Appendix 8 Compilation of Tier II summaries

Part 4 Section 7
Efficacy Data and Information

V10

suspension concentrate (SC) containing:
5-25 mg/L Mild Pepino Mosaic Virus isolate VX1
5-25 mg/L Mild Pepino Mosaic Virus isolate VC1

Data required according Annex III to the directive 91/414/EEC using OECD Guidance for Industry Data Submissions on Plant Protection Products and their Active Substances (Revision 2 May 2005)

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January 2017

pagina 1: 10.2.e
pagina 2, 8, 14, 15, 38, 41, 44, 47, 50, 52, 55, 58, 68, 69: 10.1.c Wob juncto 63.2.a Vo 1107/2009 juncto 39.2.a Vo 178/2002
pagina 71 en 72: 10.2.e, 0.1.c Wob juncto 63.2.a Vo 1107/2009 juncto 39.2.a Vo 178/2002

TABLE OF CONTENTS

INTRODUCTION

V10 is an elicitor of a suspension concentrate (SC) formulation, which contains 5-25 mg/L Mild Pepino Mosaic Virus isolate VX1 and 5-25 mg/L Mild Pepino Mosaic Virus isolate VC1, which per isolate corresponds to approximately $1.5x10^{11}$ to $7.5x10^{11}$ virus particles per ml.
V10 is to be used as post-emergent foliar applications (spray or direct rubbing on foliage) in protected cultivation of tomatoes to prevent infections by aggressive isolates of the Pepino Mosaic Virus.

This dossier concerns the registration of V10 in the Northern, Central and Southern EU Zones.

Data on 8 greenhouse efficacy trials in tomato are summarized in this Biological Assessment Dossier.

i. Mild Pepino Mosaic Virus isolate VC1

Active micro-organism	Mild Pepino Mosaic Virus strain VC 1
Function	Control of aggressive Pepino Mosaic Viruses by gene suppression (RNA silencing
Name of the organism	Pepino Mosaic Virus
Taxonomy	Potex Viruses
Species, subspecies, strain	Genus: Potexvirus Family: Alphaflexiviridae Order: Tymovirales
Origin and natural occurrence	Mild Pepino Mosaic Virus VC 1 is a mild variant of the Chilean CH2 strain which is the predominant genotype in Europe. The virus is present and replicates efficiently on Solanaceae and can survive for short times on plants of other botanical families as well.
Target organism(s)	VC1 targets the aggressive Pepino Mosaic Virus by gene suppression. The active substance VC1, prevents infection with virulent strains. Pepino mosaic virus infects *Solanum lycopersicum* (tomato)*, Solanum muricatum* (pepino).
Mode of action	Mild Pepino Mosaic Virus VC1 induces plant resistance, most likely through induction of a RNA silencing process which destroys RNAs of all closely related Pepino Mosaic Virus strains
Host specificity	Besides the target hosts tomato and pepino the Pepino Mosaic Virus can survive for short times on plants of other botanical families as well.
Life cycle	Pepino Mosaic Virus propagates on viable host tissue. After transmission the virus enters tissue through (damaged) epidermis. Viral single stranded RNA is copied in the cytoplasm of host cells. The proposed mode of action is that host cells synthesize small interfering RNA which are incorporated in a RNA–induced silencing complex. This complex destroys RNA of invading (closely related variants of) Pepino Mosaic Virus.
Infectivity, dispersal and colonisation ability	Pepino Mosaic Virus is highly infective to some plants belonging to the Solanaceae. Pepino Mosaic Virus might survive or replicate also on plants of other botanical families but without causing adverse effects.

ii. Mild Pepino Mosaic Virus isolate VX1

Active micro-organism	Mild Pepino Mosaic Virus strain VX 1
Function	Control of aggressive Pepino Mosaic Viruses by gene suppression (RNA silencing
Name of the organism	Pepino Mosaic Virus

Taxonomy	Potex Viruses
Species, subspecies, strain	Genus: Potexvirus Family: Alphaflexiviridae Order: Tymovirales
Origin and natural occurrence	Mild Pepino Mosaic Virus VX 1 is a mild variant of the Peruvian (LP) strain. The virus is present and replicates efficiently on Solanaceae and can survive for short times on plants of other botanical families as well.
Target organism(s)	VX1 targets the aggressive Pepino Mosaic Virus by gene suppression. The active substance VX1, prevents infection with virulent strains. Pepino mosaic virus infects *Solanum lycopersicum* (tomato)*, Solanum muricatum* (pepino).
Mode of action	Mild Pepino Mosaic Virus VCX induces plant resistance, most likely through induction of a RNA silencing process which destroys RNAs of all closely related Pepino Mosaic Virus strains
Host specificity	Besides the target hosts tomato and pepino the Pepino Mosaic Virus can survive for short times on plants of other botanical families as well.
Life cycle	Pepino Mosaic Virus propagates on viable host tissue. After transmission the virus enters tissue through (damaged) epidermis. Viral single stranded RNA is copied in the cytoplasm of host cells. The proposed mode of action is that host cells synthesize small interfering RNA which are incorporated in a RNA–induced silencing complex. This complex destroys RNA of invading (closely related variants of) Pepino Mosaic Virus.
Infectivity, dispersal and colonisation ability	Pepino Mosaic Virus is highly infective to some plants belonging to the Solanaceae. Pepino Mosaic Virus might survive or replicate also on plants of other botanical families but without causing adverse effects.

ii. V10

(a) Efficacy of the plant protection product

Tomato plants are protected against Pepino Mosaic Virus (PepMV) by a preceding infection with attenuated isolates of this virus (cross-protection).
Cross-protection may be due to RNA silencing activity (Ratcliff et al., 1999; Valkonen et al., 2002) induced by the protective isolate. In plants, RNA silencing has been shown to serve as a defence against virus infections. Hanssen et al., 2011 showed that Pepino Mosaic Virus differentially regulates the RNA silencing pathway in tomato, suggesting a role for a PepMV-encoded silencing suppressor.
RNA silencing is a type of gene regulation that is based on targeting and degrading specific sequences of messenger RNA (Agrios, 2004). The key characteristic of RNA silencing (Posttranscriptional Gene Silencing) is the formation of small interfering RNAs (siRNAs) that are produced by RNaseIII-like Dicer enzymes, which are incorporated into a so-called RNA-induced silencing complex, which contains an Argonaute (AGO) protein with a siRNA-binding domain and endonucleolytic activity to cleave target RNAs (Baulcombe, 2004). As a result replicated RNA of the invading virus is destroyed preventing further spread of the virus in the plant. Replicated RNA of almost identical virus isolates is also destroyed, preventing infection by these isolates.
Infection of a tomato plant with Mild Pepino Mosaic Virus isolates does not have an effect on yield loss or fruit quality (contrary to infection with aggressive isolates) but induces cross protection by RNA silencing. Multiplication of any almost identical (mild or aggressive) virus isolate (similarity between RNA of isolates ≥ 96%) that invades the plant, after RNA silencing is initiated, will therefore be prevented.

Mild Pepino Mosaic Virus isolate VX1 is an isolate of the Peruvian (LP) isolate and its RNA is for 96% identical to the RNA of the European (EU) isolate. Inoculation of a tomato plant with isolate VX1 therefore results in cross protection against isolates with origination from

the EU isolate. RNA of Mild Pepino Mosaic Virus isolate VC1 is almost identical to the RNA of the Chilean (CH2) isolate (98% similarity) which results in 100% cross protection against isolates of the Chilean isolate.

As a consequence the mechanism of cross-protection only works when tomato plants are inoculated with a mild isolate before being exposed to aggressive isolates.

References:

- Agrios, G.N., 2004. Plant Pathology. Fifth edition. Elsevier Academic Press. ISBN-10: 0-12-044565-4. Pp. 244-246.
- Baulcombe, D., 2004. RNA silencing in plants. Nature 431: 356-363.
- Hanssen, I.M., van Esse, H.P., Ballester, A-R., Hogewoning, S.W., Ortega Parra, N., Paeleman, A., Lievens, B., Bovy, A.G., Thomma, B.P.H.J., 2011. Differential Tomato Transcriptomic Responses Induced by Pepino Mosaic Virus Isolates with Differential Aggressiveness. Plant Physiology 156:301-318.
- Ratcliff, F.G., MacFarlane, S.A., Baulcombe, D.C., 1999. Gene silencing without DNA: RNA-mediated cross-protection between viruses. Plant Cell 11: 1207-1215.
- Valkonen, J.P.T., Rajamäki, M.L., Kekarainen, T., 2002. Mapping of viral genomic regions important in cross-protection between isolates of a potyvirus. Molecular Plant-Microbe Interactions 15: 683-692.

(b) Adjuvants

An abrasive should be added to the product solution to provide enough abrasion to the tomato leaves to introduce virus into the plant cells. The abrasive to be used as an additive with this formulation is synthetic sand.

(c) Pest or disease description

Pepino Mosaic virus (PepMV)

PepMV can be detected on growing plants (tomato, pepino), on tomato fruits and on tomato seeds originating from infected plants. Symptoms of PepMV can be extremely variable, ranging from latent to very severe infections. Fruit discolorations, such as marbling or flaming, are the most typical and economically significant symptoms. Occasionally, fruit cracking and malformation have been observed. In addition to fruit symptoms, leaf symptoms such as nettle heads, blistering or bubbling, chlorosis, mosaic and yellow angular leaf spots, and leaf or stem necrosis have been associated with PepMV infections. As plants mature, foliar symptoms generally disappear. Despite the variability in PepMV symptoms, PepMV can be normally detected in almost any above- and belowground part of an actively growing plant infected about 4 weeks earlier.

PepMV is very efficiently transmitted by mechanical means; i.e. fruit harvesting, pruning, and other cultural practices lead to rapid spread in protected tomato crops. In addition, bumblebees have been associated with PepMV transmission in glasshouses. A low seed transmission rate has been demonstrated; however, available evidence suggests that PepMV does not infect the embryo or endosperm but contaminates the seed coat. Long distance spread of PepMV is thought to be through contaminated seeds or infected transplants.
Transmission through recirculation of drainage water is also possible.

Like most other potexviruses, PepMV has a fairly narrow natural host range that appears to be largely restricted to Solanaceous species. In addition to tomato and the original host, pepino (*S. muricatum*), natural infections by PepMV have been reported not only from the wild tomato species *S. chilense, S. chmielewskii, S. parviflorum S. peruvianum* and potato

germplasm, but also from several weeds belonging to various plant families and growing in the vicinity of tomato glasshouses. Since the experimental host range of PepMV includes Solanaceous crop plants such as potato, tobacco, *Capsicum* peppers and eggplant, these crops may also be at risk.

Currently, four major genotypes or strain groups sharing complete nucleotide sequence identities ranging from 78% to 95% are distinguished: European (EU), Peru, Ch2 and US1. EU is the PepMV genotype that is genetically most similar (95%) to, but biologically distinct from, the Peruvian strain group and that predominated initially in European tomato crops. Since 2004, however, isolates of strain group EU seem to be replaced by, and/or to occur increasingly in mixed infections with, strain Ch2 in Europe. This latter genotype, first identified from tomato seeds originating from Chile, is genetically very distinct (79% identity) from the EU strain. Isolates of strain group US1 clearly differ genetically (identities of 78–82%) from EU, Peru and Ch2 have as yet been identified only rarely from tomato crops in the USA and Europe. There have also been reports on the occurrence in tomato of recombinant PepMV isolates which have chimeric genomes sharing striking nucleotide sequence identities with isolates of strain groups EU and Ch2.

(d) Supporting information from earlier formulations of the active substance or similar active substances

None.

(e) Further relevant data on the active substance and formulation

None.

(f) Details of intended use

Details of harmful organisms against which protection is afforded
Mild Pepino Mosaic Virus isolate VX1 is for use in horticulture as post-emergent foliar applications (spray or direct rubbing on foliage) in protected cultivation of tomatoes to prevent infections by aggressive isolates of the EU-genotype of Pepino Mosaic Virus.

Application rate
The product 'V10' contains 5-25 mg/L Mild Pepino Mosaic Virus isolates VX1 and 5-25 mg/L Mild Pepino Mosaic Virus isolates VC1.

When applied as a foliar spray the product is applied at a concentration of 2% v/v. The product is applied with a water volume of 3500 L/ha in combination with 0.8% w/v synthetic sand as an abrasive. This amounts to 0.35-1.75 VX1 and 0.35-1.75 g VC1 per ha.
When directly rubbed onto the foliage, the product is applied at a concentration of 10% v/v. The product is applied with a water volume of 8 L/ha in combination with 1.5% w/v synthetic sand as an abrasive. This amounts to 0.004-0.02 g VX1 and 0.004-0.02 g VC1 per ha.

Method of application
The product is applied by foliar spray at pressures ranging from 12-15 bar and in combination with synthetic sand to ensure infection, or rubbed directly onto the foliage in combination with synthetic sand to ensure infection.

Maximum number of applications and their timing
A single application is made when the tomato plants are young (for spraying BBCH 13-51, 10-30 cm high plants, for rubbing BBCH 13-61) and before infection with aggressive forms of PepMV have occurred.

For each application, growth stages of the crop or plants to be protected

A single application is made when the tomato plants are young. As the mode of action is based on cross protection, a single application will protect the crop for the rest of its lifetime.

For each application, development stage of the harmful organisms concerned
The product is applied preventatively, before infection.

Duration of protection afforded by each application
As the mode of action is based on cross protection, a single application will protect the crop for the rest of its lifetime.

Minimum waiting periods or other precautions between last application and sowing or planting succeeding crops
None.

Limitations on choice of succeeding crops
None.

Description of damage to rotational crops
None.

Proposed instructions for use
Spray application:
- Apply onto young tomato plants (10-30 cm in height), before or after planting. The plants should be free of pepino mosaic virus at the time of application.
- Shake well before use within the container.
- Prepare 2% dilution of the product (1 L V10 per 50 litres of water) with cold water (approximately 8 °C) and add 8 grams of synthetic sand per litre of water. Mix thoroughly.
- Apply 70 L product/ha
- Boom height should be approximately 10-15 cm above crop. Product should be sprayed at 12-15 bar (measured at spray boom).

Application by rubbing:
- Apply onto young tomato plants after planting. The plants should be free of pepino mosaic virus at the time of application.
- Shake well before use within the container.
- Prepare 10% dilution of the product (1 L V10 per 10 litres of water) with cold water (approximately 8 °C).
- The product should be applied at a dose rate of 8 L liquid per ha plant bed, after transplanting. For each worker, prepare portion of ±0.5 L in a large container (±1 litre) and add synthetic sand to each portion (15 grams per litre of water).
- The product is applied onto the crop with a sponge. Dip the sponge into the prepared liquid and rub it onto a single leaflet per plant. Up to 20 applications can be made with the sponge. If the sponge runs dry, the sponge should be dipped in to the prepared liquid again.

General:
- Use product within 3 days after delivery or purchase of the product and store at 4–10ºC.
- After dilution, use the product within 6 hours.

IIIA 6.1 Efficacy data

IIIA 6.1.1 Efficacy trials - laboratory or growth chamber tests (preliminary screening)

V10 offers protection against Pepino Mosaic Virus (PepMV) by inducing plant resistance. A preceding infection of tomato plants with attenuated isolates of the PepMV in V10 induces cross-protection due to RNA silencing. In contrary to conventional plant protection products, this product works through the RNA silencing mechanisms of the plant. Upon application with V10, the tomato plant becomes infected with the attenuated isolates of the PepMV which induces RNA silencing in the plant. As a result, the effectiveness of the product depends on the infectivity of the formulation, effective replication of the isolated VX1 and VC1 and the RNA silencing mechanisms of the plant.
The formulation V10 in combination with an abrasive should enable the virus isolates to enter the plant cells, and infect the plant. The ability of the formulation to infect tomato plants is therefore key to the effectiveness of the product.

In the intended uses for V10, 2 methods of application are claimed. V10 can be applied by foliar spray, or by rubbing the solution on leaves. The effectiveness of application by foliar spray was demonstrated in 8 efficacy trials discussed in chapter 6.1.3 of this dossier. The ability of V10 to infect tomato plants when applied by rubbing is demonstrated in a bioassay.

Over time, several changes to the V10 formulation were made for other reasons than efficacy. The efficacy trials were conducted with a V10 formulation containing borate buffer, whereas the new formulation is prepared in phosphate buffer. Furthermore, the concentration of nicotine in the formulation was lowered by including a purification step in the production process. These changes in the formulation are not expected to have an effect on the infectivity of the product, and as such no effect on the effectiveness of the product. This is demonstrated in bioassays in which the infectivity of these formulations is tested.

V10 is applied in combination with an abrasive that provides entry of the virus into plant cells. In the efficacy trials, V10 was applied in combination with carborundum. Due to a new classification for carborundum as a possible carcinogen, the abrasive to be used as an additive with this formulation is changed to synthetic sand. In multiple bioassays it was demonstrated that both carborundum and synthetic sand are equally effective as abrasive.

