

European Commission



**Draft Assessment Report prepared according to the Commission Regulation
(EU) N° 1107/2009**

**Pepino Mosaic Virus, EU strain, mild
isolate Abp1
Pepino Mosaic Virus, CH2 strain, mild
isolate Abp2
Product data: AbioProtect®
Volume 3 – Annex B.3 Data on application and
efficacy**

Rapporteur Member State: Spain

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Version History

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|------------|--|
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Introduction

This dossier is submitted by Abiopep S.L., Spain, for the approval of two new microbial active ingredients (Microbial Pest Control Agents) MCPAs: *Pepinomosaic virus* (PepMV), European (EU) strain, mild isolate Abp1 and PepMV, Chilean (CH2) strain, mild isolate Abp2, under the Regulation (EC) 1107/2009 of the European Parliament.

PepMV belongs to the genus *Potexvirus* of the *Alphaflexiviridae* family; it is widespread in Europe and in fact is a major disease in greenhouse tomato crops worldwide.

The cross-protection effect and thus the actual activity is obtained by infection of the plants with the mild isolates of the virus: PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2. Viral cross-protection in plants is known as an acquired immunity phenomenon, where a mild virus isolate can protect plants against economic damage caused by a severe challenge isolate of the same virus. The mode of action of cross-protection has been explained in a relatively complete general manner by a model based on a combination of RNA silencing and coat-protein-mediated resistance. Mild isolates will induce in tomato crop a symptomless infection without damage to the fruit, while an aggressive isolate will induce symptoms leading to economic losses in the crop.

PepMV is a plant virus, which can only replicate in living plant cells and the virus can only be produced in plants. Tomato is the most suitable host for PepMV, so production of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, is performed in tomato plants.

The preparation (Microbial Pest Control Product) MPCP AbioProtect® is a suspension concentrate formulated with equivalent amounts of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2. The MPCP is envisaged as a preventive treatment in greenhouse (protective) tomato production against aggressive isolates of PepMV to be applied in a close compartment near or inside the final destination greenhouse in a single application to tomato seedlings (BBCH 13-15). Abiopep employs trained and qualified personnel to conduct product application and the product is never applied by third parties.

GAP Table: Details of all national GAPs within each zone**MPCP/PPP (product name/code)** AbioProtect®**Formulation:** Type:SC^(a-b)**MPCA: active ingredient 1****PepMV, EU strain, mild isolate Abp1****Conc. of as 1:** at least 2.5×10^{11} genome copies/L**MPCA: active ingredient 2****PepMV, CH2 strain, mild isolate Abp2****Conc. of as 2:** at least 2.5×10^{11} genome copies/L**Zone(s):**EU**Professional use** ☒**Non professional use** ☐

| 1 | 2 | 3 | 4 | 5 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|------------|--------------------|--|--------------------------|---|---|--|--|---|--|------------------------|----------------------|--|
| Use- No | Member state(s) | Crop and/ or situation (crop destination/purpose of crop) (c) | F G or I (d) | Pests or Group of pests controlled Additionally: developmental stages of the pest or pest group (e) | Application | | | Application rate per treatment | | | PHI (days) (j) | Remarks e.g. g. safener/synergist per ha (k) |
| | | | | | Method Kind (f-g) | Timing/ Growth stage of crop & season (h) | 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 (i) | kg, L a.s /ha a) max rate per appl. b) max. total rate per crop/season | Water L/ha min/ max | | |
| 1 | All | <i>Solanum lycopersicum</i> (tomato) (LYPES) | G | Pepino mosaic virus (PEPMVO, PepMV) | Low volume spraying (aerial spraying with an airbrush 75 psi/ 5171.07 mbar/ 517.10 kPa) | Seedlings immediately before planting (BBCH 13-15) Jan-Dec | a) 1 per use b) 1 per crop cycle | a) 0.1–1.6 L/ha (0.05–0.8 L/ha PepMV Abp1 and 0.05–0.8 L/ha of PepMV Abp2) b) 0.1 – 1.6 L/ha per crop cycle cycle | At least 1.25 – 2.0 x 10^{12} genome copies/ha of Abp1 and At least 1.25–2.0 x 10^{12} genome copies/ha of Abp2 | 4–7.84 L/ha | NA | - |

Remarks:

- a) e.g. wettable powder (WP), emulsifiable concentrate (EC), granule (GR).
- b) GCPF Codes - GIFAP Technical Monograph No 2, 1989.
- c) For crops, the EU and Codex classifications (both) should be used; where relevant, the use situation should be described (e.g. fumigation of a structure).
- d) Outdoor or field use (F), glasshouse application (G) or indoor application (I).
- e) e.g. biting and suckling insects, soil born insects, foliar fungi, weeds.
- f) Method, e.g. high volume spraying, low volume spraying, spreading, dusting, drench.
- g) Kind, e.g. overall, broadcast, aerial spraying, row, individual plant, between the plants - type of equipment used must be indicated.

