-03-2013	19.8	70.24	1717				
27-03-2013	19.9	70.68	1438				
28-03-2013	19.4	71.73	842				
29-03-2013	19.6	71.65	278				
30-03-2013	19.2	72.23	824				
31-03-2013	19.4	73.72	934				
01-04-2013	19.9	71.33	1734				
02-04-2013	20.1	67.76	1626				
03-04-2013	19.7	73.29	1239				
04-04-2013	19.1	74.02	577				
05-04-2013	19.5	74.26	922				
06-04-2013	19.7	71.25	1549				
07-04-2013	20.1	71.41	1614				

APPENDIX 4 RAW DATA OF THE ASSESSMENTS

Leaf symptoms

			brandnetel gem % bladopp	brandnetel perc pl	mozaïek gem % bladopp	mozaïek perc pl	gele stip perc pl	gele stip gem % bladopp	bladnecr perc pl
mild	agr	ras							
geen	agEU		18,67	90,56	23,61	92,22	26,11	1,16	100,00
			30,33	90,00	37,28	94,44	21,11	2,79	100,00
			30,39	97,78	35,67	97,78	4,44	0,04	100,00
VX	agEU		2,47	16,67	4,31	34,44	3,33	0,00	0,00
			4,39	23,33	4,44	23,89	8,33	0,01	13,89
			2,56	18,89	3,67	31,67	3,89	0,00	1,67
			0,97	6,67	1,39	11,67	7,78	0,01	2,78

[illegible]

		brandnetel gem % bladopp	brandnetel perc pl	mozaïek gem % bladopp	mozaïek perc pl	gele stip perc pl	gele stip gem % bladopp	bladnecr perc pl	bladnecr gem % bladopp
mild	agr								
geen	agEU	26,5	92,8	32,2	94,8	17,2	1,3	100,0	27,9
VX	agEU	2,6	16,4	3,5	25,4	5,8	0,0	4,6	0,2

stengelnecr perc pl	stengelnecr gem % bladopp	chlorose perc pl	chlorose gem % bladopp	bladmisl perc pl	bladmisl gem score	#bladreductie	#gewasstand
92,6	9,3	0,0	0,0	85,2	1,5	41,5	4,9
0,0	0,0	0,0	0,0	0,6	0,0	0,8	8,0

Setting and flowering

datum	22-2-2013
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gemiddelde decimale bloei		ras			
mild	ag				Eindtotaal
geen	agEU	4,17	4,17	4,47	4,27
VX	agEU	3,80	4,27	4,37	3,67
Eindtotaal		3,98	4,22	4,42	3,67

datum	8-3-2013
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gemiddelde decimale bloei		ras			
mild	ag				Eindtotaal
geen	agEU	5,53	5,27	6,07	5,62
VX	agEU	5,53	5,47	5,73	5,47
Eindtotaal		5,53	5,37	5,90	5,47

toename bloei tussen 22 feb 8 mrt

toename decimale bloei		ras			
mild	ag				Eindtotaal
geen	agEU	1,37	1,10	1,60	0,00
VX	agEU	1,73	1,20	1,37	1,80
Eindtotaal		1,55	1,15	1,48	1,80

Verschil bloei-gezet op 22 febr 2013

		ras			
mild	ag				Eindtotaal
geen	agEU	1,20	0,43	0,77	0,00
VX	agEU	0,47	0,67	0,60	0,50
Eindtotaal		0,83	0,55	0,68	0,50

Verschil bloei-gezet op 8 mrt 2013

		ras			
mild	ag				Eindtotaal
geen	agEU	0,60	0,30	1,03	0,00
VX	agEU	0,60	0,40	0,63	0,80
Eindtotaal		0,60	0,35	0,83	0,80

datum	22-2-2013
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Gemiddelde decimale zetting		ras			
mild	ag				Eindtotaal
geen	agEU	2,97	3,73	3,70	3,47
VX	agEU	3,33	3,60	3,77	3,17
Eindtotaal		3,15	3,67	3,73	3,17

datum	8-3-2013
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Gemiddelde decimale zetting		ras			
mild	ag				Eindtotaal
geen	agEU	4,93	4,97	5,03	4,98
VX	agEU	4,93	5,07	5,10	4,67
Eindtotaal		4,93	5,02	5,07	4,67

toename zetting tussen 22 feb 8 mrt

toename decimale zetting		ras			
mild	ag				Eindtotaal
geen	agEU	1,97	1,23	1,33	0,00
VX	agEU	1,60	1,47	1,33	1,50
Eindtotaal		1,78	1,35	1,33	1,50