The following trials were conducted:

Target(s)	Crop(s)	Country	Years	Type of trial	# trials	GEP, non-GEP	Remarks
PEPMVO	Tomato	NL	2013	bioassay	1	non-GEP	concentration range
			2014	bioassay	2	non-GEP	effect of buffer
			2015	bioassay	4	non-GEP	effect of abrasive
			2016	bioassay	2	non-GEP	effect of purification
	TOTAL				9		

Bioassays were conducted to the Standard Operating Procedure of [redacted]. Bioassays were performed in greenhouses with tomato seedlings of approximately 18 days with at least two true leaves of more than 1 cm^2. Seedlings were inoculated by rubbing virus suspension with abrasive on leaves (unless stated otherwise). Per sample 10 seedlings were used. 2 seedlings were used as negative control. After approximately 14 days, from each plantlet 2 or 3 (non-inoculated) leaflets were sampled and assessed on virus

infection using ELISA. A quality criterion of 70% was set. If 70% of the plants test positive for virus infection, the infectivity is deemed sufficient. In practise, if 70% of the plants are infected after 2 weeks, through cross contamination via contact between plants, crop handling, maintenance to the crop, etc., the whole crop will be infected within 4 weeks after inoculation.

Infectivity of VX1 and VC1 applied by rubbing
The efficacy of the product depends on the effective replication of the isolates VX1 and VC1 in the crop and thus on the infectivity of the product.
The infectivity of VX1 and VC1 when applied by rubbing was tested in a bioassay performed in a laboratory study conducted in the Netherlands in 2013 (report VAL507, 6.1.1/02)
Tomato seedlings were inoculated with mild PepMV at different dilutions. Virus suspension to which the abrasive carborundum was added was rubbed on tomato leaves of seedlings after which the plants were incubated under normal growing conditions for 2 weeks. After this incubation, the plantlets were checked for infectivity by ELISA (validated method for determination).

The infectivity of two products containing 10-50 mg/L Mild Pepino Mosaic Virus isolate VX1 or VC1 were tested at different concentrations. The products were diluted 10, 50, 100, 500 or 1000 times in PBS buffer which was equivalent to a concentration of respectively 10%, 2%, 1%, 0.2% and 0.1%. For both of the isolates each dilution was applied in combination with carborundum onto 10 plantlets. After two weeks, the plantlets were checked for infection.

Table 6.1.1-1 Infectivity of VX1 and VC1

Dilution (concentration)	PepMV positive plants (%)	
	VX1	VC1
10 (10%)	100	100
50 (2%)	100	100
100 (1%)	100	100
500 (0.2%)	50	70
1000 (0.1%)	20	60

These results indicate that a concentration between 1% and 10% will be sufficiently infective when carborundum suspension containing mild isolates of PepMV is rubbed on tomato leaves of seedlings. As the amount of Mild Pepino Mosaic Virus isolate may vary between 10 to 50 mg/L between different batches, and also taking into account that the product is already applied at a relatively low dose rate when applied as proposed (0.8 L/ha), the proposed concentration has been set at 10% to guarantee adequate infection of the tomato plants in all cases.

Effect of buffer on infectivity
Before 2014, batches with VX1, VC1 and V10 were prepared in a borate buffer. The efficacy trials summarized in this dossier were conducted in 2012 and 2013 with a borate buffer suspension. From 2014, batches with VX1, VC1 and V10 are prepared in a phosphate buffer.

Since the isolates VX1 and VC1 will replicate within the crop, the effectiveness of the product depends on the infectivity of the virus isolates VC1 and VX1. There are no

indications that the mechanism of effectivity (cross-protection; RNA-silencing) will be influenced by the initial buffer.

Multiple bioassays were commissioned by the applicant of batches of VC1 and VX1 in phosphate buffer (Report IIIA 6.1.1/03 Bioassay VC1 and Report IIIA 6.1.1/04 Bioassay VX1 are examples). In these bioassays tomato plants were inoculated with the suspensions after which the plants were incubated under normal growing conditions for 2 weeks. After this incubation, the plantlets were checked for infectivity by ELISA (validated method for determination). In each test all plants tested positive for PepMV, demonstrating that the suspensions were infectious.

Based on the results from the bioassays, the infectivity of VX1 and VC1 in phosphate buffer is not expected to be different to the infectivity of VX1 and VC1 in borate buffer. Both meet the set quality criterion.

With respect to crop safety, no adverse effects are expected from phosphate buffer as it is considered in life sciences as a very mild buffer for plants and is widely used in plant experiments without adverse effects.

Effect of nicotine concentration
The active ingredients of V10, VX1 and VC1 are produced in tobacco plants. As a result the formulation also contains nicotine. Due to possible risk for human health, the nicotine concentration in the formulation was lowered by means of concentration, centrifugation and dialysis. After dilution, the nicotine concentration in V10 is limited to a maximum of 0.1 mg/L. The infectivity of virus isolates VC1 and VX1 after these purification steps was tested in bioassays conducted in the Netherlands in 2016 (reports 6.1.1/05 and 06).

Five different batches of VC1 virus suspension and VX1 virus suspension were used for the inoculation of tomato seedlings. Inoculation was made using the abrasive carborundum. After 14 days, the plantlets were checked for infectivity by ELISA.

All plants inoculated with the different batches from isolate VC1 tested positive for VC1. A range of 70-90% of the plants inoculated with the different batches from isolate VX1 tested positive for VX1.

Table 6.1.1-2 Infectivity of VX1 and VC1 after purification

Isolate	Batch	PepMV positive plants (%)
VC1	VC1-A-150616	100
	VC1-B-150616	100
	VC1-C-150616	100
	VC1-D-150616	100
	VC1-E-150616	100
VX1	VX1-A-150616	90
	VX1-B-150616	80
	VX1-C-150616	90
	VX1-D-150616	90
	VX1-E-150616	70

Based on the results from the bioassays, the infectivity of VX1 and VC1 is not expected to be affected by the extra purification steps. Both isolates meet the set quality criterion of 70% infected plants.

Effect of abrasive on infectivity
To ensure entry of the virus into plant cells, an abrasive is added to the formulation. Formerly carborundum was used for this purpose, however due to a new classification as a possible carcinogen, the abrasive to be used as an additive with this formulation is changed to synthetic sand. A bioassay was conducted to demonstrate that both carborundum and synthetic sand are equally effective as abrasive.
Four bioassays were conducted in the Netherlands in 2015 (report 6.1.1/07), in which the infectivity of V10 was tested with either carborundum or synthetic sand as an abrasive.
Groups of 6500 young tomato plants (10-30 cm in height) were inoculated per treatment. 2% V10 was applied with 0.8% w/v carborundum or 0.8% w/v synthetic sand.
After 2-3 weeks, 10 plants per group were tested for the presence of virus using ELISA. To determine the presence of VX1 and VC1, real-time PCR was used (according to protocol SPV A518, 6.1.1/08).

All plants inoculated using carborundum as abrasive tested positive for infection. 80-100% of plants inoculated using synthetic sand tested positive for infection. In all the infected plants both VX1 and VC1 were detected by qRT-PCR. Though the number of plants infected using synthetic sand that tested positive for infection in 2 of the bioassays was slightly lower as compared to those infected using carborundum, the difference can be considered as normal variation for the mechanical transmission of viruses.
Both carborundum and synthetic sand meet the quality control requirement of an infectivity of at least 70%.

Table 6.1.1-3 Infectivity of V10 with carborundum or synthetic sand as abrasives

Week of inoculation	Cultivar	PepMV positive plants (%)	
		2% V10 + 0.8% carborundum	2% V10 + 0.8% synthetic sand
Wk 18, 2015	Komeett	100	100
Wk 18, 2015	Komeett	100	100
Wk 20, 2015	Robino	100	80
Wk 28, 2015	Komeett	100	80

Based on the results of the bioassays, it can be expected that the infectivity obtained with synthetic sand as an abrasive is comparable to the infectivity obtained with carborundum as abrasive.

IIIA 6.1.2 Efficacy trials - small scale field or greenhouse tests (preliminary screening)

Benefit of the co-formulation
V10 is a combination of 2 attenuated virus isolates, namely Mild Pepino Mosaic Virus isolate VX1 and Mild Pepino Mosaic Virus isolate VC1.
Introduction of Mild Pepino Mosaic Virus isolate VX1 in tomato plants results in cross protection against isolates with origination from the EU isolate. Introduction of Mild Pepino Mosaic Virus isolate VC1 in tomato plants results in cross protection against isolates of the Chilean isolate (CH2). The EU isolate and the CH2 isolate are the main occurring genotypes of PepMV in Europe. Additionally, EU isolate infections occur often mixed with CH2 isolate infections.

Preventative treatment with the combination of VX1 and VC1 ensures protection against the main occurring PepMV in Europe.

This was demonstrated in 1 efficacy trial (I-12-6702-2) conducted in the Netherlands in 2012. In this trial, the effectiveness of V10 (VX1 + VC1) was compared to both active substances

solo. The products were applied by spraying at 2% in combination with carborundum. Artificial infection was made using aggressive PepMV isolates EU and CH, isolates from respectively the European PepMV and Chile PepMV.

The different aggressive isolates of the PepMV can cause different symptoms in plants. In this trial it was observed that plants treated with VC1 solo showed a greater severity of nettle head, mosaic, leaf necrosis and stem necrosis on leaves as compared to the plants treated with V10 (VC1+ VX1). In plants treated with VX1 solo the severity of leaf and stem necrosis was increased as compared to the plants treated with V10. Likewise in fruits, symptoms such as blotches, damage, deformation and calyx damage in plants treated with VC1 solo occurred more often than in plants treated with V10. Symptoms in plants treated with VX1 occurred only rarely and even to a lesser extent as compared to plants treated with V10.
Yield of plants treated with V10 was higher than that of plants treated with VC1 solo and comparable to plants treated with VX1 solo.
Stunting in plants treated with V10 was significantly lower than that observed in plants treated withVC1 solo and in plants treated with VX1 solo.
Crop condition of plants treated with VC1 solo and plants treated with VX1 solo was lessened as compared to plants treated with V10.

Table 6.1.2 Benefit of the co-formulation. Effectiveness of V10 compared to VC1 and VX1 solo after infection with PepMV EU and CH

Number of trials	Plant part	Symptom/ assessment	Untreated infected	2% VC1 infected	2% VX1 infected	2% V10 infected
1	leaves (% area affected)	nettle head	27	16	5	7
		mosaic	27	16	5	5
		leaf necrosis	72	37	15	0
		stem necrosis	49	29	14	0
	leaves	deformation (scale of 1-3)[1]	2.1	1.7	0.3	0
	flowers	number of flowers	1.0	3.8	3.8	4.2
		number of trusses	1.0	3.0	3.5	3.7
	fruits (% fruits affected)	blotch	100	27.3	0.9	5.6
		marble	21	6	0	2
		damaged	100	58.3	3.6	18.1
		deformed	100	70.2	4.5	15.3
		canker	9.5	0.6	0.1	0.1
		damage calyx	100	92.5	0	49.2
	fruits	yield number of fruits (% relative to untreated)	100 (0.8)	22400	34375	28875
		total weight (% relative to untreated)	100 (0.02)	19650	56500	47500
		fruit weight (% relative to untreated)	100 (5.8)	510	824	848
	whole plant	stunting (%)	80.4	50.0	23.5	9.4
		crop condition (scale of 1-10)[2]	2-8	4-6.5	5-8	6.5-7.5

[1] Deformation of leaves on a scale of 1-3 with 1: light deformation and 3: severe deformation.
[2] Crop condition on a scale of 1-10 with 1: crop dead and 10: excellent crop

Overall, the combination of VX1 and VC1 in V10 ensures protection against the 2 main occurring PepMV genotypes in Europe.

IIIA 6.1.3 Efficacy trials - operational, large scale

Testing facility or organization

Data to support the label claims for V10 which are summarized in this biological dossier were generated in a total of 8 efficacy trials conducted in the Netherlands in 2012 and 2013.

Target(s)	Crop(s)	Country	Years	Type of trial	# trials	GEP, non-GEP
PEPMVO	Tomato	NL	2012	E + Y + CS	5	GEP
			2013	E + Y + CS	3	GEP
	TOTAL				8	

E = efficacy, Y = yield, CS = crop safety

All trials were carried out by the officially recognized organisation for efficacy testing De Bredelaar. All trials were conducted to the principles of *Good Experimental Practise* (GEP).

Further details of the individual trials conducted are provided in Table IIIA 6.1.3-1.

Table IIIA 6.1.3-1 Trials sites and application details in summary form – V10 in tomato

Type of trials: effectiveness / phytotoxicity / other :	Effectiveness/phytotoxicity
Identity of the product under test (commercial name (s), active substance (s), content, formulation type (s) :	V10 (5-25 mg/L Mild PepMV isolate VX1, 5-25 mg/L Mild PepMV isolate VC1)
Crop :	Tomato (*Solanum lycopersicum,* LYPES)
Harmful organism (common name, scientific name, Bayer Code) or intended use :	Pepino Mosaic Virus (PepMV) (PEPMVO)
Responsible body for reporting trial (name, address and telephone number) :	Valto B.V. De Lier, Leehove 81, 2678 MB De Lier, the Netherlands
Date of submission :	2015

Test report	Testing Unit GEP	Trial location Crop cultivar F/G; N/A	Test method Plot size Sample size	Application details		Remarks
				Method	Application date	
I-12-6701-1	[redacted] GEP	[redacted] Tomato *Endeavour, Levanzo, Brioso, Komeett* G; A	PP 1/135(3) , PP 1/152(3), PP 1/181(3) 20 plants 20 plants	A1: Foliar spray 1.1-1.6 L/m^2 A2: Inoculation, rubbing	A1: 22.03.2012: BBCH 12-13 A2: 12.04.2012: BBCH 51	
I-12-6701-2	[redacted] GEP	[redacted] Tomato *Endeavour, Levanzo, Brioso, Komeett* G; A	PP 1/135(3) , PP 1/152(3), PP 1/181(3) 20 plants 20 plants	A1: Foliar spray 1.1-1.6 L/m^2 A2: Inoculation, rubbing	A1: 22.03.2012: BBCH 12-13 A2: 12.04.2012: BBCH 51	
I-12-6702-1	[redacted] GEP	[redacted] Tomato *Endeavour, Levanzo, Brioso, Komeett* G; A	PP 1/135(3) , PP 1/152(3), PP 1/181(3) 20 plants 20 plants	A1: Foliar spray 0.26-0.44 L/m^2 A2: Inoculation, rubbing	A1: 02.10.2012: BBCH 13-14 A2: 23.10.2012: BBCH 61-62	
I-12-6702-2	[redacted] GEP	[redacted] Tomato *Endeavour, Levanzo, Brioso, Komeett* G; A	PP 1/135(3) , PP 1/152(3), PP 1/181(3) 20 plants 20 plants	A1: Foliar spray 0.26-0.44 L/m^2 A2: Inoculation, rubbing	A1: 02.10.2012: BBCH 13-14 A2: 24.10.2012*: BBCH 61-62	Inoculation with ag EU was made at 15.11.2012
I-12-6703-1	[redacted] GEP	[redacted] Tomato *Plaisance, Merlice, Roterno* G; A	PP 1/135(3) , PP 1/152(3), PP 1/181(3) 20 plants 20 plants	A1: Foliar spray 0.5 L/m^2 A2: Inoculation, rubbing	A1: 23-30.11.2012: BBCH 13-14 A2: 01.02.2013: BBCH 61-62	
I-13-6701-1	[redacted] GEP	[redacted] Tomato *Endeavour, Levanzo, Brioso, Komeett* G; A	PP 1/135(3) , PP 1/152(4), PP 1/181(4) 20 plants 20 plants	A1: Foliar spray 0.27-0.49 L/m^2 A2: Inoculation, rubbing	A1: 01.02.2013: BBCH 12-13 A2: 21.03.2013: BBCH 51	

Test report	Testing Unit GEP	Trial location Crop cultivar F/G; N/A	Test method Plot size Sample size	Application details		Remarks
				Method	Application date	
I-13-6701-2	[redacted] GEP	[redacted] Tomato *Endeavour, Levanzo, Brioso, Komeett* G; A	PP 1/135(3) , PP 1/152(4), PP 1/181(4) 20 plants 20 plants	A1: Foliar spray 0.27-0.49 L/m^2 A2: Inoculation, rubbing	A1: 01.02.2013: BBCH 12-13 A2: 22.03.2013: BBCH 51	
I-13-6701-3	[redacted] GEP	[redacted] Tomato *Endeavour, Levanzo, Brioso, Komeett* G; A	PP 1/135(3) , PP 1/152(4), PP 1/181(4) 26 plants 26 plants	A1: Foliar spray 0.27-0.49 L/m^2 A2: Inoculation, rubbing	A1: 01.02.2013: BBCH 12-13 A2: 21.03.2013: BBCH 51	

MATERIALS AND METHODS

Sites
The trials were carried out indoors in greenhouses

Experimental details
A total of 8 trials carried out against Pepino Mosaic Virus in tomato. The trials were conducted in greenhouses in the Netherlands in 2012 and 2013. All trials were conducted in accordance to GEP and EPPO guidelines.
Trial details are given below:

Guidelines	General guidelines	PP 1/135, PP 1/152, PP 1/181
	Specific guidelines	-
Experimental design	Plot design	randomized block design
	Plot size	20 plants (7 trials), 26 plants (1 trial)
	Number of replications	3 (1 trial), 4 (7 trials)
Crop	Trials per crop	Tomato: 8 trials
	Varieties per crop	Endavour, Levanzo, Brioso, Komeett, Plaisance, Merlice, Roterno
Application	Crop stage (BBCH) at application	BBCH 12-14
	Timing	preventative, at crop height of 10-30 cm
	Number of applications Intervals between applications	1
	Spray volumes	0.26-1.6 L/m^2
Assessment	Assessment types	Symptoms on plant: 1. Nettle head 2. Mosaic 3. Necrosis leaf 4. Necrosis stem 5. Yellow spot 6. Chlorosis Symptoms on fruits 1. Blotch 2. Marble 3. Damage 4. Deformed 5. Open 6. Blossom end rot 7. Damage calyx 8. Damage stem trusses Number of flowers and trusses setting Crop condition Stunting Yield (number of fruits, total weight, fruit weight)
	Assessment dates	Symptoms on plant: 7-10 assessments per trial Symptoms on fruits: as fruits develop Number of flowers and trusses setting: 1-2 assessments per trial Crop condition: 7-10 assessments per trial For assessment dates is referred to the BAD Table 6.1.3-4).
Other relevant information	Pests	PepMV (PEPMVO) strains: agEU: aggressive European strain agCH: aggressive Chile strain

	Natural / artificial infestation	artificial inoculation at BBCH 51-62, at 21-70 days after application ELISA and RT-PCR testing was used to verify virus infection
	Field / Greenhouse	greenhouse

Assessments on virus symptoms on the apical leaves and foliage were generally carried out weekly. Plants were assessed on the severity of symptoms. Per plot the percentage nettle head, mosaic, yellow spots, leaf necrosis, stem necrosis and chlorosis was recorded. Furthermore, flowering and setting of trusses was assessed and fruits were evaluated on viral symptoms.