- h) Growth stage at last treatment (BBCH Monograph, Growth stages of mono- and dicotyledonous plants, 2^o edit 2001, Blackwell, ISBN 3-8263-3152-4), including where relevant, information on season at time of application.
- i) The minimum and maximum number of application possible under practical conditions of use must be provided.
- j) PHI - minimum pre-harvest interval.
- k) Remarks may include: Extent of use/economic importance/restrictions.

B.3 DATA ON APPLICATION AND EFFICACY

B.3.1 Field of use envisaged

AbioProtect® is for use in horticulture, in protected (greenhouse) tomato crops as an elicitor against aggressive isolates of PepMV.

B.3.2 Mode of action

AbioProtect® is formulated with equivalent amounts of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2. It is used to infect (vaccinate) tomato plants and therefore protect them against a wide range of aggressive isolates of PepMV by cross-protection.

AbioProtect® is sprayed on to the tomato seedlings and the viral particles penetrate the plant mechanically, as PepMV is very efficiently mechanically transmitted; in fact, contaminated hands, clothing or tools facilitate PepMV transmission (Van der Vlugt, 2009). Crop workers can transmit the virus simply by brushing against affected plants and during crop nursing activities such as pruning and harvesting (Ferguson, 2001). After penetration AbioProtect® PepMV isolates infect the plant systemically and prevent infection by other isolates of PepMV by cross-protection.

Cross-protection is a natural phenomenon in which prior systemic infection with one virus (the protector virus) prevents or interferes with subsequent infection by another isolate of the same virus or a closely related virus (the challenging virus) (Natsuaki, 2014). The phenomenon was first reported with *Tobacco mosaic virus* (TMV) in 1929 (McKinney, 1929). Since then, cross-protection has been demonstrated for many plant viruses including sap-transmissible viruses such as *Potato virus X* (PVX), non-sap-transmissible *Potato leafroll virus* (PLRV), *Citrus tristeza virus* (CTV), other RNA viruses, DNA viruses, and viroids (Gal-On and Shibolet, 2006; Pennazio *et al.* 2001).

Viral cross-protection in plants is known as an acquired immunity phenomenon, where a mild virus isolate can protect plants against economic damage caused by a severe challenge isolate of the same virus.

Mechanisms for specificity can act either at the initial plant/virus interaction, or during the replication of the challenge virus. In the initial interaction, the challenge virus could be inhibited from uncoating, and the replication would never be initiated. If replication is initiated, a number of mechanisms may be impairing it (i) the initial translation could be blocked, (ii) the transcription could be blocked and (iii) the production of genome-length viral nucleic acid could be inhibited. Finally, even if challenge virus managed to replicate, its movement from cell to cell could be prevented. Explanation of cross-protection by one hypothesis alone is unlikely and it is plausible that different mechanisms may be operating in different virus groups (Sherwood, 1987). A model based on a combination of RNA silencing and coat-protein-mediated resistance can explain the cross-protection phenomenon in a relatively complete general manner (Gal-On and Shibolet, 2006), though alternative models have been proposed recently (Zhang *et al.*, 2016).

Cross-protection using attenuated viruses offers a promising strategy for biological control of plant viral diseases. Vaccination of a tomato plant with AbioProtect® does not have any effect on yield or fruit quality (contrary to infection with aggressive isolates) but induces cross-protection. Multiplication of any aggressive isolate from the EU strain or the CH2 strain of PepMV would be prevented. Cross-protection only works when tomato plants are inoculated with the mild isolates before being exposed to the aggressive isolates.

B.3.3 Details of intended use

AbioProtect® is to be used against aggressive isolates of PepMV in greenhouse tomato crops.

It is not necessary to wait any specific interval before treatment with any chemical pesticide, the treatment is to be applied alone by Abiopep trained and qualified personnel.