Fruit symptoms








			Data			
mild	agressief	ras	Sum of marmering	Sum of wankleur	Sum of beschadiging	Sum of misvorming
geen	agEU			4	69	73
				2	81	88
				8	57	73
VX	agEU			0	13	15
				0	8	9
				0	0	12
				0	5	16

Sum of neusrot	Sum of open vrucht	Sum of aantvruchten	
	0	0	363
	0	3	326
	0	0	326
	0	1	321
	0	0	296
	0	1	350
	0	0	316








Som van trossen		ras			
mild	agressief				Eindtotaal
geen	agEU	82	78	74	234
VX	agEU	73	66	76	69
					284

Fruit yield						
Som van vruchten		datum				
mild	ag	ras	02-22-13	03-01-13	03-08-13	Eindtotaal
geen	agEU		48	79	93	220
			57	62	74	193
			61	80	91	232
VX	agEU		59	66	91	216
			59	73	94	226
			65	73	93	231
			55	60	91	206

# trossen	ras				
vak					Eindtotaal
7	54	57	59		170
8	53	57	57	49	216
Eindtotaal	107	114	116	49	386

Som van gewicht			datum			
mild	agressief	ras	25-3-2013	2-4-2013	5-4-2013	12-4-2013
geen	agEU		0,3	4,06	3,15	1,07
			0,29	3,69	0,56	2,59
			2,06	0,91	0	1,2
VX	agEU		0,5	3,62	0	4,37
			0,52	1,68	0	4,12
			0,49	0,62	2,31	3,93
			0	0,7	0,77	3,82

16-4-2013	19-4-2013	23-4-2013	26-4-2013	29-4-2013	Eindtotaal
3,51	3,2	8,54	2,77	9,7	36,3
0,78	1,73	7,2	6,9	5,84	29,58
5,4	3,62	6,22	0	5,4	24,81
3,74	2,79	6,22	0,41	6,22	27,87
7,44	1,99	5,08	3,33	5,38	29,54
3,71	2,11	5,52	0,31	4,04	23,04
5,02	2,12	6,86	1,3	7,86	28,45

Som van trossen			datum			
mild	agressief	ras	25-3-2013	2-4-2013	5-4-2013	12-4-2013
geen	agEU		1	14	9	3
			1	13	2	8
			14	4	0	4
VX	agEU		2	14	0	13
			2	6	0	11
			6	3	8	14
			0	4	4	14

16-4-2013	19-4-2013	23-4-2013	26-4-2013	29-4-2013	Eindtotaal
9	8	17	6	15	82
4	6	18	16	10	78
15	10	16	0	11	74
10	6	15	1	12	73
17	4	11	6	9	66
11	6	16	1	11	76
12	5	14	3	13	69

Som van vruchten		ras			
mild	agressief				Eindtotaal
geen	agEU	363	326	326	1015
VX	agEU	321	296	350	1283

Som van gewicht		ras			
mild	agressief				Eindtotaal
geen	agEU	36,3	29,58	24,81	90,69
VX	agEU	27,87	29,54	23,04	108,9

vruchten / tros		ras			
mild	agressief				Eindtotaal
geen	agEU	4,260362	3,816667	3,379972	3,819
VX	agEU	3,902177	3,77857	4,440657	4,006694

Gemiddeld vruchtgewicht		ras			
mild	agressief				Eindtotaal
geen	agEU	96,48559	89,02335	56,68598	80,73164
VX	agEU	76,4242	90,23728	62,60573	75,97784

productie per m2		ras			
mild	agressief				gemiddeld
geen	agEU	5,082	4,1412	3,4734	12,6966
VX	agEU	3,9018	4,1356	3,2256	15,246

geoogste trossen/plant		ras			
mild	agressief				gemiddeld
geen	agEU	4,1	3,9	3,7	3,9
VX	agEU	3,65	3,3	3,8	3,55

Som van gewicht (kg)		ras				
mild	agressief					Eindtotaal
geen	agEU	36,3	29,58	24,81		90,69
VX	agEU	27,87	29,54	23,04	28,45	108,9