Crop condition was assessed weekly on a scale of 10-1 with:
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

Formulations applied and application rates
Details of the formulations tested are given in Table IIIA 6.1.3-2. Details of application rates are provided in Table IIIA 6.1.3-3.

Table IIIA 6.1.3-2 Formulations included in efficacy trials

Product	Authorisation Number(s)	Active substance	a.s. content	Formulation type
V10	-	Mild PepMV isolate VX1 Mild PepMV isolate VC1	5-25 mg/L 5-25 mg/L	SC
VC1	-	Mild PepMV isolate VC1	5-25 mg/L	SC
VX1	-	Mild PepMV isolate VX1	5-25 mg/L	SC

Application methods
Application of products was made with a high-pressure spraying arm. The spray volume ranged between 0.26 and 1.6 L/m^2. Carborundum was added to the spray solution to provide enough abrasion to introduce virus into the plant cells.

Inoculation with the aggressive virus strains was carried out by rubbing the virus suspension (sap from the upper leaves of tomato plants infected with virus) on two leaves of each plant.

Two-three weeks after application, leaf samples (one sample per plant) were tested using ELISA to ensure the absence of virus in the control treatment and to check whether the plants were 100% infected with the mild and the aggressive, 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.

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

Details of application rates and timings are provided in Table IIIA 6.1.3-3.

Details of assessments and assessment timings are provided in Table IIIA 6.1.3-4.

Table IIIA 6.1.3-3a Rates of application and timing of applications - V10 in tomato

Test report	Treatment				Inoculation		
	Product	**Active substance**	**Rate**	**Application timing**	**Product**	**Active substance**	**Application timing**
I-12-6701-1	untreated	-		-	agEU agCH	PepMV EU PepMV CH	A2: 12.04.2012
	untreated	-		-	-	-	-
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	A1: 22.03.2012	agEU agCH	PepMV EU PepMV CH	A2: 12.04.2012
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	A1: 22.03.2012	-	-	-
	VC1	Mild PepMV VC1	2%	A1: 22.03.2012	agCH	PepMV CH	A2: 12.04.2012
	VX1	Mild PepMV VX1	2%	A1: 22.03.2012	agEU	PepMV EU	A2: 12.04.2012
I-12-6701-2	untreated	-		-	agEU agCH	PepMV EU PepMV CH	A2: 12.04.2012
	untreated	-		-	-	-	-
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	A1: 22.03.2012	agEU agCH	PepMV EU PepMV CH	A2: 12.04.2012
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	A1: 22.03.2012	-	-	-
	VC1	Mild PepMV VC1	2%	A1: 22.03.2012	agCH	PepMV CH	A2: 12.04.2012
	VX1	Mild PepMV VX1	2%	A1: 22.03.2012	agEU	PepMV EU	A2: 12.04.2012
I-12-6702-1	untreated	-		-	agEU agCH	PepMV EU PepMV CH	A2: 23.10.2012
	untreated	-		-	-	-	
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	A1: 02.10.2012	agEU agCH	PepMV EU PepMV CH	A2: 23.10.2012
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	A1: 02.10.2012	-	-	
	VC1	Mild PepMV VC1	2%	A1: 02.10.2012	agCH	PepMV CH	A2: 23.10.2012
	VX1	Mild PepMV VX1	2%	A1: 02.10.2012	agEU	PepMV EU	A2: 23.10.2012

Test report	Treatment				Inoculation		
	Product	**Active substance**	**Rate**	**Application timing**	**Product**	**Active substance**	**Application timing**
I-12-6702-2	untreated	-		-	agEU agCH	PepMV EU PepMV CH	A2: 24.10.2012
	untreated	-		-	-	-	
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	A1: 02.10.2012	agEU agCH	PepMV EU PepMV CH	A2: 24.10.2012-15.11.2012
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	A1: 02.10.2012	-	-	
	VC1	Mild PepMV VC1	2%	A1: 02.10.2012	agEU agCH	PepMV CH	A2: 24.10.2012-15.11.2012
	VX1	Mild PepMV VX1	2%	A1: 02.10.2012	agEU agCH	PepMV EU	A2: 24.10.2012-15.11.2012
I-12-6703-1	untreated	-		-	agEU	PepMV EU	
	VX1	Mild PepMV VX1	2%	A1: 23-30.11.2012	agEU	PepMV EU	A2: 01.02.2013
I-13-6701-1	untreated	-		-	agEU agCH	PepMV EU PepMV CH	A2: 21.03.2013
	untreated	-		-	-	-	
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	A1: 01.02.2013	agEU agCH	PepMV EU PepMV CH	A2: 21.03.2013
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	A1: 01.02.2013	-	-	
	VC1	Mild PepMV VC1	2%	A1: 01.02.2013	agCH	PepMV CH	A2: 21.03.2013
	VX1	Mild PepMV VX1	2%	A1: 01.02.2013	agEU	PepMV EU	A2: 21.03.2013
I-13-6701-2	untreated	-		-	agEU agCH	PepMV EU PepMV CH	A2: 22.03.2013
	untreated	-		-	-	-	
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	A1: 01.02.2013	agEU agCH	PepMV EU PepMV CH	A2: 22.03.2013
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	A1: 01.02.2013	-	-	
	VC1	Mild PepMV VC1	2%	A1: 01.02.2013	agCH	PepMV CH	A2: 22.03.2013

Test report	Treatment				Inoculation		
	Product	**Active substance**	**Rate**	**Application timing**	**Product**	**Active substance**	**Application timing**
	VX1	Mild PepMV VX1	2%	A1: 01.02.2013	agEU	PepMV EU	A2: 22.03.2013
I-13-6701-3	untreated	-		-	agEU agCH	PepMV EU PepMV CH	A2: 21.03.2013
	untreated	-		-	-	-	
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	A1: 01.02.2013	agEU agCH	PepMV EU PepMV CH	A2: 21.03.2013
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	A1: 01.02.2013	-	-	
	VC1	Mild PepMV VC1	2%	A1: 01.02.2013	agCH	PepMV CH	A2: 21.03.2013
	VX1	Mild PepMV VX1	2%	A1: 01.02.2013	agEU	PepMV EU	A2: 21.03.2013

Carborundum was added to the spray solutions to provide enough abrasion to introduce the virus into the plant cells.

Virus isolates:
VX1 mild Peruvian strain
VC1 mild Chile-2 strain
agEU aggressive European strain
agCH aggressive Chile strain

Table IIIA 6.1.3-4a Details of assessments carried out in efficacy tests - V10 in tomato

Test Report	Date of Assessment	Assessment type
I-12-6701-1	05.04.2012	nettle head, mosaic, necrosis leaf, necrosis stem
	11.04.2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spots, crop vigour
	24.04.2012	nettle head, mosaic, necrosis leaf, necrosis stem, chlorosis, crop vigour
	03.05.2012	nettle head, mosaic, necrosis leaf, necrosis stem, crop vigour
	11.05.2012	nettle head, mosaic, necrosis leaf, necrosis stem, flowering, setting of trusses, crop vigour
	16.05.2012	nettle head, mosaic, necrosis leaf, necrosis stem, crop vigour
	04.06.2012	nettle head, mosaic, necrosis leaf, necrosis stem, flowering setting of trusses, crop vigour
	22.06.2012	nettle head, mosaic, necrosis leaf, necrosis stem, crop vigour
	as fruits developed	blotchy fruits, marble fruits, damaged fruits, deformed fruits, canker fruits, open fruits, damage calyx, damage stem trusses
	-	yield
I-12-6701-2	25.04.2012	mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour
	02.05.2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour
	09.05.2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, setting of trusses, flowering, crop vigour
	23.05.2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, setting of trusses, flowering, crop vigour
	30.05.2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour
	13.06.2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour
	20.06.2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour
	27.06.2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour
	04.07.2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour
	as fruits develop	blotchy fruits, marble fruits, damaged fruits, deformed fruits, canker fruits, open fruits, damage calyx
	-	yield
I-12-6702-1	23.10.2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	05.11.2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	13.11.2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	20.11.2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	26.11.2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	10.12.2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	23.12.2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	as fruits develop	blotchy fruits, marble fruits, damaged fruits, deformed fruits, canker fruits, open fruits, damage calyx, damage stem trusses
	-	yield

Test Report	Date of Assessment	Assessment type
I-12-6702-2	wk 43, 2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	wk 45, 2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	wk 46, 2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	wk 47, 2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour, flowering, setting of trusses
	wk 48, 2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour, flowering, setting of trusses
	wk 50, 2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour, flowering, setting of trusses
	wk 52, 2012	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour, flowering, setting of trusses
	wk 2, 2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	wk 3, 2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	wk 4, 2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	as fruits develop	blotchy fruits, marble fruits, damaged fruits, deformed fruits, canker fruits, open fruits, damage calyx
	-	yield
I-12-6703-1	14.02.2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	22.02.2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour, flowering and setting of trusses
	01.03.2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	08.03.2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour, flowering and setting of trusses
	25.03.2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	05.04.2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	15.04.2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	22.04.2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	29.04.2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	as fruits develop	blotchy fruits, marble fruits, damaged fruits, deformed fruits, canker fruits, open fruits, damage calyx
	-	yield
I-13-6701-1	15.03.2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	29.03.2013	flowering and setting of trusses
	12.04.2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	19.04.2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	26.04.2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour

Test Report	Date of Assessment	Assessment type
	03.05.2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	24.05.2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, crop vigour
	as fruits develop	blotchy fruits, marble fruits, damaged fruits, deformed fruits, blossom end rot, open fruits, damage stem trusses
	-	yield
I-13-6701-2	wk 14, 2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour, flowering and setting of trusses
	wk 16, 2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour
	wk 17, 2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour, flowering and setting of trusses
	wk 18, 2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour
	wk 19, 2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour
	wk 20, 2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour
	wk 21. 2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour
	wk 22, 2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour
	wk 23, 2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour
	wk 24, 2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour
	as fruits develop	blotchy fruits, marble fruits, damaged fruits, deformed fruits, blossom end rot, open fruits, damage calyx
	-	yield
I-13-6701-3	12.04.2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour
	19.04.2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour
	wk 16, 2013	flowering and setting of trusses
	26.04.2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour
	03.05.2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour
	10.05.2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour
	24.05.2013	nettle head, mosaic, necrosis leaf, necrosis stem, yellow spot, chlorosis, crop vigour
	as fruits develop	blotchy fruits, marble fruits, damaged fruits, deformed fruits, blossom end rot, open fruits, damage stem trusses
	-	yield

IIIA 6.1.3.1 Summary and evaluation of individual trials results – V10 in tomato

Proposed label claim:
V10 is applied preventatively on young tomato plants (BBCH 13-51, plant height of 10-30 cm) by spraying at 2% or it is applied on tomato plants (BBCH 13-61) by rubbing the suspension on leaves of the plants at 10%.
V10 is to be applied in combination with carborundum (8 g carborundum/1 L spray liquid for spray application and 15 g carborundum/1 L liquid for the rubbing application).

Efficacy
The effectiveness of mild Pepino Mosaic Virus against aggressive Pepino Mosaic Virus (PEPMVO) was tested in a total of 8 efficacy trials conducted in greenhouses in the Netherlands in 2012 and 2013. In 7 trials the effectiveness of the product V10 was tested. In 1 trial the effectiveness of only isolate VX1 was tested.

V10
The product V10, a combination of mild PepMV isolates VC1 and VX1, was tested in 7 out of 8 trials. The product was applied by spraying at 2% in combination with carborundum.
Artificial infection was made using aggressive PepMV isolates EU and CH, isolates from respectively the European PepMV and Chile PepMV.

Assessment was made on viral symptoms on the leaves and on fruits, on yield and crop stand. The effect of V10 on infected tomato plants was compared to untreated infected plants.

Symptoms on leaves
Assessments were made on nettle head, mosaic, leaf necrosis, stem necrosis, yellow spots and chlorosis. In Table 6.1.3.1-1 an overview of the symptoms on leaves is given. Yellow spots and chlorosis occurred only rarely and are therefore not summarized here.

Table 6.1.3.1-1 Symptoms on leaves

Symptom	Number of trials	% area of leaves affected Mean (Min-Max)	
		Untreated infected	2% V10 infected
nettle head	7	49.3 (26-71.9)	7.4 (3-10)
mosaic	6	15.1 (0-63)	2.4 (0-7)
leaf necrosis	7	44.9 (6.7-72)	2.7 (0-10.4)
stem necrosis	7	20.2 (0.1-49)	0.5 (0-1.5)

Treatment with V10 achieved a substantial and often significant reduction of PepMV symptoms on leaves of tomato plants in relation to the untreated infected plants.
The percentage area of the leaves that was affected after treatment with V10 was low (maximal 10%) as compared to the untreated infected plants (maximal 72%).

Symptoms on fruits
Assessments were made on blotchy, marble, damaged, deformed, cankerous and open fruits, on damage to the calyx, stem trusses and on blossom end rot. Assessments on blotchy,

marble, damaged and deformed fruits were made in all trials. In 1 trial no symptoms on fruits were observed in any of the objects. This trial was excluded from the summary.
In Table 6.1.3.1-2 an overview of the symptoms on fruits is given. Cankerous and open fruits were observed only rarely and are therefore not summarized here.

Table 6.1.3.1-2 Symptoms on fruits

Symptom	Number of trials	% of fruits affected Mean (Min-Max)	
		Untreated infected	2% V10 infected
blotchy	6	27.9 (1.2-100)	2.8 (0.4-6)
marble	6	4.7 (0.3-21)	0.6 (0-0.9)
damaged	6	19.5 (0-100)	4.9 (0-18.1)
deformed	6	21.2 (0-100)	3.4 (0-15.3)
damage to calyx	3	61.2 (16-100)	16.4 (0-49.2)
damage to stem trusses	4	36.5 (0-75)	0
blossom end rot	3	5 (0-11.8)	1.3 (0-3.7)

PepMV symptoms on fruits were much reduced after treatment with V10 in relation to fruits of untreated infected plants. The percentage fruits with symptoms in the V10 treated plants was generally low (generally maximal 18%, with exception of 49% calyx damage in 1 trial) as compared to fruits with symptoms in the untreated infected plants (maximal 100%).

Yield
All trials were taken to yield. In Table 6.1.3.1-3 an overview of yield and relative yield in relation to the untreated infected objects is given.

Table 6.1.3.1-3 Yield

Assessment	Number of trials	Yield Mean (Min-Max)	Relative yield (%) Mean (Min-Max)
		Untreated infected	2% V10 infected
Number of fruits	7	396 (0.8-872)	4249 (95-28875)
	6*	462 (8-872)	145 (95-353)
Total weight (kg)	7	44.3 (0.02-97)	8436 (73-47500)
	5*	62 (36-97)	111 (73-139)
Fruit weight (g)	7	79 (5.8-131)	223 (108-848)
	6*	91 (28-131)	119 (108-129)

* The trial(s) with very low yields in the untreated objects were excluded from the summary to prevent distortion of the data.

With the exception of 1 trial, in which the number of fruits of plants treated with V10 was slightly lower (relative yield of 95%) and the total weight lower (relative yield of 73%), treatment with V10 resulted in a substantial increase, often significant, in the number of fruits and the total weight of harvested fruits in relation to the untreated objects.
Over all trials the individual fruit weight was increased after treatment with V10 in relation to the untreated infected objects.

Flowering, setting of trusses
In all trials assessment was made on the number of flowers and the number of setting trusses. Though in 2 trials a significant increase in the number of flowers and setting trusses was observed in relation to the untreated infected objects, the effect was not consistent throughout the trials.

Stunting
Stunting was assessed in all trials. In Table 6.1.3.1-4 an overview of stunting is given.

Table 6.1.3.1-4 Stunting

Number of trials	Stunting (%) Mean (Min-Max)	
	Untreated infected	2% V10 infected
7	39.3 (0-80.4)	3.4 (0-9.4)

In 2 trials no stunting was observed in any of the objects. In the remaining 5 trials, treatment with V10 resulted in a significant decrease in stunting in relation to the untreated infected objects.