B.3.4 Application rate

AbioProtect® is used at 5 L/10,000 plants equivalent to 1 ha in standard tomato greenhouse practice, in cases where a very high risk of PepMV infection exist the rate could reach 8 L/ha.

B.3.5 Content of micro-organism in material used (e.g. in dilutes spray, baits or treated seed)

AbioProtect® is formulated with a minimum content of at least 5×10^{11} PepMV genome copies (viral units)/L, that is a minimum content of at least 2.5×10^{11} genome copies (viral units) of PepMV, EU strain, mild isolate Abp1/L and a minimum content of at least 2.5×10^{11} genome copies (viral units) of PepMV, CH2 strain, mild isolate Abp2/L.

B.3.6 Method of application

AbioProtect® is applied using spraying equipment such as an airbrush, adjusting the application pressure to 75 psi, at a distance between 25-30 cm from the tomato seedlings, in a close facility of inside the greenhouse right before transplanting to the final destination tomato greenhouse plot. The volume is 5-8 L of AbioProtect® per 10,000 tomato plants (equivalent to 1 ha).

B.3.7 Number and timing of applications

| Crop | Nº of applications | Timing of applications | Duration of protection |
|---------------------|--------------------|---|------------------------|
| Tomato (greenhouse) | 1 per crop cycle | Tomato seedlings (3-5 leaves) BBCH 13-15 (all seasons) | Whole crop cycle |

B.3.8 Necessary waiting periods or other precautions to avoid phytopathogenic effects on succeeding crops

Not applicable.

B.3.9 Proposed instructions for use

Applied onto tomato seedlings before transplanting to the commercial greenhouse in appropriate enclosed area, inside the greenhouse.

Adjust airbrush pressure to 75 psi.

Shake well before used within the container.

Apply 5 L/10,000 plants (1 ha) at a distance between 25-30 cm from the plants.

Optimum protection is achieved when transplanting is fulfilled in the same day of treatment.

Plants should be kept in the shadow until transplanting to the greenhouse.

After preparation keep the product at 4-10°C and used within 6 hours.

Full details of the proposed instruction for use are included in the draft label and leaflet provided as part of Document C.

B.3.10 Efficacy data

The representative uses of Abiopep applied for in this dossier are included in the GAP table above.

B.3.10.1 Preliminary tests

Gomez *et al.* (2009) showed that tomato plants infected with *Pepinomosaic virus* (PEPMVO, PepMV) isolates from the European (EU) strain together with isolates from the Chilean (CH2) strain (mixed infections) were symptomless. Therefore, to test the possibility of using mixed infections with mild isolates of PepMV from those two strains in cross-protection, several research trials were conducted using PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 in tomato plants against infection with an aggressive PepMV isolate.

A first small preliminary trial with 2 tomato cultivars, Pera and Kumato, was conducted in April-September 2012 in an experimental greenhouse at CEBAS-CSIC facilities in Murcia (Spain). Plants were inoculated with different treatments including mild isolate Abp1, mild isolate Abp2 and both mild isolates Abp1 and Abp2 simultaneously, as well as the corresponding controls. Treatments were later challenged with an aggressive PepMV isolate. Plant vigor and fruit production was observed and determined that the plants inoculated with both mild isolates simultaneously and subsequently challenged with the aggressive isolate, showed vigor and fruit production similar to the control without inoculation and not challenged. Results were confirmed in a second trial during April-September 2013 (a summary of those trials could be found in Aranda *et al.*, 2016a).

Another preliminary trial to assess symptom performance was conducted in 2012 in a greenhouse in a commercial tomato production area in *Región de Murcia* (southeast Spain) with a history of high incidence of PepMV infections. The greenhouse was divided in two parts. The tomato seedlings for one part were inoculated with both PepMV mild isolates (Abp1 and Abp2) simultaneously at the moment of transplanting from the nursery and the tomato seedlings for the other part, were kept un-inoculated as control. Approximately 12-14 weeks after inoculation, the fruits of the un-inoculated control started to show PepMV symptoms, with a high percentage of plants affected, while the fruits of the inoculated plants remained symptomless or with very mild transient symptoms (a summary of those trials could be found in Aranda *et al.*, 2016a).