Crop condition
Crop condition was assessed weekly in all trials. Crop condition was assessed on a scale of 1-10 with 1: crop dead and 10: excellent crop. As would be expected, the crop condition of untreated infected plants worsened as the virus symptoms increased. The lowest crop condition per trial in the untreated infected objects ranged between crop dead and bad/very bad crop condition (scores of 1-3.5). The crop condition of plants treated with V10 was substantially better than that of the untreated infected objects. Crop condition of plants treated with V10 was generally good. The lowest crop stand per trial ranged between moderate/ reasonable crop condition to reasonable/ good crop condition (score range of 6.5-7.8).

Based on the presented data it can be concluded that spray application of V10 is effective in the prevention of the European and Chile strains of the Pepino Mosaic Virus.

<u>VC1 and VX1</u>
In the efficacy trials, the effectiveness of the mild PepMV isolates VC1 and VX1 were also tested separately.
Plants treated with VC1 were artificially inoculated with the aggressive PepMV isolate CH (Chile PepMV) in 6 trials.
Plants treated with VX1 were artificially inoculated with the aggressive PepMV isolate EU (European PepMV) in 7 trials.

Assessment was made on viral symptoms on the leaves and on fruits, on yield and crop stand.

The severity and incidence of virus symptoms on the plants treated with the isolates separately, VC1 inoculated with agCH and VX1 inoculated with agEU, were generally somewhat lower as compared to the plants treated with the combination of the isolates (V10 inoculated with agCH+agEU). However the severity of the separate virus infections of the aggressive CH and EU isolates was not tested (untreated inoculated with agCH and untreated inoculated with agEU).
In 1 trial (I-13-6701-1), the effect of treatment with VX1 against PepMV isolate EU was compared to untreated plants infected with PepMV isolate EU. VX1 was effective in the reduction of symptoms in leaves and in fruits, the reduction of stunting and a positive effect on crop condition in relation to the untreated objects infected with agEU. However no positive effect on yield was observed.

Rubbing
Application of V10 by rubbing the virus suspension on tomato leaves was not tested in the efficacy trials. However, in these trials, the inoculation of the aggressive virus isolates was made by rubbing. In these trials, as was confirmed by ELISA testing, all plants were successfully infected with the aggressive virus isolates.
Also, it was demonstrated in a bioassay that this method was effective in infecting tomato seedlings with VX1 and VC1 virus suspension (please refer to IIIA 6.1.1).
Furthermore, mechanical inoculation of viruses by rubbing the virus suspension on leaves is a well-known and regularly used method for virus inoculation in scientific research.

Minimum effective dose
No minimum effective dose trials are available for the product V10. However, since the isolates VX1 and VC1 will replicate within the crop, efficacy of the product will be independent of the concentration of the applied product once the plant is infected. The effectiveness of the product depends on the infectivity of the virus isolates VC1 and VX1. As a result, standard minimum effective dose trials are not considered useful.

In a bioassay conducted on tomato seedlings it was shown that a concentration of 1-10% was sufficiently infective when applied by rubbing on tomato leaves (please refer to IIIA 6.1.1).

No reliable data is available for determination of the minimum required concentration of V10 during spray applications. Successful infection of the crop by spray application will largely depend on the mechanical damage inflicted to the crop through which the infection can occur. The rate at which the crop is infected is therefore determined by many different factors such as the distance of the spray boom from the crop and the spray pressure at application, the amount of abrasive in the spray solution, the age of the crop and the crop cultivar. To ensure good infection of the crop under varying conditions the proposed dose rate was set at 2%. The product is applied at a high spray volume of 3500 L/ha, with 0.15% w/v synthetic sand to ensure that enough mechanical damage occurs to the crop to facilitate infection and that no blockage of the spray nozzles occurs.
The proposed dose rate of 2% V10 has demonstrated to be effective in the prevention of PepMV virus in the efficacy trials.

Table IIIA 6.1.3-6 Summary of effectiveness data for V10 in tomato

	Symptom 'nettle head'– % area of leaves affected					
Treatment	**Untreated**	**Untreated**	**V10 at 2%**	**V10 at 2%**	**VC1at 2%**	**VX1 at 2%**
Inoculation	**agEU+agCH**	**-**	**agEU+agCH**	**-**	**agCH**	**agEU**
Test report						
I-12-6701-1	48.9	0.0	7.3	6.7	8.1	3.1
I-12-6701-2	71.9	0.0	7.8	2.5	5.2	6.6
I-12-6702-1	84	1	8	9	6	2
I-12-6702-2	27.0	0.0	7.0	7.7	16.0***	5.2***
I-12-6702-3	24.2*	-	-	-	-	2.8
I-13-6701-1	26.0	0.0	3.0	3.0	2.0	0.0
I-13-6701-2	58	0	10	5	2	0
I-13-6701-3	29	2 **	9	4	5	4
Mean (n=7)	**49.3**	**0.4**	**7.4**	**5.4**		
Mean (n=6)	**53.0**	**0.5**	**7.5**	**5.0**	**4.7**	**2.6**

* Untreated was inoculated with only agEU.
** Contaminated with V10
*** Accidently inoculated with both agEU and agCH

	Symptom 'mosaic'– % area of leaves affected					
Treatment	**Untreated**	**Untreated**	**V10 at 2%**	**V10 at 2%**	**VC1at 2%**	**VX1 at 2%**
Inoculation	**agEU+agCH**	**-**	**agEU+agCH**	**-**	**agCH**	**agEU**
Test report						
I-12-6701-1	0.7	0	0.6	1.9	2.2	0
I-12-6702-1	0	0	1.8	2.5	2.1	0
I-12-6702-2	27	0	5	5	16***	5***
I-12-6702-3	29.6*	0	-	-	-	3.8
I-13-6701-1	0	0	0	0	0	0
I-13-6701-2	63	0	7	6	5	3
I-13-6701-3	0	0**	0	0	0	0
Mean (n=6)	**15.1**	**0**	**2.4**	**2.6**	**-**	**-**
Mean (n=5)	**12.7**	**0**	**1.9**	**2.1**	**1.9**	**0.6**

* Untreated was inoculated with only agEU.
** Contaminated with V10
*** Accidently inoculated with both agEU and agCH

Table IIIA 6.1.3-6 Summary of effectiveness data for V10 in tomato

	Symptom 'necrosis leaf'– % area of leaves affected					
Treatment	**Untreated**	**Untreated**	**V10 at 2%**	**V10 at 2%**	**VC1at 2%**	**VX1 at 2%**
Inoculation	**agEU+agCH**	**-**	**agEU+agCH**	**-**	**agCH**	**agEU**
Test report						
I-12-6701-1	6.7	0	0	0	0	0
I-12-6701-2	59.4	0	4.5	0	7.9	6.7
I-12-6702-1	68	0	3	0	0	0
I-12-6702-2	72	0	0	0	37***	15***
I-12-6703-1	24.3*	-	-	-	-	0.2
I-13-6701-1	27.1	0.2	0	0.2	0	0
I-13-6701-2	64.8	0	10.4	0	0	0.5
I-13-6701-3	16	0**	1	0	0	0
Mean (n=7)	**44.9**	**0**	**2.7**	**0**	**-**	**-**
Mean (n=6)	**40.3**	**0**	**3.2**	**0**	**1.3**	**1.2**

* Untreated was inoculated with only agEU.
** Contaminated with V10
*** Accidently inoculated with both agEU and agCH

	Symptom 'necrosis stem'– % area of leaves affected					
Treatment	**Untreated**	**Untreated**	**V10 at 2%**	**V10 at 2%**	**VC1at 2%**	**VX1 at 2%**
Inoculation	**agEU+agCH**	**-**	**agEU+agCH**	**-**	**agCH**	**agEU**
Test report						
I-12-6701-1	0.1	0	0	0	0	0
I-12-6701-2	12.5	0	1.5	0	1	0.4
I-12-6702-1	29	0	1	0	0	0
I-12-6702-2	49	0	0	0	29***	14***
I-12-6703-1	9.2*	-	-	-	-	0
I-13-6701-1	5.7	0	0	0	0	0
I-13-6701-2	26	0	1	0	0	0
I-13-6701-3	19	0**	0	0	0	0
Mean (n=7)	**20.2**	**0**	**0.5**	**0**	**-**	**-**
Mean (n=6)	**15.4**	**0**	**0.6**	**0**	**0.2**	**0.1**

* Untreated was inoculated with only agEU.
** Contaminated with V10
*** Accidently inoculated with both agEU and agCH

Table IIIA 6.1.3-6 Summary of effectiveness data for V10 in tomato

	Symptom 'blotchy'– % of fruits affected					
Treatment	**Untreated**	**Untreated**	**V10 at 2%**	**V10 at 2%**	**VC1at 2%**	**VX1 at 2%**
Inoculation	**agEU+agCH**	**-**	**agEU+agCH**	**-**	**agCH**	**agEU**
Test report						
I-12-6701-1	11	2	1	1	0	1
I-12-6701-2	44	1	6	4	6	3
I-12-6702-2	100	0	5.6	0.2	27.3***	0.9***
I-12-6703-1	20*	-	-	-	-	2
I-13-6701-1	1.2	0	1.0	0.9	1.2	0.3
I-13-6701-2	2.5	0.1	0.4	0.6	0.5	0.2
I-13-6701-3	8.7	3.6**	2.9	0.9	1.7	2.5
Mean (n=6)	**27.9**	**1.1**	**2.8**	**1.3**	**-**	**-**
Mean (n=5)	**13.5**	**1.3**	**2.3**	**1.5**	**1.9**	**1.4**

* Untreated was inoculated with only agEU.
** Contaminated with V10
*** Accidently inoculated with both agEU and agCH

	Symptom 'marble'– % of fruits affected					
Treatment	**Untreated**	**Untreated**	**V10 at 2%**	**V10 at 2%**	**VC1at 2%**	**VX1 at 2%**
Inoculation	**agEU+agCH**	**-**	**agEU+agCH**	**-**	**agCH**	**agEU**
Test report						
I-12-6701-1	1.5	1.6	0	0	0	0.5
I-12-6701-2	0.8	0	0.5	0.1	0	0.2
I-12-6702-2	21	0	2	0	6***	0***
I-12-6703-1	1.4*	-	-	-	-	0
I-13-6701-1	2.3	0	0.9	1.4	0.6	0.1
I-13-6701-2	0.3	0	0	0	0.1	0
I-13-6701-3	2.1	0.9**	0.1	0	0	1.9
Mean (n=6)	**4.7**	**0.4**	**0.6**	**0.3**	**-**	**-**
Mean (n=5)	**1.4**	**0.5**	**0.3**	**0.3**	**0.1**	**0.5**

* Untreated was inoculated with only agEU.
** Contaminated with V10
*** Accidently inoculated with both agEU and agCH

Table IIIA 6.1.3-6 Summary of effectiveness data for V10 in tomato

	Symptom damaged fruits– % of fruits affected					
Treatment	**Untreated**	**Untreated**	**V10 at 2%**	**V10 at 2%**	**VC1at 2%**	**VX1 at 2%**
Inoculation	**agEU+agCH**	**-**	**agEU+agCH**	**-**	**agCH**	**agEU**
Test report						
I-12-6701-1	0.2	0	0	0	0	0
I-12-6701-2	11.2	2.6	8.9	5.9	7.3	5.0
I-12-6702-2	100	1.7	18.1	0.8	58.3***	3.6***
I-12-6703-1	23.9*	-	-	-	-	3.9
I-13-6701-1	0	0	0	0	0	0
I-13-6701-2	4.6	0.6	1.3	0.8	1.1	0.8
I-13-6701-3	1.1	1.2**	0.9	0.9	0.5	1.3
Mean (n=6)	**19.5**	**1.0**	**4.9**	**1.4**	**-**	**-**
Mean (n=5)	**3.4**	**0.9**	**2.2**	**1.5**	**1.8**	**1.4**

* Untreated was inoculated with only agEU.
** Contaminated with V10
*** Accidently inoculated with both agEU and agCH

	Symptom deformed fruits– % of fruits affected					
Treatment	**Untreated**	**Untreated**	**V10 at 2%**	**V10 at 2%**	**VC1at 2%**	**VX1 at 2%**
Inoculation	**agEU+agCH**	**-**	**agEU+agCH**	**-**	**agCH**	**agEU**
Test report						
I-12-6701-1	0.4	0	0	0	0	0
I-12-6701-2	23.7	0.4	5.2	1.6	3.7	1.4
I-12-6702-2	100	0.3	15.3	0.1	70.2***	4.5***
I-12-6703-1	19.8*	-	-	-	-	2.5
I-13-6701-1	1.0	0.1	0	0	0	0
I-13-6701-2	2.1	0.1	0.1	0.1	0.1	0.1
I-13-6701-3	0	0**	0	0	0	0
Mean (n=6)	**21.2**	**0.2**	**3.4**	**0.3**	**-**	**-**
Mean (n=5)	**5.4**	**0.1**	**1.1**	**0.3**	**0.8**	**0.3**

* Untreated was inoculated with only agEU.
** Contaminated with V10
*** Accidently inoculated with both agEU and agCH

Table IIIA 6.1.3-6 Summary of effectiveness data for V10 in tomato

	Symptom damage to calyx– % of fruits affected					
Treatment	**Untreated**	**Untreated**	**V10 at 2%**	**V10 at 2%**	**VC1at 2%**	**VX1 at 2%**
Inoculation	**agEU+agCH**	**-**	**agEU+agCH**	**-**	**agCH**	**agEU**
Test report						
I-12-6701-1	67.6	0	0	0	0	0
I-12-6702-2	100	0	49.2	0	92.5**	0**
I-12-6703-1	67*	-	-	-	-	0
I-13-6701-2	16	0	0	0	0	0
Mean (n=3)	**61.2**	**0**	**16.4**	**0**	**-**	**-**
Mean (n=2)	**41.8**	**0**	**0**	**0**	**0**	**0**

* Untreated was inoculated with only agEU.
** Accidently inoculated with both agEU and agCH

	Symptom damage to stem trusses – % of fruits affected					
Treatment	**Untreated**	**Untreated**	**V10 at 2%**	**V10 at 2%**	**VC1at 2%**	**VX1 at 2%**
Inoculation	**agEU+agCH**	**-**	**agEU+agCH**	**-**	**agCH**	**agEU**
Test report						
I-12-6701-1	70	0	0	0	0	0
I-12-6701-2	75	0	0	0	0	0
I-13-6701-1	0	0	0	0	0	0
I-13-6701-3	0.9	0*	0	0	0	0.3
Mean (n=4)	**36.5**	**0**	**0**	**0**	**0**	**0.1**

* Contaminated with V10

	Symptom blossom end rot– % of fruits affected					
Treatment	**Untreated**	**Untreated**	**V10 at 2%**	**V10 at 2%**	**VC1at 2%**	**VX1 at 2%**
Inoculation	**agEU+agCH**	**-**	**agEU+agCH**	**-**	**agCH**	**agEU**
Test report						
I-13-6701-1	3.2	0	0.3	0.2	0.2	0
I-13-6701-2	0	0	0	0	0	0
I-13-6701-3	11.8	2.9*	3.7	2.9	5.4	7.9
Mean (n=3)	**5**	**1.0**	**1.3**	**1.0**	**1.9**	**2.6**

* Contaminated with V10

Table IIIA 6.1.3-6 Summary of effectiveness data for V10 in tomato

	Yield (#)	Yield (number of fruits) - % relative to untreated				
Treatment	**Untreated**	**Untreated**	**V10 at 2%**	**V10 at 2%**	**VC1at 2%**	**VX1 at 2%**
Inoculation	**agEU+agCH**	**-**	**agEU+agCH**	**-**	**agCH**	**agEU**
Test report						
I-12-6701-1	515	105	106	106	105	106
I-12-6701-2	413	122	113	116	118	119
I-12-6702-1	8	356	353	360	371	357
I-12-6702-2	0.8	35588	28875	35275	22400	34375
I-12-6703-1	337*	-	-	-	-	95
I-13-6701-1	541	101	103	99	101***	99***
I-13-6701-2	425	101	101	96	100	99
I-13-6701-3	872	80**	95	88	93	88
Mean (n=7)	**396**	**5208**	**4249**	**5163**	**-**	**-**
Mean (n=6) (without I-12-6702-2)	**462**	**144**	**145**	**144**	**-**	**-**
Mean (n=5) (without I-12-6702-2)	**447**	**153**	**154**	**153**	**157**	**154**

* Untreated was inoculated with only agEU.
** Contaminated with V10
*** Accidently inoculated with both agEU and agCH

Table IIIA 6.1.3-6 Summary of effectiveness data for V10 in tomato

	Yield (kg)	Yield (total weight) - % relative to untreated				
Treatment	**Untreated**	**Untreated**	**V10 at 2%**	**V10 at 2%**	**VC1at 2%**	**VX1 at 2%**
Inoculation	**agEU+agCH**	**-**	**agEU+agCH**	**-**	**agCH**	**agEU**
Test report						
I-12-6701-1	46.1	123	123	123	124	115
I-12-6701-2	37.6	149	139	140	137	138
I-12-6702-1	0.1	12300	11000	11500	11800	11700
I-12-6702-2	0.02	59400	47500	57850	19650	56500
I-12-6703-1	30*	-	--	-	-	90
I-13-6701-1	93.6	100	108	114	110***	107***
I-13-6701-2	36	114	111	103	108	108
I-13-6701-3	97	74**	73	88	98	89
Mean (n=7)	**44.3**	**12031**	**8436**	**9988**	**-**	**-**
Mean (n=5) (without I-6702-1&2)	**62**	**122**	**111**	**114**	**-**	**-**
Mean (n=4) (without I-6702-1&2)	**54**	**129**	**112**	**114**	**117**	**113**

* Untreated was inoculated with only agEU.
** Contaminated with V10
*** Accidently inoculated with both agEU and agCH

Table IIIA 6.1.3-6 Summary of effectiveness data for V10 in tomato

	Yield (g)	Yield (fruit weight) - % relative to untreated				
Treatment	**Untreated**	**Untreated**	**V10 at 2%**	**V10 at 2%**	**VC1at 2%**	**VX1 at 2%**
Inoculation	**agEU+agCH**	**-**	**agEU+agCH**	**-**	**agCH**	**agEU**
Test report						
I-12-6701-1	109	117	116	116	118	109
I-12-6701-2	102	127	123	125	104	115
I-12-6702-1	28	136	129	129	129	132
I-12-6702-2	5.8	812	848	803	510	824
I-12-6703-1	89.8*	-	-	-	-	95
I-13-6701-1	85	122	119	128	121***	121***
I-13-6701-2	93	113	116	113	116	111
I-13-6701-3	131	95**	108	104	107	109
Mean (n=7)	**79**	**217**	**223**	**217**	**-**	**-**
Mean (n=6) (without I-12-6702-2)	**91**	**118**	**119**	**119**	**-**	**-**
Mean (n=5) (without I-12-6702-2)	**93**	**118**	**118**	**117**	**115**	**115**

* Untreated was inoculated with only agEU.
** Contaminated with V10
*** Accidently inoculated with both agEU and agCH

	Yield (number of fruits)		Yield (total weight)		Yield (fruit weight)	
	# of fruits	**% relative to untreated**	**kg fruits**	**% relative to untreated**	**g/fruit**	**% relative to untreated**
Treatment	**Untreated**	**V10 at 2%**	**Untreated**	**V10 at 2%**	**Untreated**	**V10 at 2%**
Inoculation	**-**	**-**	**-**	**-**	**-**	**-**
Test report						
I-12-6701-1	542	100	56.6	100	128	98
I-12-6701-2	503	95	56.1	94	130	98
I-12-6702-1	355.5	101	12.3	93	38	95
I-12-6702-2	284.7	99	11.9	97	47.1	97
I-13-6701-1	548	98	43.1	110	93.8	114
I-13-6701-2	431	114	41	90	105	100
Mean (n=6)	**444**	**98**	**36.8**	**97**	**90.3**	**101**

Table IIIA 6.1.3-6 Summary of effectiveness data for V10 in tomato

	Stunting (%)					
Treatment	**Untreated**	**Untreated**	**V10 at 2%**	**V10 at 2%**	**VC1at 2%**	**VX1 at 2%**
Inoculation	**agEU+agCH**	**-**	**agEU+agCH**	**-**	**agCH**	**agEU**
Test report						
I-12-6701-1	40	0	0	1	1	0
I-12-6701-2	71.9	0	7.8	2.5	5.2	6.6
I-12-6702-1	75	0	5	8	3	0
I-12-6702-2	80.4	3	9.4	9	50***	23.5***
I-12-6703-1	41.5*	-	-	-	-	0.8
I-13-6701-1	8.1	0	1.7	0.8	0.8	0
I-13-6701-2	0	0	0	0	0	0
I-13-6701-3	0	0**	0	0	0	0
Mean (n=7)	**39.3**	**0.4**	**3.4**	**3.0**	**-**	**-**
Mean (n=6)	**32.5**	**0**	**2.4**	**2.1**	**1.7**	**1.1**

* Untreated was inoculated with only agEU.
** Contaminated with V10
*** Accidently inoculated with both agEU and agCH
*** Accidently inoculated with both agEU and agCH

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Type of trials: effectiveness / phytotoxicity / other : Effectiveness/phytotoxicity
Identity of the product under test (commercial name (s), active substance (s), content, formulation type (s) : V10 (5-25 mg/L Mild PepMV isolate VX1, 5-25 mg/L Mild PepMV isolate VC1)
Crop : Tomato (*Solanum lycopersicum,* LYPES)
Harmful organism (common name, scientific name, Bayer Code) or intended use : Pepino Mosaic Virus (PepMV) (PEPMVO)
Responsible body for reporting trial (name, address and telephone number) : Valto B.V. De Lier, Leehove 81, 2678 MB De Lier, the Netherlands
Date of submission : 2015

Test report	Testing Unit GEP	Trial location Crop cultivar F/G; N/A	Test method Plot size Sample size	Application details		Experimental Design Replicates
				Method	Date ; Growth Stage;	
I-12-6701-1	[redacted] GEP	[redacted] Tomato *Endeavour, Levanzo, Brioso, Komeett* G; A	PP 1/135(3) , PP 1/152(3), PP 1/181(3) 20 plants 20 plants	A1: Foliar spray 1.1-1.6 L/m^2 A2: Inoculation, rubbing	A1: 22.03.2012: BBCH 12-13 A2: 12.04.2012: BBCH 51	RBD 4

Test report	Treatment			Inoculation	Average pest severity on leaves (%) (average of 8 assessments from 05.04.2012-22.06.2012)			
	Product	Active substance	Rate		Nettle head	Mosaic	Necrosis leaf	Necrosis stem
I-12-6701-1	untreated	-		agEU + agCH	48.9 a	0.7 b	6.7 a	0.07 a
	untreated	-		-	0.0 e	0.0 c	0.0 b	0.00 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	7.3 bc	0.6 b	0.0 b	0.00 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	6.7 c	1.9 a	0.0 b	0.00 b
	VC1	Mild PepMV VC1	2%	agCH	8.1 b	2.2 a	0.0 b	0.00 b
	VX1	Mild PepMV VX1	2%	agEU	3.1 d	0.0 c	0.0 b	0.00 b

Additionally assessment was made on yellow spots and chlorosis on leaves, but these symptoms occurred only little and are therefore not shown.

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Test report	Treatment			Inoculation	Number of flowers		Number of trusses setting		Symptoms of fruits (% of fruits affected)	
	Product	Active substance	Rate		11.05.2012	04.06.2012	11.05.2012	04.06.2012	damage calyx	damage stem trusses
I-12-6701-1	untreated	-		agEU + agCH	4.8 ab	8.6 bc	4.2 ab	7.7 bc	67.6 a	70.0 a
	untreated	-		-	5.0 a	9.0 a	4.4 a	8.3 a	0.0 b	0.0 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	4.9 a	8.9 ab	4.2 ab	8.0 ab	0.0 b	0.0 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	4.6 b	8.6 bc	4.0 b	7.9 c	0.0 b	0.0 b
	VC1	Mild PepMV VC1	2%	agCH	4.8 ab	8.8 abc	4.0 b	8.0 ab	0.0 b	0.0 b
	VX1	Mild PepMV VX1	2%	agEU	4.9 a	9.0 a	4.1 b	8.1 ab	0.0 b	0.0 b

Test report	Treatment			Inoculation	Symptoms of fruits (% of fruits affected)					
	Product	Active substance	Rate		blotchy	marble	damaged	deformed	canker	open
I-12-6701-1	untreated	-		agEU + agCH	11 a	1.5 a	0.2 a	0.4 a	0.5 a	0.1 a
	untreated	-		-	2 b	1.6 a	0.0 b	0.0 b	0.8 a	0.0 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	1 b	0.0 b	0.0 b	0.0 b	0.4 a	0.0 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	1 b	0.0 b	0.0 b	0.0 b	0.7 a	0.0 b
	VC1	Mild PepMV VC1	2%	agCH	0 b	0.0 b	0.0 b	0.0 b	1.0 a	0.0 b
	VX1	Mild PepMV VX1	2%	agEU	1 b	0.5 ab	0.0 b	0.0 b	0.5 a	0.0 b

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Test report	Treatment			Inoculation	Yield (% relative)[1]		
	Product	Active substance	Rate		number of fruits	total weight	fruit weight
I-12-6701-1	untreated	-		agEU + agCH	100 b (515)	100 b (46.1)	100 c (109)
	untreated	-		-	105 a	123 a	117 a
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	106 a	123 a	116 ab
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	106 a	123 a	116 ab
	VC1	Mild PepMV VC1	2%	agCH	105 a	124 a	118 a
	VX1	Mild PepMV VX1	2%	agEU	106 a	115 a	109 b

[1] Between brackets respectively the number of fruits, total weight in kg and fruit weight in g.

Test report	Treatment			Inoculation	Damage (1-10)[1]	Stunting (%)	Crop condition (1-10)[2]	
	Product	Active substance	Rate				min	max
I-12-6701-1	untreated	-		agEU + agCH	8.5 b	40 a	1.0	10.0
	untreated	-		-	10 a	0 c	7.8	10.0
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	10 a	0 c	7.8	9.0
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	10 a	1 b	7.8	10.0
	VC1	Mild PepMV VC1	2%	agCH	10 a	1 b	8.0	9.0
	VX1	Mild PepMV VX1	2%	agEU	10 a	0 c	7.8	10.0

[1] Crop damage on a scale of 1-10 with 10: no damage, 1: crop dead
[2] Crop condition on a scale of 1-10 with 1: crop dead and 10: excellent crop

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Test report	Testing Unit GEP	Trial location Crop cultivar F/G; N/A	Test method Plot size Sample size	Application details		Experimental Design Replicates
				Method	Date ; Growth Stage;	
I-12-6701-2	[redacted] GEP	[redacted] Tomato *Endeavour, Levanzo, Brioso, Komeett* G; A	PP 1/135(3) , PP 1/152(3), PP 1/181(3) 20 plants 20 plants	A1: Foliar spray 1.1-1.6 L/m^2 A2: Inoculation, rubbing	A1: 22.03.2012: BBCH 12-13 A2: 12.04.2012: BBCH 51	RBD 4

Test report	Treatment			Inoculation	Average pest severity on leaves (%) (average of 8-9 assessments from 25.04.2012-04.07.2012)			
	Product	Active substance	Rate		Nettle head	Necrosis leaf	Necrosis stem	Chlorosis
I-12-6701-2	untreated	-		agEU + agCH	71.9 a	59.4 a	12.5 a	2.0 a
	untreated	-		-	0.0 f	0.0 d	0.0 d	0.0 d
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	7.8 b	4.5 c	1.5 b	1.1 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	2.5 e	0.0 d	0.0 d	0.6 bc
	VC1	Mild PepMV VC1	2%	agCH	5.2 d	7.9 b	1.0 b	0.8 bc
	VX1	Mild PepMV VX1	2%	agEU	6.6 c	6.7 b	0.4 c	0.3 c

Additionally assessment was made on yellow spots on leaves, but these symptoms occurred only little and are therefore not shown.

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Test report	Treatment			Inoculation	Number of trusses setting	
	Product	Active substance	Rate		10.05.2012	24.05.2012
I-12-6701-2	untreated	-		agEU + agCH	2.2 a	5.0 a
	untreated	-		-	1.6 b	4.4 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	1.8 b	4.6 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	1.8 b	4.7 b
	VC1	Mild PepMV VC1	2%	agCH	1.8 b	4.6 b
	VX1	Mild PepMV VX1	2%	agEU	1.7 b	4.6 b

Test report	Treatment			Inoculation	Symptoms of fruits (% of fruits affected)						
	Product	Active substance	Rate		blotchy	marble	damaged	deformed	canker	open	damage stem trusses
I-12-6701-2	untreated	-		agEU + agCH	44 a	0.8 a	11.2 a	23.7 a	4.1 a	0.2 ab	75.0 a
	untreated	-		-	1 b	0.0 b	2.6 cd	0.4 cd	1.1 bc	0.4 ab	0.0 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	6 b	0.5 ab	8.9 ab	5.2 b	2.2 bc	0.1 b	0.0 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	4 b	0.1 b	5.9 bc	1.6 cd	2.0 bc	0.5 ab	0.0 b
	VC1	Mild PepMV VC1	2%	agCH	6 b	0.0 b	7.3 bc	3.7 bc	3.2 ab	0.1 b	0.0 b
	VX1	Mild PepMV VX1	2%	agEU	3 b	0.2 ab	5.0 bcd	1.4 cd	1.9 bc	0.7 a	0.0 b

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Test report	Treatment			Inoculation	Yield (% relative)[1]		
	Product	Active substance	Rate		number of fruits	total weight	fruit weight
I-12-6701-2	untreated	-		agEU + agCH	100 b (413)	100 c (37.6)	100 a (102)
	untreated	-		-	122 a	149 a	127 a
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	113 ab	139 ab	123 a
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	116 a	140 ab	125 a
	VC1	Mild PepMV VC1	2%	agCH	118 a	137 b	104 a
	VX1	Mild PepMV VX1	2%	agEU	119 a	138 ab	115 a

[1] Between brackets respectively the number of fruits, total weight in kg and fruit weight in g.

Test report	Treatment			Inoculation	Stunting (%)	Crop condition (1-10)[1]	
	Product	Active substance	Rate			min	max
I-12-6701-2	untreated	-		agEU + agCH	71.9 a	3.0	5.0
	untreated	-		-	0.0 f	8.0	9.0
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	7.8 b	7.0	8.0
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	2.5 e	7.0	9.0
	VC1	Mild PepMV VC1	2%	agCH	5.2 d	7.0	8.0
	VX1	Mild PepMV VX1	2%	agEU	6.6 c	7.0	8.0

[1] Crop condition on a scale of 1-10 with 1: crop dead and 10: excellent crop

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Test report	Testing Unit GEP	Trial location Crop cultivar F/G; N/A	Test method Plot size Sample size	Application details		Experimental Design Replicates
				Method	Date ; Growth Stage;	
I-12-6702-1	[redacted] GEP	[redacted] Tomato *Endeavour, Levanzo, Brioso, Komeett* G; A	PP 1/135(3) , PP 1/152(3), PP 1/181(3) 20 plants 20 plants	A1: Foliar spray 0.26-0.44 L/m^2 A2: Inoculation, rubbing	A1: 02.10.2012: BBCH 13-14 A2: 23.10.2012: BBCH 61-62	RBD 4

Test report	Treatment			Inoculation	Average pest severity on leaves (%) (average of 7 assessments from 23.10.2012-23.12.2012)			
	Product	Active substance	Rate		Nettle head	Mosaic	Necrosis leaf	Necrosis stem
I-12-6702-1	untreated	-		agEU + agCH	84 a	0.0 b	68 a	29 a
	untreated	-		-	1 d	0.0 b	0 c	0 c
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	8 b	1.8 a	3 b	1 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	9 b	2.5 a	0 c	0 c
	VC1	Mild PepMV VC1	2%	agCH	6 c	2.1 a	0 c	0 c
	VX1	Mild PepMV VX1	2%	agEU	2 d	0.0 b	0 c	0 c

Additionally assessment was made on yellow spots on leaves, but these symptoms occurred only little and equally over all objects are therefore not shown. Chlorosis was not observed in this trial.

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Test report	Treatment			Inoculation	Number of flowers		Number of trusses setting	
	Product	Active substance	Rate		20.11.2012	18.12.2012	20.11.2012	18.12.2012
I-12-6702-1	untreated	-		agEU + agCH	1.0 b	1.0 b	1.0 b	1.0 b
	untreated	-		-	1.6 a	3.7 a	2.1 a	3.5 a
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	1.5 a	3.6 a	2.0 a	3.5 a
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	1.4 a	3.5 a	2.0 a	3.3 a
	VC1	Mild PepMV VC1	2%	agCH	1.5 a	3.5 a	2.1 a	3.4 a
	VX1	Mild PepMV VX1	2%	agEU	1.6 a	3.7 a	2.1 a	3.4 a

Damage to fruits was assessed, yet hardly no PepMV symptoms were observed during the trial (results not shown).

Test report	Treatment			Inoculation	Yield (% relative)[1]		
	Product	Active substance	Rate		number of fruits	total weight	fruit weight
I-12-6702-1	untreated	-		agEU + agCH	100 b (8)	100 b (0.1)	100 b (28)
	untreated	-		-	356 a	12300 a	136 a
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	353 a	11000 a	129 a
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	360 a	11500 a	129 a
	VC1	Mild PepMV VC1	2%	agCH	371 a	11800 a	129 a
	VX1	Mild PepMV VX1	2%	agEU	357 a	11700 a	132 a

[1] Between brackets respectively the number of fruits, total weight in kg and fruit weight in g.

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Test report	Treatment			Inoculation	Stunting (%)	Crop condition (1-10)[1]	
	Product	Active substance	Rate			min	max
I-12-6702-1	untreated	-		agEU + agCH	75 a	1.3	8.0
	untreated	-		-	0 e	7.0	8.0
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	5 c	6.5	8.0
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	8 b	6.5	8.0
	VC1	Mild PepMV VC1	2%	agCH	3 d	6.5	8.0
	VX1	Mild PepMV VX1	2%	agEU	0 e	7.0	8.0

[1] Crop condition on a scale of 1-10 with 1: crop dead and 10: excellent crop

No crop damage was observed in the trial.