AbioProtect® the formulation containing equivalent amounts of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, was first registered in Spain in 2014 according to Orden APA/1470/2007 number 2536, followed by a registration according to RD 951/2014 until October 2015. In 2016 a temporary exemption provided for in Article 53 of Regulation (EC) 1107/2009 have been granted for the use of AbioProtect® for the protection of greenhouse tomato cultivation against damage by aggressive PepMV from both the EU strain and the CH2 strain, and especially adapted for the specific phytosanitary situation in Spain. The application rate for those treatments during that period was set at 5 L/ha (10,000 tomato seedlings), a standard cropping practice for tomato greenhouses in southeast Spain grow on average 10,000 plants/ha.

A dose trial was conducted in 2016-2017 with GEP certification.

Report MP 6.1/01 Field study to evaluate the crop safety and the efficiency and velocity of the infection of the Plant Protection Product (PPP) AbioProtect® applied at different doses in tomato crop (Southern Spain, 2016). Prats (2017c). (Unpublished report). Study Code: ACEx/1276/AB.

Guideline: PP 1/152(3), PP 1/181(3) and PP/135 (3).

GEP: Fully GEP compliance

The aim of the study was to evaluate the efficiency and velocity of the infection of AbioProtect® applied at different doses in tomato crops, as well as to evaluate the treated crop for appearance of phytotoxicity effects.

Methodology

The trial lasted from October 10th until November 15th, 2016.

The test product, AbioProtect®, was applied at three rates (3, 5 and 8 L/ha). At a concentration of at least 5×10^{11} genome copies of PepMV/L (at least 2.5×10^{11} genome copies of PepMV, EU strain, mild isolate Abp1/L and at least 2.5×10^{11} genome copies of PepMV, CH2 strain, mild isolate Abp2/L).

AbioProtect® was applied with an airbrush on tomato plants before planting. Distance 25-30 cm.

Assessments of phytotoxicity were conducted at 7, 14, 21, 28 and 35 days after the application.

Analyses of AbioProtect® presence (presence of virus, PepMV-EU and PepMV-CH2 strains) were conducted in each plant throughout the trial to evaluate the efficiency and velocity of the infection of the Plant Protection Product (PPP).

Findings

No problems were encountered during application of the product under test.

No crop phytotoxicity symptoms were observed in the trial at any of the assessment timings, so the three doses of AbioProtect® were safe to the crop.

The following tables (Table MP 6.1/01 and Table MP 6.1/02) summarized the results of the trial. More information is presented in the individual trial report.

Table MP 6.1/01 Mean of % phytotoxicity on tomato (LYPES) after treatment with AbioProtect® (PepMV-EU (Abp1) and PepMV-CH2 (Abp2)). Treatment means with no letters in common are significantly different, LSD test ($P \leq 0.05$).

| Trt · No. | Product | Appr ate (L fp/ ha) | A1 7DAAp | A2 14DAAp | A3 21DAAp | A4 28DAAp | A5 35DAAp |
|------------------|------------------------|---------------------------------|---------------|---------------|---------------|---------------|---------------|
| | | | Phytotoxicity | Phytotoxicity | Phytotoxicity | Phytotoxicity | Phytotoxicity |
| 1 | CONTROL (untreated) | - | 0.0 a | 0.0 a | 0.0 a | 0.0 a | 0.0 a |
| 2 | ABIOPROTE CT® | 3 | 0.0 a | 0.0 a | 0.0 a | 0.0 a | 0.0 a |
| 3 | ABIOPROTE CT® | 5 | 0.0 a | 0.0 a | 0.0 a | 0.0 a | 0.0 a |
| 4 | ABIOPROTE CT® | 8 | 0.0 a | 0.0 a | 0.0 a | 0.0 a | 0.0 a |
| Date type | | | No transf. | No transf. | No transf. | No transf. | No transf. |
| LSD | | | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| CV | | | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |
| p(F), treatments | | | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |