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Test report	Testing Unit GEP	Trial location Crop cultivar F/G; N/A	Test method Plot size Sample size	Application details		Experimental Design Replicates
				Method	Date ; Growth Stage;	
I-12-6702-2	[redacted] GEP	[redacted] Tomato *Endeavour, Levanzo, Brioso, Komeett* G; A	PP 1/135(3) , PP 1/152(3), PP 1/181(3) 20 plants 20 plants	A1: Foliar spray 0.26-0.44 L/m^2 A2: Inoculation, rubbing	A1: 02.10.2012: BBCH 13-14 A2: 24.10.2012 and 15.11.2012: BBCH 61-62	RBD 4

Test report	Treatment			Inoculation*	Average pest severity on leaves (%) (average of 10 assessments from wk43, 2012 – wk4, 2013)				Deformation of leaves (1-3)[1]
	Product	Active substance	Rate		Nettle head	Mosaic	Necrosis leaf	Necrosis stem	
I-12-6702-2	untreated	-		agEU + agCH	27.0 a	27 a	72 a	49 a	2.1 a
	untreated	-		-	0.0 e	0 d	0 d	0 d	0.0 f
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	7.0 c	5 c	0 d	0 d	0.0 e
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	7.7 c	5 c	0 d	0 d	0.1 d
	VC1	Mild PepMV VC1	2%	agEU + agCH	16.0 b	16 b	37 b	29 b	1.7 b
	VX1	Mild PepMV VX1	2%	agEU + agCH	5.2 d	5 c	15 c	14 c	0.3 c

[1] Deformation of leaves on a scale of 1-3 with 1: light deformation and 3: severe deformation.
* Inoculation with ag EU was made later at 15.11.2012

Additionally assessment was made on yellow spots and chlorosis on leaves, but these symptoms occurred only little and are therefore not shown.

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Test report	Treatment			Inoculation	Number of flowers	Number of trusses setting
	Product	Active substance	Rate			
I-12-6702-241	untreated	-		agEU + agCH	1.0 d	1.0 d
	untreated	-		-	4.5 a	4.2 a
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	4.2 ab	3.7 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	4.2 b	3.7 b
	VC1	Mild PepMV VC1	2%	agEU + agCH	3.8 c	3.0 c
	VX1	Mild PepMV VX1	2%	agEU + agCH	3.8 c	3.5 b

Test report	Treatment			Inoculation	Symptoms of fruits (% of fruits affected)					
	Product	Active substance	Rate		blotchy	marble	damaged	deformed	canker	damage calyx
I-12-6702-2	untreated	-		agEU + agCH	100 a	21 a	100 a	100 a	9.5 a	100 a
	untreated	-		-	0.0 d	0 ac	1.66 de	0.33 e	0.0 ac	0.0 d
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	5.58 c	2 ab	18.06 c	15.34 c	0.102 ab	49.24 c
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	0.18 d	0 ac	0.75 e	0.08 e	0.0 ac	0.0 d
	VC1	Mild PepMV VC1	2%	agEU + agCH	27.33 b	6 a	58.31 b	70.15 b	0.633 a	92.54 b
	VX1	Mild PepMV VX1	2%	agEU + agCH	0.91 d	0 ac	3.64 d	4.53 d	0.173 a	0.0 d

No open fruits were observed in any of the objects.

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Test report	Treatment			Inoculation	Yield (% relative)[1]		
	Product	Active substance	Rate		number of fruits	total weight	fruit weight
I-12-6702-2	untreated	-		agEU + agCH	100 c (0.8)	100 d (0.02)	100 c (5.8)
	untreated	-		-	35588 a	59400 a	812 ab
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	28875 ab	47500 b	848 a
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	35275 a	57850 a	803 ab
	VC1	Mild PepMV VC1	2%	agEU + agCH	22400 b	19650 c	510 b
	VX1	Mild PepMV VX1	2%	agEU + agCH	34375 a	56500 ab	824 ab

[1] Between brackets respectively the number of fruits, total weight in kg and fruit weight in g.

Test report	Treatment			Inoculation	Stunting (%)	Crop condition (1-10)[1]	
	Product	Active substance	Rate			min	max
I-12-6702-2	untreated	-		agEU + agCH	80.4 a	2	8
	untreated	-		-	3.0 f	6.4	8
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	9.4 d	6.5	7.5
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	9.0 e	6	7.5
	VC1	Mild PepMV VC1	2%	agEU + agCH	50.0 b	4	6.5
	VX1	Mild PepMV VX1	2%	agEU + agCH	23.5 c	5	8

[1] Crop condition on a scale of 1-10 with 1: crop dead and 10: excellent crop

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Test report	Testing Unit GEP	Trial location Crop cultivar F/G; N/A	Test method Plot size Sample size	Application details		Experimental Design Replicates
				Method	Date ; Growth Stage;	
I-12-6703-1	[redacted] GEP	[redacted] Tomato *Plaisance, Merlice, Roterno* G; A	PP 1/135(3) , PP 1/152(3), PP 1/181(3) 20 plants 20 plants	A1: Foliar spray 0.5 L/m^2 A2: Inoculation, rubbing	A1: 23-30.11.2012: BBCH 13-14 A2: 01.02.2013: BBCH 61-62	RBD 3

Test report	Treatment			Inoculation*	Average pest severity on leaves (%) (average of 9 assessments from 14.02.2013-29.04.2013)				
	Product	Active substance	Rate		Nettle head	Mosaic	Necrosis leaf	Necrosis stem	Yellow spots
I-12-6703-1	untreated	-		agEU	24.2 a	29.57 a	24.27 a	9.238 a	0.03591 a
	VX1	Mild PepMV VX1	2%	agEU	2.83 b	3.75 b	0.19 b	0 a	0.00556 b

No chlorosis was observed in the trial.

Test report	Treatment			Inoculation*	Number of flowers		Number of trusses setting	
	Product	Active substance	Rate		22.02.2013	08.03.2013	22.02.2013	08.03.2013
I-12-6703-1	untreated	-		agEU	4.2 a	5.16 a	3.5 a	4.9 a
	VX1	Mild PepMV VX1	2%	agEU	4.1 a	5.56 a	3.5 a	5.0 a

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Test report	Treatment			Inoculation*	Symptoms of fruits (% of fruits affected)					
	Product	Active substance	Rate		blotchy	marble	damaged	deformed	open	damage calyx
I-12-6703-1	untreated	-		agEU	20 a	1.4 a	23.9 a	19.8 a	0.3 a	67 a
	VX1	Mild PepMV VX1	2%	agEU	2 b	0.0 b	3.9 b	2.5 b	0.2 a	0 b

No cankerous fruits were observed.

Test report	Treatment			Inoculation	Yield (% relative)[1]		
	Product	Active substance	Rate		number of fruits	total weight	fruit weight
I-12-6703-1	untreated	-		agEU	100 a (337)	100 a (30)	100 a (89.8)
	VX1	Mild PepMV VX1	2%	agEU	95 a	90 a	95 a

[1] Between brackets respectively the number of fruits, total weight in kg and fruit weight in g.

Test report	Treatment			Inoculation	Stunting (%)	Crop condition (1-10)[1]	
	Product	Active substance	Rate			min	max
I-12-6703-1	untreated	-		agEU	41.5 a	4.3	5.2
	VX1	Mild PepMV VX1	2%	agEU	0.8 b	7.4	9.0

[1] Crop condition on a scale of 1-10 with 1: crop dead and 10: excellent crop

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Test report	Testing Unit GEP	Trial location Crop cultivar F/G; N/A	Test method Plot size Sample size	Application details		Experimental Design Replicates
				Method	Date ; Growth Stage;	
I-13-6701-1	[redacted] GEP	[redacted] Tomato *Endeavour, Levanzo, Brioso, Komeett* G; A	PP 1/135(3) , PP 1/152(4), PP 1/181(4) 20 plants 20 plants	A1: Foliar spray 0.27-0.49 L/m^2 A2: Inoculation, rubbing	A1: 01.02.2013: BBCH 12-13 A2: 21.03.2013: BBCH 51	RBD 4

Test report	Treatment			Inoculation*	Average pest severity on leaves (%) (average of 7 assessments from 15.03.2013-24.05.2013)		
	Product	Active substance	Rate		Nettle head	Necrosis leaf	Necrosis stem
I-13-6701-1	untreated	-		agEU + agCH	26 a	27.1 a	5.7 a
	untreated	-		-	0 c	0.2 b	0.0 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	3 b	0.0 c	0.0 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	3 b	0.2 b	0.0 b
	VC1	Mild PepMV VC1	2%	agEU + agCH	2 b	0.0 c	0.0 b
	VX1	Mild PepMV VX1	2%	agEU + agCH	0 c	0.0 c	0.0 b

Additionally assessment was made on mosaic, yellow spots and chlorosis, yet these symptoms were not observed during the trial.

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Test report	Treatment			Inoculation*	Number of flowers	Number of trusses setting
	Product	Active substance	Rate			
I-13-6701-1	untreated	-		agEU + agCH	2.7 a	3.4 a
	untreated	-		-	2.58 ab	3.3 a
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	1.88 d	2.6 a
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	2.20 bc	2.9 b
	VC1	Mild PepMV VC1	2%	agEU + agCH	2.13 bcd	2.9 bc
	VX1	Mild PepMV VX1	2%	agEU + agCH	2.37 bc	3.0 b

Test report	Treatment			Inoculation*	Symptoms of fruits (% of fruits affected)			
	Product	Active substance	Rate		blotchy	marble	deformed	blossom end rot
I-13-6701-1	untreated	-		agEU + agCH	1.16 a	2.25 a	1.01 a	3.2 a
	untreated	-		-	0.00 b	0.00 cd	0.05 b	0.0 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	1.02 a	0.94 ab	0.00 c	0.3 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	0.93 a	1.39 ab	0.00 c	0.2 b
	VC1	Mild PepMV VC1	2%	agEU + agCH	1.19 a	0.59 bc	0.00 c	0.2 b
	VX1	Mild PepMV VX1	2%	agEU + agCH	0.32 ab	0.09 bcd	0.00 c	0.0 b

No damaged or open fruits nor damage to stem trusses were observed in this trial.

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Test report	Treatment			Inoculation*	Yield (% relative)[1]			
	Product	Active substance	Rate		number of fruits	total weight	fruit weight	total weight including non-harvested fruits
I-13-6701-1	untreated	-		agEU + agCH	100 b (541)	100 bc (41.6)	100 b (93.6)	100 c (85)
	untreated	-		-	101 ab	104 bc	100 b	122 ab
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	103 a	111 ab	108 ab	119 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	99 b	113 a	114 a	128 a
	VC1	Mild PepMV VC1	2%	agEU + agCH	101 ab	111 ab	110 a	121 ab
	VX1	Mild PepMV VX1	2%	agEU + agCH	99 b	107 abc	107 ab	121 ab

[1] Between brackets respectively the number of fruits, total weight in kg and fruit weight in g.

Test report	Treatment			Inoculation*	Stunting (%)	Crop condition (1-10)[1]	
	Product	Active substance	Rate			min	max
I-13-6701-1	untreated	-		agEU + agCH	8.1 a	2.5	8
	untreated	-		-	0.0 d	7	8
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	1.7 d	7	8
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	0.8 c	7	8
	VC1	Mild PepMV VC1	2%	agEU + agCH	0.8 c	7	8
	VX1	Mild PepMV VX1	2%	agEU + agCH	0.0 d	7	8

[1] Crop condition on a scale of 1-10 with 1: crop dead and 10: excellent crop

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Test report	Testing Unit GEP	Trial location Crop cultivar F/G; N/A	Test method Plot size Sample size	Application details		Experimental Design Replicates
				Method	Date ; Growth Stage;	
I-13-6701-2	[redacted] GEP	[redacted] Tomato *Endeavour, Levanzo, Brioso, Komeett* G; A	PP 1/135(3) , PP 1/152(4), PP 1/181(4) 20 plants 20 plants	A1: Foliar spray 0.27-0.49 L/m^2 A2: Inoculation, rubbing	A1: 01.02.2013: BBCH 12-13 A2: 22.03.2013: BBCH 51	RBD 4

Test report	Treatment			Inoculation*	Average pest severity on leaves (%) (average of 10 assessments from wk 14, 2013-wk 24, 2013)			
	Product	Active substance	Rate		Nettle head	Mosaic	Necrosis leaf	Necrosis stem
I-13-6701-2	untreated	-		agEU + agCH	58 a	63 a	64.8 a	26 a
	untreated	-		-	0 e	0 e	0.0 d	0 c
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	10 b	7 b	10.4 b	1 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	5 c	6 b	0.0 d	0 c
	VC1	Mild PepMV VC1	2%	agEU + agCH	2 d	5 c	0.0 d	0 c
	VX1	Mild PepMV VX1	2%	agEU + agCH	0 e	3 d	0.5 c	0 c

Additionally assessment was made on yellow spots and chlorosis, yet these symptoms occurred only very little (results not shown).

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Test report	Treatment			Inoculation*	Number of flowers		Number of trusses setting	
	Product	Active substance	Rate		wk 14	wk 17	wk 14	wk 17
I-13-6701-2	untreated	-		agEU + agCH	4.5 a	6.4	4.2 a	6.9 a
	untreated	-		-	4.8 a	7.6	4.2 a	6.9 a
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	4.4 a	7.3	3.4 b	6.9 a
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	4.2 a	7.6	4.2 a	6.5 a
	VC1	Mild PepMV VC1	2%	agEU + agCH	4.6 a	7.1	3.6 b	6.9 a
	VX1	Mild PepMV VX1	2%	agEU + agCH	4.7 a	7.1	4.1 a	6.4 a

Test report	Treatment			Inoculation*	Symptoms of fruits (% of fruits affected)				
	Product	Active substance	Rate		blotchy	marble	damaged	deformed	damage calyx
I-13-6701-2	untreated	-		agEU + agCH	2.5 a	0.25 a	4.56 a	2.1 a	16 a
	untreated	-		-	0.1 b	0.0 c	0.62 c	0.1 b	0 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	0.4 b	0.0 c	1.25 b	0.1 c	0 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	0.6 b	0.0 c	0.78 bc	0.1 c	0 b
	VC1	Mild PepMV VC1	2%	agEU + agCH	0.5 b	0.05 b	1.06 bc	0.1 c	0 b
	VX1	Mild PepMV VX1	2%	agEU + agCH	0.2 b	0.0 c	0.76 bc	0.1 c	0 b

Additionally assessments were made on blossom end rot and open fruits. No blossom end rot was observed in this trial. Open fruits were observed only very little (results not shown).

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Test report	Treatment			Inoculation*	Yield (% relative)[1]		
	Product	Active substance	Rate		number of fruits	total weight	fruit weight
I-13-6701-2	untreated	-		agEU + agCH	100 a (425)	100 a (36)	100 b (93)
	untreated	-		-	101 a	114 a	113 a
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	101 a	111 a	116 a
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	96 a	103 a	113 a
	VC1	Mild PepMV VC1	2%	agEU + agCH	100 a	108 a	116 a
	VX1	Mild PepMV VX1	2%	agEU + agCH	99 a	108 a	111 a

[1] Between brackets respectively the number of fruits, total weight in kg and fruit weight in g.

Test report	Treatment			Inoculation*	Crop condition (1-10)[1]	
	Product	Active substance	Rate		min	max
I-13-6701-2	untreated	-		agEU + agCH	3.5	5
	untreated	-		-	8	9
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	7.5	8.5
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	7.5	8
	VC1	Mild PepMV VC1	2%	agEU + agCH	7.5	8.5
	VX1	Mild PepMV VX1	2%	agEU + agCH	7.5	8.5

[1] Crop condition on a scale of 1-10 with 1: crop dead and 10: excellent crop

No stunting was observed in this trial.

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Test report	Testing Unit GEP	Trial location Crop cultivar F/G; N/A	Test method Plot size Sample size	Application details		Experimental Design Replicates
				Method	Date ; Growth Stage;	
I-13-6701-3	[redacted] GEP	[redacted] Tomato *Endeavour, Levanzo, Brioso, Komeett* G; A	PP 1/135(3) , PP 1/152(4), PP 1/181(4) 26 plants 26 plants	A1: Foliar spray 0.27-0.49 L/m^2 A2: Inoculation, rubbing	A1: 01.02.2013: BBCH 12-13 A2: 21.03.2013: BBCH 51	RBD 4

Test report	Treatment			Inoculation*	Average pest severity on leaves (%) (average of 6 assessments from 12.04.2013-24.05.2013)		
	Product	Active substance	Rate		Nettle head	Necrosis leaf	Necrosis stem
I-13-6701-3	untreated	-		agEU + agCH	29 a	16 a	19 a
	untreated*	-		-	2 d	0 c	0 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	9 b	1 b	0 b
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	4 c	0 c	0 b
	VC1	Mild PepMV VC1	2%	agEU + agCH	5 c	0 c	0 b
	VX1	Mild PepMV VX1	2%	agEU + agCH	4 c	0 c	0 b

* contaminated with V10

Additionally assessment was made on mosaic, yellow spots and chlorosis, yet these symptoms occurred not at all or only very little (results not shown).

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Test report	Treatment			Inoculation*	Number of flowers	Number of trusses setting
	Product	Active substance	Rate			
I-13-6701-3	untreated	-		agEU + agCH	6.61 a	7.26 a
	untreated*	-		-	6.36 abc	6.95 abc
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	6.13 bcd	6.75 bc
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	6.01 bcd	6.68 bc
	VC1	Mild PepMV VC1	2%	agEU + agCH	6.23 bcd	6.91 bc
	VX1	Mild PepMV VX1	2%	agEU + agCH	6.46 ab	7.07 ab

Test report	Treatment			Inoculation*	Symptoms of fruits (% of fruits affected)				
	Product	Active substance	Rate		blotchy	marble	damaged	blossom end rot	damage stem trusses
I-13-6701-3	untreated	-		agEU + agCH	8.7 a	2.1 a	1.09 ab	11.78 a	0.94 a
	untreated*	-		-	3.6 b	0.9 b	1.21 ab	2.87 c	0 c
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	2.9 b	0.1 c	0.85 ab	3.69 c	0 c
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	0.9 c	0.0 c	0.88 ab	2.90 c	0 c
	VC1	Mild PepMV VC1	2%	agEU + agCH	1.7 bc	0.0 c	0.45 b	5.40 bc	0 c
	VX1	Mild PepMV VX1	2%	agEU + agCH	2.5 bc	1.9 a	1.28 a	7.87 b	0.31 b

* contaminated with V10

No open or deformed fruits were observed in this trial.

Table IIIA 6.1.3-6a Effectiveness data for V10 in tomato

Test report	Treatment			Inoculation*	Yield (% relative)[1]		
	Product	Active substance	Rate		number of fruits	total weight	fruit weight
I-13-6701-3	untreated	-		agEU + agCH	100 a (872)	100 a (97)	100 bc (131)
	untreated*	-		-	80 b	74 c	95 bc
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	95 a	73 ab	108 ab
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	88 ab	88 b	104 ab
	VC1	Mild PepMV VC1	2%	agEU + agCH	93 a	98 ab	107 ab
	VX1	Mild PepMV VX1	2%	agEU + agCH	88 ab	89 ab	109 a

* contaminated with V10
[1] Between brackets respectively the number of fruits, total weight in kg and fruit weight in g.

Test report	Treatment			Inoculation*	Crop condition (1-10)[1]	
	Product	Active substance	Rate		min	max
I-13-6701-3	untreated	-		agEU + agCH	2.8	6.0
	untreated*	-		-	7.0	8.0
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	agEU + agCH	6.8	8.0
	V10	Mild PepMV VX1 Mild PepMV VC1	2%	-	7.0	8.0
	VC1	Mild PepMV VC1	2%	agEU + agCH	7.3	8.0
	VX1	Mild PepMV VX1	2%	agEU + agCH	6.0	8.0

* contaminated with V10
[1] Crop condition on a scale of 1-10 with 1: crop dead and 10: excellent crop

No stunting was observed in this trial.

IIIA 6.1.4 Effects on yield and quality

IIIA 6.1.4.1 Effects on quality of plants or plant products

The Pepino Mosaic Virus causes symptoms on tomato plants and the forming fruits. The quality of plants and fruits can be severely compromised by the virus. This was demonstrated in 7 efficacy trials, where symptoms such as nettle head, mosaic, leaf necrosis and stem necrosis were observed on the leaves. Fruits were blotchy, marble, damaged, deformed, cankerous and open and damage to the calyx, stem trusses and blossom end rot were observed as a result of PepMV infection in the untreated objects.
The symptoms on leaves and fruits of plants treated with V10 and inoculated with aggressive PepMV isolates CH and EU were much reduced as compared to untreated plants inoculated with aggressive PepMV isolates CH and EU (for results see chapter 6.1.3).

To establish the effect of V10 on quality in absence of the aggressive virus, plants treated with V10 (and not inoculated with aggressive PepMV) were compared to untreated plants in 7 efficacy trials. However in 1 of these trials ELISA testing demonstrated that the untreated non-inoculated plants were infected with V10. Therefore this trial is excluded from the summary.

The product V10, a combination of mild PepMV isolates VC1 and VX1, was applied by spraying at 2% in combination with carborundum.

Assessment was made on viral symptoms on leaves and on fruits.

Table 6.1.4.1-1 Symptoms on leaves

Symptom	Number of trials	% area of leaves affected Mean (Min-Max)	
		Untreated not infected	2% V10 not infected
nettle head	6	0.2 (0-1)	5.7 (2.5-7.7)
mosaic	5	0	3.1 (0-6)
leaf necrosis	6	0 (0-0.2)	0 (0-0.2)
stem necrosis	6	0	0

Table 6.1.4.1-1 Symptoms on fruits

Symptom	Number of trials	% of fruits affected Mean (Min-Max)	
		Untreated not infected	2% V10 not infected
blotchy	5	0.6 (0-2)	1.3 (0.2-4)
marble	5	0.3 (0-1.6)	0.3 (0-1.4)
damaged	5	1.0 (0-2.6)	1.5 (0-5.9)
deformed	5	0.2 (0-0.4)	0.4 (0-1.6)
damage to calyx	3	0	0
damage to stem trusses	3	0	0
blossom end rot	2	0	0.1

			(0-0.2)

The percentage area of leaves that was affected following treatment with V10 was very low, though some minor symptoms were observed. These can be ascribed to the fact that V10 contains attenuated virus and may cause a very mild virus reaction in plants and to the fact that carborundum was added to the spray solution as an abrasive.
The percentage of fruits affected following treatment with V10 was very low and often comparable to that observed in the untreated objects. Some symptoms may be due to a very mild virus reaction from the attenuated virus.

Overall, treatment with V10 largely prevents the adverse effects on tomato plants and fruits caused by the Pepino Mosaic Virus. In absence of aggressive PepMV infection, treatment with V10 can cause mild PepMV symptoms on tomato plants, but does not affect yield (see chapter 6.1.4.3). A warning sentence to this extent is placed on the label.

IIIA 6.1.4.2 Effects on transformation processes

Not applicable for use on tomatoes.

IIIA 6.1.4.3 Effects on yield of treated plants or plant products

The Pepino Mosaic Virus causes symptoms on tomato plants and the forming fruits. The yield of fruits can be severely compromised by the virus. This was demonstrated in 7 efficacy trials, where yield of the untreated objects was reduced as a result of PepMV infection. The yields of plants treated with V10 and inoculated with aggressive PepMV isolates CH and EU were increased as compared to untreated plants inoculated with aggressive PepMV isolates CH and EU (for results see chapter 6.1.3).

To establish the effect of V10 on yield in absence of the aggressive virus, plants treated with V10 (and not inoculated with aggressive PepMV) were compared to untreated plants in the 7 efficacy trials. However in 1 of these trials ELISA testing demonstrated that the untreated non-inoculated plants were infected with V10. Therefore this trial is excluded from the summary.

The product V10, a combination of mild PepMV isolates VC1 and VX1, was applied by spraying at 2% in combination with carborundum.

In table 6.1.4.3-1 an overview of yield and relative yield in relation to the untreated non-infected objects is given.

Table 6.1.4.3-1 Yield

Assessment	**Number of trials**	**Yield Mean (Min-Max)**	**Relative yield (%) Mean (Min-Max)**
		Untreated not infected	**2% V10 not infected**
Number of fruits	6	444 (284.7-542)	98 (95-114)
Total weight (kg)	6	36.8 (11.9-56.6)	97 (90-110)
Fruit weight (g)	6	90.3 (38-130)	101 (95-114)

Overall, in absence of aggressive PepMV infection, no effect on yield of treatment with V10 was observed in relation to the untreated objects.

IIIA 6.2 Adverse effects

IIIA 6.2.1 Phytotoxicity to target plants (including different cultivars) or to target plant products

The Pepino Mosaic Virus can be detected on growing plants, on tomato fruits and on tomato seeds originating from infected plants. Symptoms of PepMV can result in serious decreases in crop condition. This was demonstrated in 7 efficacy trials, where symptoms such as nettle head, mosaic, leaf necrosis and stem necrosis were observed on the leaves of the untreated objects. Fruits were blotchy, marble, damaged, deformed, cankerous and open and damage to the calyx, stem trusses, blossom end rot and stunting were observed as a result of PepMV infection in the untreated objects.

The symptoms on leaves and fruits and stunting of plants treated with V10 and inoculated with aggressive PepMV isolates CH and EU were much reduced as compared to untreated plants inoculated with aggressive PepMV isolates CH and EU. As a result the crop condition of plants treated with V10 (inoculated with aggressive PepMV isolates CH and EU) was much better as compared to the untreated objects (inoculated with aggressive PepMV isolates CH and EU). Furthermore, yield was increased in relation to the untreated objects (inoculated with aggressive PepMV isolates CH and EU). For results see chapter 6.1.3.

To establish the effect of V10 on the crop in absence of the aggressive virus, plants treated with V10 (and not inoculated with aggressive PepMV) were compared to untreated plants in the 7 efficacy trials. However in 1 of these trials ELISA testing demonstrated that the untreated non-inoculated plants were infected with V10. Therefore this trial is excluded from the summary.

The product V10, a combination of mild PepMV isolates VC1 and VX1, was applied by spraying at 2% in combination with carborundum.

In section IIIA 6.1.4.1 it was concluded that, in absence of aggressive PepMV infection, treatment with V10 can cause mild PepMV symptoms on tomato plants (see Tables 6.1.4.1-1 and 2). However these effects did not have an effect on yield (see Table 6.1.4.3-1).
In plants treated with V10 yet not inoculated with aggressive PepMV, some minor stunting was observed (see Table 6.2.1-1), that was not observed in the untreated plants. In one trial stunting was also observed in the untreated uninfected objects, however this was not due to virus infection as the ELISA test was negative for infection.
The crop condition of plants treated with V10 (not inoculated with aggressive PepMV) was always moderate to excellent (scores of 6-10), yet was generally slightly lower than that observed in the untreated plants (not inoculated with aggressive PepMV) (scores of 6.4-10)

Table 6.2.1-1 Stunting

Number of trials	Stunting (%) Mean (Min-Max)	
	Untreated not infected	**2% V10 not infected**
6	0.5 (0-3)	3.6 (0-9)

Overall, treatment with V10 largely prevents the adverse effects on tomato plants and fruits caused by the Pepino Mosaic Virus. In absence of aggressive PepMV infection, treatment with V10 can cause mild PepMV symptoms on tomato plants, but does not affect yield (see chapter 6.1.4.3). A warning sentence to this extent is placed on the label.

IIIA 6.2.2 Adverse effects on health of target animals

This is not an EC data requirement.

IIIA 6.2.3 Adverse effects on site of application (discoloration, corrosion, etc.)

This is not an EC data requirement.

IIIA 6.2.4 Adverse effects on beneficial and other non-target organisms

V10 contains attenuated virus, similar to the naturally occurring Pepino Mosaic Virus. Adverse effects on beneficial and other non-target organism are not foreseen.

IIIA 6.2.5 Adverse effects on parts of plants used for propagating purposes (e.g. seeds, cuttings, runners)

The Pepino Mosaic Virus can be detected on growing plants, on tomato fruits and on tomato seeds. A low seed transmission rate has been demonstrated; however, available evidence suggests that PepMV does not infect the embryo or endosperm but contaminates the seed coat (Krinkels, 2011[1]) .
As V10 contains attenuated PepMV strains, these are also expected to be transmitted through seed on a low level, yet are not expected to influence the embryo or endosperm, thereby not interfering with germination.

IIIA 6.2.6 Impact on succeeding crops

V10 is applied to young tomato plants in the greenhouse. The product is to be applied within 6 hours after dilution to ensure infectivity. The risk that the virus isolates in the product are still infective in succeeding crops is therefore negligible. Furthermore, the host-range of the virus is limited.

IIIA 6.2.7 Impact on other plants including adjacent crops

Tomato is usually grown in separate compartments of a greenhouse. Spray drift to crops in other compartments can practically be ruled out.

IIIA 6.3 Economics

This is not an EC data requirement.

IIIA 6.4 Consideration of Benefits

IIIA 6.4.1 Survey of alternative pest control measures (chemical and non-chemical)

This is not an EC data requirement.

IIIA 6.4.2 Compatibility with current management practices including IPM

This is not an EC data requirement.

IIIA 6.4.3 Contribution to risk reduction

This is not an EC data requirement.

IIIA 6.4.4 Information on the possible occurrence of the development of resistance or cross-resistance

V10 contains attenuated virus isolates of the Pepino Mosaic Virus and undergoes sequence analysis to check for and to rule out mutations in the production. The isolates in V10, mild PepMV isolate VX1

[1] Krinkels, M., 2001. Pepino mosaic virus causes sticky problem. Prophyta: The Annual, May 2001, 30-33.

and mild PepMV isolate VC1, are almost identical to respectively the European PepMV isolate and the Chilean isolate.
The virus is controlled via RNA silencing using the attenuated virus. Resistance is therefore not applicable.

IIIA 6.5 Other/special studies

None.

IIIA 6.6 Summary and evaluation of data presented

Introduction
V10 is an elicitor of a suspension concentrate (SC) formulation, which contains 5-25 mg/L Mild Pepino Mosaic Virus isolate VX1 and 5-25 mg/L Mild Pepino Mosaic Virus isolate VC1, which per isolate corresponds to approximately $1.5x10^{11}$ to $7.5x10^{11}$ virus particles per ml.
V10 is to be used as post-emergent foliar applications (spray or direct rubbing on foliage) in protected cultivation of tomatoes to prevent infections by aggressive isolates of the Pepino Mosaic Virus.
Tomato plants are protected against Pepino Mosaic Virus (PepMV) by a preceding infection with attenuated isolates of this virus (cross-protection).

V10 is applied preventatively on young tomato plants (BBCH 13-51, plant height of 10-30 cm) by spraying at 2% or it is applied on tomato plants (BBCH 13-61) by rubbing the suspension on leaves of the plants at 10%.
V10 is to be applied in combination with the abrasive synthetic sand (8 g synthetic sand/1 L spray liquid for spray application and 15 g synthetic sand/1 L liquid for the rubbing application).

Preliminary trials
V10 offers protection against Pepino Mosaic Virus (PepMV) by inducing plant resistance. A preceding infection of tomato plants with attenuated isolates of the PepMV in V10 induces cross-protection due to RNA silencing. In contrary to conventional plant protection products, this product works through the RNA silencing mechanisms of the plant. Upon application with V10, the tomato plant becomes infected with the attenuated isolates of the PepMV which induces RNA silencing in the plant. As a result, the effectiveness of the product depends on the infectivity of the formulation, effective replication of the isolated VX1 and VC1 and the RNA silencing mechanisms of the plant.
The formulation V10 in combination with an abrasive should enable the virus isolates to enter the plant cells, and infect the plant. The ability of the formulation to infect tomato plants is therefore key to the effectiveness of the product.

In the intended uses for V10, two methods of application are claimed. V10 can be applied by foliar spray, or by rubbing the solution on leaves. The effectiveness of application by foliar spray was demonstrated in 8 efficacy trials. The ability of V10 to infect tomato plants when applied by rubbing is demonstrated in a bioassay. The results indicate that a concentration between 1% and 10% will be sufficiently infective when carborundum suspension containing mild isolates of PepMV is rubbed on tomato leaves of seedlings. As the amount of Mild Pepino Mosaic Virus isolate may vary between 10 to 50 mg/L between different batches, and also taking into account that the product is already applied at a relatively low dose rate when applied as proposed (0.8 L/ha), the proposed concentration has been set at 10% to guarantee adequate infection of the tomato plants in all cases.

Over time, several changes to the V10 formulation were made for other reasons than efficacy. The efficacy trials were conducted with a V10 formulation containing borate buffer, whereas the new formulation is prepared in phosphate buffer. Furthermore, the concentration of nicotine in the formulation was lowered by including a purification step in the production process. These changes in the formulation are not expected to have an effect on the infectivity of the product, and as such no

effect on the effectiveness of the product. This is demonstrated in bioassays in which the infectivity of these formulations is tested.
Multiple bioassays were commissioned by the applicant of batches of VC1 and VX1 in phosphate buffer. In each test all plants tested positive for PepMV, demonstrating that the suspensions were infectious.
Five different batches of VC1 virus suspension and VX1 virus suspension with a nicotine concentration below 0.1 mg/L due to an extra purification step in the production process, demonstrated to be infectious to tomato plants. All plants inoculated with the different batches from isolate VC1 tested positive for VC1. A range of 70-90% of the plants inoculated with the different batches from isolate VX1 tested positive for VX1.

V10 is applied in combination with an abrasive that provides entry of the virus into plant cells. In the efficacy trials, V10 was applied in combination with carborundum. Due to a new classification for carborundum as a possible carcinogen, the abrasive to be used as an additive with this formulation is changed to synthetic sand. In different bioassays it was demonstrated that both carborundum and synthetic sand are effective as abrasive. V10 was applied by high pressure spraying in combination with either carborundum or synthetic sand. Both carborundum and synthetic sand met the quality control requirement of an infectivity of at least 70%.
Based on the results of the bioassays, it can be expected that the infectivity obtained with synthetic sand as an abrasive is comparable to the infectivity obtained with carborundum as abrasive.