L fp/ha litre of formulated product /ha

DAAp: days after application

Table MP 6.1/02 Mean of % plants with presence of AbioProtect® (PepMV-EU and PepMV-CH2 strain) on tomato (LYPES). Treatment means with no letters in common are significantly different, LSD test ($P \leq 0.05$)

| Tr · No | Product | Ap p rat e (L fp/ ha) | A3 21DAp | | A4 28DAp | | A5 35DAp | |
|------------------|------------------------|---|--------------|---------------|--------------|---------------|--------------|---------------|
| | | | PepMV- EU | PepMV- CH2 | PepMV- EU | PepMV- CH2 | PepMV- EU | PepMV- CH2 |
| 1 | CONTROL (untreated) | - | 0.0 b | 0.0 c | 0.0 b | 0.0 c | 0.0 b | 0.0 c |
| 2 | ABIOPROT ECT® | 3 | 55.6 ab | 44.4 b | 79.4 a | 49.2 b | 81.0 a | 65.1 b |
| 3 | ABIOPROT ECT® | 5 | 88.9 a | 49.2 b | 96.8 a | 57.1 b | 96.8 a | 68.3 ab |
| 4 | ABIOPROT ECT® | 8 | 88.9 a | 74.6 a | 96.8 a | 81.0 a | 98.4 a | 87.3 a |
| Date type | | | No transf. | No transf. | No transf. | No transf. | No transf. | No transf. |
| LSD | | | 61.89 | 21.13 | 36.13 | 15.26 | 36.93 | 19.33 |
| CV | | | 46.81 | 22.16 | 23.36 | 14.38 | 23.6 | 15.46 |
| p(F), treatments | | | 0.0445 | 0.0028 | 0.0046 | 0.0005 | 0.0049 | 0.0009 |

L fp/ha litre of formulated product /ha

DAp: days after application

Conclusions

The plant protection product AbioProtect® is safe for use in tomato crops.

The Plant Protection Product AbioProtect® showed a good efficiency and velocity of infection in the tomato plants in general.

The component Abp1 (EU strain of PepMV) showed a better efficiency of infection than the component Abp2 (CH2 strain). The high dose of AbioProtect® (T4 8 L/ha) presented the best results, followed by T3 (5 L/ha). In the case of EU strain, treatments 3 and 4 obtained similar results, but in CH2 strain treatment 4 showed an incidence of plants infected 20-25% higher than treatment 3. The low dose (T2 3 L/ha) obtained a lower incidence and velocity of infection.

Considering the results of the dose trial and according to previous experience of the Applicant, the dose for applications is set up at 5 L/ha (10,000 tomato plants) of AbioProtect®. In tomato production areas with a record of high incidence of aggressive PepMV infections it could be increased up to 8 L/ha.

B.3.10.2 Testing effectiveness

The formulation AbioProtect®, formulated with equivalent amounts of PepMV EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, has been tested in greenhouse trials which demonstrated its effectiveness and appropriate crop safety against infection by PepMV aggressive isolates from both the EU and the CH2 strains. The trials data supporting effectiveness against this target comprise 6 trials conducted in greenhouses in different locations in Spain from 2014-2017. All trials were carried out in accordance with the principles of Good Experimental Practices (GEP), and are certified by the officially recognized organization. The trials were conducted in protected tomato crops; therefore, the data are representative for the entire EU. Further details of the

individual trials conducted are provided in Table MP 6.2/01 and in the corresponding individual trials (Documents K-MP 6.2/01/02/03, /04, /05 and /06 (Céspedes, 2015b; Prats, 2017a, 2017b and 2017c).

In three of these trials the efficacy of AbioProtect® was tested against aggressive isolates of the PepMV EU strain, (isolated from a commercial greenhouse tomato crop in Alicante (Spain) on September 2015, aggressive EU) and of the PepMV CH2 strain (isolated from a commercial greenhouse tomato crop in Granada (Spain) on March 2014, aggressive CH2). Additionally, the efficacy of the separate isolates was tested against both aggressive isolates.

In one of the six trials, the efficacy of AbioProtect® was tested against the aggressive CH2 isolate of PepMV. In this trial also the efficacy of the separate isolates of PepMV was tested against the aggressive CH2 isolate.

In the remaining 2 trials the efficacy of AbioProtect® was tested against the aggressive CH2 isolate of PepMV, without testing the efficacy of the independent isolates.

In all the trials the formulation has been applied at the proposed dose rate of 5 L/ha, containing $>5 \times 10^{11}$ genome copies of PepMV/L ($>2.5 \times 10^{11}$ genome copies of PepMV, EU strain, mild isolate Abp1/L and $>2.5 \times 10^{11}$ genome copies of PepMV, CH2 strain, mild isolate Abp2/L). The separate isolates Abp1 and Abp2, were each applied at a dose of $>2.5 \times 10^{11}$ genome copies /L. In all the trials, the treatments were compared with a control treatment that was artificially infected with either isolate aggressive EU or isolate aggressive CH2. No standard reference materials are available for this type of use and were not included.