V10 is a combination of 2 attenuated virus isolates, namely Mild Pepino Mosaic Virus isolate VX1 and Mild Pepino Mosaic Virus isolate VC1.
Preventative treatment with the combination of VX1 and VC1 ensures protection against the main occurring PepMV in Europe. This was demonstrated in 1 efficacy trial (I-12-6702-2) conducted in the Netherlands in 2012. In this trial, the effectiveness of V10 (VX1 + VC1) was compared to both active substances solo. Artificial infection was made using aggressive PepMV isolates EU and CH, isolates from respectively the European PepMV and Chile PepMV.
The different aggressive isolates of the PepMV can cause different symptoms in plants. In this trial it was observed that plants treated with VC1 solo showed a greater severity of nettle head, mosaic, leaf necrosis and stem necrosis on leaves as compared to the plants treated with V10 (VC1+ VX1). In plants treated with VX1 solo the severity of leaf and stem necrosis was increased as compared to the plants treated with V10. Likewise in fruits, symptoms such as blotches, damage, deformation and calyx damage in plants treated with VC1 solo occurred more often than in plants treated with V10. Symptoms in plants treated with VX1 occurred only rarely and even to a lesser extent as compared to plants treated with V10.
Yield of plants treated with V10 was higher than that of plants treated with VC1 solo and comparable to plants treated with VX1 solo.
Stunting in plants treated with V10 was significantly lower than that observed in plants treated withVC1 solo and in plants treated with VX1 solo.
Crop condition of plants treated with VC1 solo and plants treated with VX1 solo was lessened as compared to plants treated with V10.
Overall, the combination of VX1 and VC1 in V10 ensures protection against the 2 main occurring PepMV genotypes in Europe.

Effectiveness
The effectiveness of mild Pepino Mosaic Virus against aggressive Pepino Mosaic Virus (PEPMVO) was tested in a total of 8 efficacy trials conducted in greenhouses in the Netherlands in 2012 and 2013.

<u>V10</u>
The product V10, a combination of mild PepMV isolates VC1 and VX1, was tested in 7 out of 8 trials. The product was applied by spraying at 2% in combination with carborundum.
Artificial infection was made using aggressive PepMV isolates EU and CH, isolates from respectively the European PepMV and Chile PepMV.

Assessment was made on viral symptoms on leaves and on fruits, on yield and crop stand. The effect of V10 on infected tomato plants was compared to untreated infected plants.

The Pepino Mosaic Virus causes symptoms on tomato plants and the forming fruits. This was demonstrated in the efficacy trials, where symptoms such as nettle head, mosaic, leaf necrosis and stem necrosis were observed on the leaves. Fruits were blotchy, marble, damaged, deformed, cankerous and open and damage to the calyx, stem trusses and blossom end rot were observed as a result of PepMV infection.
The symptoms on leaves and fruits of plants treated with V10 and inoculated with aggressive PepMV isolates CH and EU were much reduced as compared to untreated plants inoculated with aggressive PepMV isolates CH and EU.
The yields of plants treated with V10 and inoculated with aggressive PepMV isolates CH and EU were increased as compared to untreated plants inoculated with aggressive PepMV isolates CH and EU.

Spray application of V10 is effective in the protection of tomato plants from the European and Chile strains of the Pepino Mosaic Virus.

Rubbing
Application of V10 by rubbing the virus suspension on tomato leaves was not tested in the efficacy trials. However, in these trials, the inoculation of the aggressive virus isolates was made by rubbing. In these trials, as was confirmed by ELISA testing, all plants were successfully infected with the aggressive virus isolates.
Also, it was demonstrated in a bioassay that this method was effective in infecting tomato seedlings with VX1 and VC1 virus suspension (please refer to IIIA 6.1.1).
Furthermore, mechanical inoculation of viruses by rubbing the virus suspension on leaves is a well-known and regularly used method for virus inoculation in scientific research.

Minimum effective dose
No minimum effective dose trials are available for the product V10. However, since the isolates VX1 and VC1 will replicate within the crop, efficacy of the product will be independent of the concentration of the applied product once the plant is infected. The effectiveness of the product depends on the infectivity of the virus isolates VC1 and VX1. As a result, standard minimum effective dose trials are not considered useful.

In a bioassay conducted on tomato seedlings it was shown that a concentration of 1-10% was sufficiently infective when applied by rubbing on tomato leaves (please refer to IIIA 6.1.1).

No reliable data is available for determination of the minimum required concentration of V10 during spray applications. Successful infection of the crop by spray application will largely depend on the mechanical damage inflicted to the crop through which the infection can occur. The rate at which the crop is infected is therefore determined by many different factors such as the distance of the spray boom from the crop and the spray pressure at application, the amount of abrasive in the spray solution, the age of the crop and the crop cultivar. To ensure good infection of the crop under varying conditions the proposed dose rate was set at 2%. The product is applied at a high spray volume of 3500 L/ha, with 0.15% w/v synthetic sand to ensure that enough mechanical damage occurs to the crop to facilitate infection and that no blockage of the spray nozzles occurs.
The proposed dose rate of 2% V10 has demonstrated to be effective in the prevention of PepMV virus in the efficacy trials.

Quality
To establish the effect of V10 on quality in absence of the aggressive virus, plants treated with V10 (and not inoculated with aggressive PepMV) were compared to untreated plants in the 7 efficacy trials.

However in 1 of these trials ELISA testing demonstrated that the untreated non-inoculated plants were infected with V10. Therefore this trial is excluded from the summary.
The product V10, a combination of mild PepMV isolates VC1 and VX1, was applied by spraying at 2% in combination with carborundum.
Overall, treatment with V10 largely prevents the adverse effects on tomato plants and fruits caused by the Pepino Mosaic Virus. In absence of aggressive PepMV infection, treatment with V10 can cause mild PepMV symptoms on tomato plants, but does not affect yield. A warning sentence to this extent is placed on the label.

Yield
To establish the effect of V10 on yield in absence of the aggressive virus, plants treated with V10 (and not inoculated with aggressive PepMV) were compared to untreated plants in the 7 efficacy trials. However in 1 of these trials ELISA testing demonstrated that the untreated non-inoculated plants were infected with V10. Therefore this trial is excluded from the summary.
The product V10, a combination of mild PepMV isolates VC1 and VX1, was applied by spraying at 2% in combination with carborundum.

In absence of aggressive PepMV infection, no effect on yield of treatment with V10 was observed in relation to the untreated objects.

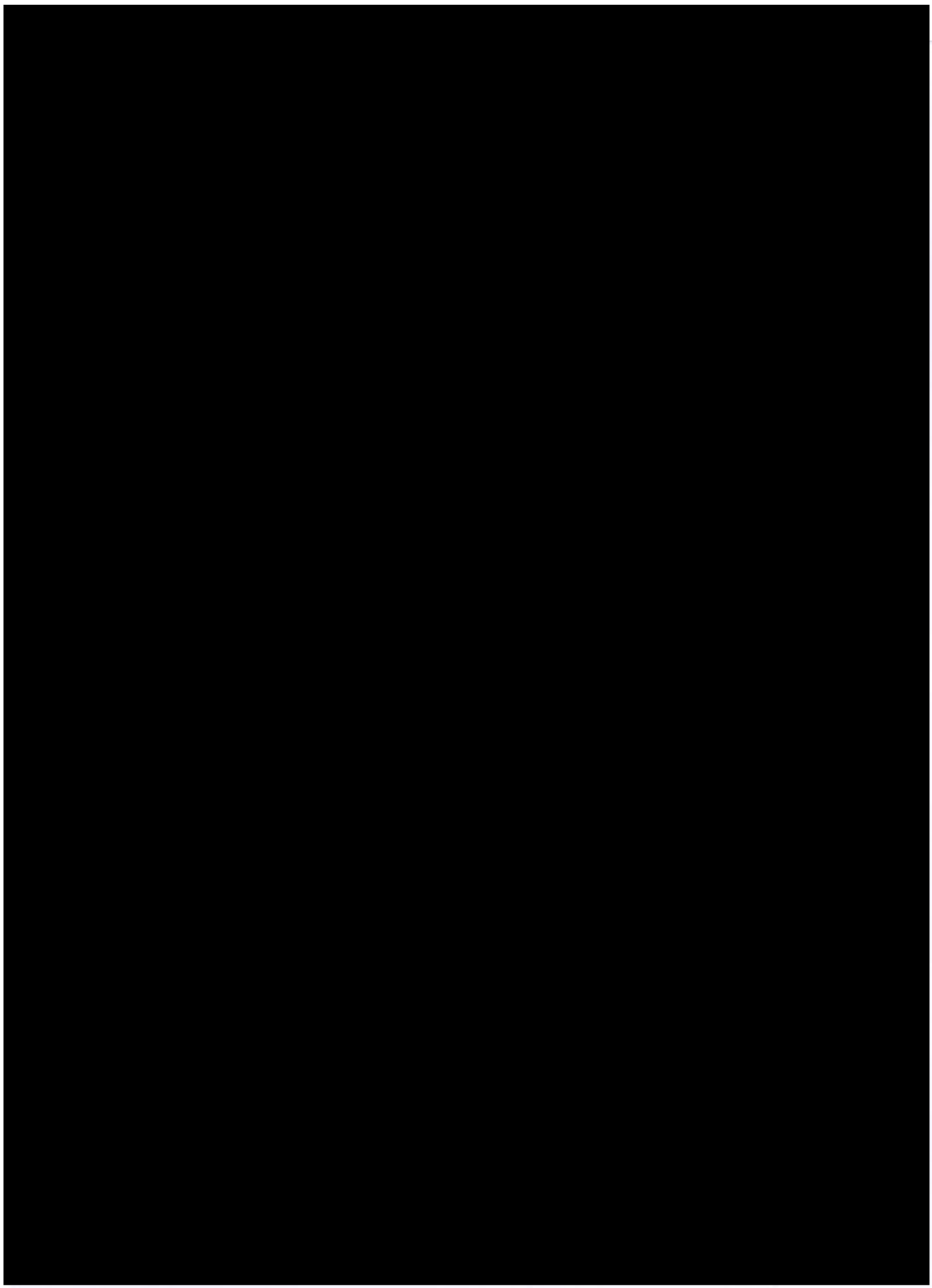
Crop safety
To establish the effect of V10 on the crop in absence of the aggressive virus, plants treated with V10 (and not inoculated with aggressive PepMV) were compared to untreated plants in the 7 efficacy trials. However in 1 of these trials ELISA testing demonstrated that the untreated non-inoculated plants were infected with V10. Therefore this trial is excluded from the summary.
The product V10, a combination of mild PepMV isolates VC1 and VX1, was applied by spraying at 2% in combination with carborundum.

In absence of aggressive PepMV infection, treatment with V10 can cause minor PepMV symptoms on tomato plants and to a lesser extent on tomato fruits, resulting in a negative effect on the crop. However these effects did not have an effect on yield.
In plants treated with V10 yet not inoculated with aggressive PepMV, some minor stunting was observed, that was not observed in the untreated plants. In one trial stunting was also observed in the untreated uninfected objects, however this was not due to virus infection as the ELISA test was negative for infection.
The crop condition of plants treated with V10 (not inoculated with aggressive PepMV) was always moderate to excellent (scores of 6-10), yet was generally slightly lower than that observed in the untreated plants (not inoculated with aggressive PepMV) (scores of 6.4-10)

Overall, treatment with V10 largely prevents the adverse effects on tomato plants and fruits caused by the Pepino Mosaic Virus. In absence of aggressive PepMV infection, treatment with V10 can cause mild PepMV symptoms on tomato plants, but does not affect yield. A warning sentence to this extent is placed on the label.

IIIA 6.7 List of test facilities including the corresponding certificates

Organization	Address	GEP
[redacted]	[redacted]	GEP



Appendix 1a: Reference list

Annex point	Author	Year	Title	Source
IIIA 6.0/01	Agrios, G.N	2004	Defense through RNA silencing by pathogen-derived genes	Plant Pathology. Fifth edition. Elsevier Academic Press. ISBN-10: 0-12-044565-4. Pp. 244-246
IIIA 6.0/02	Baulcombe, D	2004	RNA silencing in plants	Nature 431: 356-363
IIIA 6.0/03	Hanssen, I.M., van Esse, H.P., Ballester, A-R., Hogewoning, S.W., Ortega Parra, N., Paeleman, A., Lievens, B., Bovy, A.G., Thomma, B.P.H.J	2011	Differential Tomato Transcriptomic Responses Induced by Pepino Mosaic Virus Isolates with Differential Aggressiveness	Plant Physiology 156:301-318
IIIA 6.0/04	Ratcliff, F.G., MacFarlane, S.A., Baulcombe, D.C	1999	Gene silencing without DNA: RNA-mediated cross-protection between viruses	Plant Cell 11: 1207-1215
IIIA 6.0/05	Valkonen, J.P.T., Rajamäki, M.L., Kekarainen, T	2002	Mapping of viral genomic regions important in cross-protection between isolates of a potyvirus	Molecular Plant-Microbe Interactions 15: 683-692
IIIA 6.0/06	Krinkels, M	2001	Pepino mosaic virus causes sticky problem	The Annual, May 2001, 30-33

Appendix 1b: List of data submitted in support of the evaluation

Annex point	Author	Year	Title Source Company, Report No. GEP status, (un)published	Data protection claimed Y/N	Owner
IIIA 6.1.1/01	[redacted]	2013	Bio-assay infectivity PepMV SPV A519 non GEP, unpublished	Y	Valto B.V
IIIA 6.1.1/02	[redacted]	2013	Validation of bio-assay to determine the infectivity of pepino mosaic virus [redacted] non GEP, unpublished	Y	Valto B.V
IIIA 6.1.1/03	Anonymous	2015	Microbiologisch Analyserapport. [redacted] non GEP, unpublished	Y	Valto B.V
IIIA 6.1.1/04	Anonymous	2014	Microbiologisch Analyserapport. [redacted] non GEP, unpublished	Y	Valto B.V
IIIA 6.1.1/05	[redacted]	2016	Infectivity of VC1 batches. [redacted] non GEP, unpublished	Y	Valto B.V
IIIA 6.1.1/06	[redacted]	2016	Infectivity of VX1 batches. [redacted] non GEP, unpublished	Y	Valto B.V
IIIA 6.1.1/07	[redacted]	2017	Comparison of infectivity efficiency after high-pressure spraying of V10 with carborundum and with synthetic sand [redacted] non GEP, unpublished	Y	Valto B.V
IIIA 6.1.1/08	[redacted]	2012	Protocol for the determination of indices of VC1, VX1 and PepMv by qRT-PCR [redacted] non GEP, unpublished	Y	Valto B.V
IIIA 6.1.3/01	[redacted]	2012	Control of pepino mosaic virus in tomato. [redacted] GEP, unpublished	Y	Valto B.V
IIIA 6.1.3/02	[redacted]	2012	Control of pepino mosaic virus in tomato. [redacted] GEP, unpublished	Y	Valto B.V

Annex point	Author	Year	Title Source Company, Report No. GEP status, (un)published	Data protection claimed Y/N	Owner
IIIA 6.1.3/03	[redacted]	2012	Control of pepino mosaic virus in tomato. [redacted] GEP, unpublished	Y	Valto B.V
IIIA 6.1.3/04	[redacted]	2013	Control of pepino mosaic virus in tomato. [redacted] GEP, unpublished	Y	Valto B.V

Appendix 2: GAP Tables

PPP (product name/code) **V10** **Formulation: Type:** **SC**
active substance 1 **Mild PepMV isolate VX1** **Conc. of as 1:** **5-25 mg/L**
active substance 2 **Mild PepMV isolate VC1** **Conc. of as 2:** **5-25 mg/L**

Zone(s): **EU** **professional use** ☒ **non professional use** ☐

1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*10*	*11*	*12*	*13*	*14*
Use-No.	**Member state(s)**	**Crop and/ or situation (crop destination / purpose of crop)**	**F G or I**	**Pests or Group of pests controlled** (additionally: developmental stages of the pest or pest group)	**Application**			**Application rate**			**PHI** (days)	**Remarks:** e.g. g safener/synergist per ha
					Method / Kind	Timing / Growth stage of crop & season	Max. number (min. interval between applications) a) per use b) per crop/ season	kg, L product / ha a) max. rate per appl. b) max. total rate per crop/season	g, kg a.s./ha a) max. rate per appl. b) max. total rate per crop/season	Water L/ha min / max		
1	NL, BE, AT, DE, UK, FR, ES, IT, PL, SE	*Solanum lycopersicum* (tomato)	G	Pepino mosaic virus	Downward spraying (12-15 bar)	Young tomato plants (BBCH 13-51, 10-30 cm high) Jan-Dec	a) 1 per crop cycle b) 8 per 12 months (8 crops cycles per year)	a) 70 L product / ha per appl. b) 560 L product / ha per 12 months	a) 1.75 g VC1/ha per crop cycle 1.75 g VX1/ha per crop cycle b) 14 g VC1/ha per 12 months 14 g VX1/ha per 12 months	3500 L/ha per appl.		1-8 crop cycles per 12 months V10 is applied in combination with 800 grams of synthetic sand per 100 litres of spray liquid
2	NL, BE, AT, DE, UK, FR, ES, IT, PL, SE	*Solanum lycopersicum* (tomato)	G	Pepino mosaic virus	Rubbing individual plants	Young tomato plants (BBCH 13-61) Jan-Dec	a) 1 per crop cycle b) 8 per 12 months (8 crops cycles per year)	a) 0.8 L product / ha per appl. b) 6.4 L product / ha per 12 months	a) 0.02 g VC1/ha per crop cycle 0.02 g VX1/ha per crop cycle b) 0.16 g VC1/ha per 12 months 0.16 g VX1/ha per 12 months	8L/ha per appl.		1-8 crop cycles per 12 months V10 is applied in combination with 15 grams of synthetic sand per 1 litre of liquid