Overall AbioProtect® and the separate active ingredients proved effective in preventing infection with the virulent isolates. PepMV related symptoms on leaves and fruits were strongly reduced in the plants infected with mild virus isolates and then challenged with the aggressive viral isolates compared with the non-treated plants. Moreover, compared to the untreated plots challenged with aggressive isolates, aggressive EU and/or aggressive CH2, the plants treated with AbioProtect® and the separate active ingredients resulted in higher fruit yield.

Table MP 6.2/01 Summary of efficacy trials sites and application details

Type of trials:

EPPO Guidelines:

Identity of the product under test:

Crop:

Harmful organism (common name, scientific name) or intended use:

Responsible body for reporting trial (name, address):

Date of submission:

effectiveness and phytotoxicity

PP1/152(3); PP1/181(3); PP1/135(3)

AbioProtect®

Tomato (LYPES)

Pepino mosaic virus (PEPMVO, PepMV) aggressive isolates EU and CH2 strains

Agrocolor S.L. Ctra. de Ronda, 11-bajo. 04004 Almeria, Spain.

July 2017

| Test Report (1) year | Testing unit address | Trial location | Test Method Plot size Sample size | Tomato cultivar | Application | | | Remarks |
|------------------------|---|----------------------------------|---|-----------------|--|-----------------------------|---|---|
| | | | | | Method and equipment | Treatment application time | Harmful organism incidence | |
| LPA/2014-23/Ca 2014 | Estación Experimental CajaMar “Las Palmerillas” Tel 950 58 05 48 | El Ejido 04710 Almeria, Spain | 4 m ² (2 m x 2 m) (1 row per plot, 4 plants per plot) 182 plots | Caniles | Foliar (entire plants) Hand sprayer | Planting time BBCH 13-15 | 20 days after treatment. Crop stage: 17-18 BBCH. Selected plots were treated manually with aggressive PepMV challenge | Assessments of phytotoxicity*: - PepMV symptoms in crop (bright yellow mosaic in leaves) - PepMV symptoms in fruits (discoloration, open necrotic fruits) - Production (total and marketable, number of fruits and kg) |
| LPA/2014-23/Ve 2014 | Estación Experimental CajaMar “Las Palmerillas” Tel 950 58 05 48 | El Ejido 04710 Almeria, Spain | 4 m ² (2 m x 2 m) (1 row per plot, 4 plants per plot) 80 plots | Ventero | Foliar (entire plants) Hand sprayer | Planting time BBCH 13-15 | 20 days after treatment. Crop stage: 17-18 BBCH. Selected plots were treated manually with aggressive PepMV challenge | Assessments of phytotoxicity*: - PepMV symptoms in crop (bright yellow mosaic in leaves) - PepMV symptoms in fruits (discoloration, open necrotic fruits) - Production (total and marketable, number of fruits and kg) |
| LPA/2014-23/An 2014 | Estación Experimental CajaMar “Las Palmerillas” Tel 950 58 05 48 | El Ejido 04710 Almeria, Spain | 4 m ² (2 m x 2 m) (1 row per plot, 4 plants per plot) 80 plots | Angele | Foliar (entire plants) Hand sprayer | Planting time BBCH 13-15 | 20 days after treatment. Crop stage: 17-18 BBCH. Selected plots were treated manually with aggressive PepMV challenge | Assessments of phytotoxicity*: - PepMV symptoms in crop (bright yellow mosaic in leaves) - PepMV symptoms in fruits (discoloration, open necrotic fruits) - Production (total and marketable, number of fruits and kg) |
| ACEX1274/ | Instituto de Investigación y | La Mojonera | 5 m ² (2 m x 25 m) (1 row per plot, 5 | Pitenza | Foliar (entire | Planting time | 24 days after treatment. Crop | Assessments of phytotoxicity*: |

| Test Report (1) year | Testing unit address | Trial location | Test Method Plot size Sample size | Tomato cultivar | Application | | | Remarks |
|----------------------|--|---|---|-----------------|--|-----------------------------|---|--|
| | | | | | Method and equipment | Treatment application time | Harmful organism incidence | |
| AB 2016 | Formación Agraria y Pesquera, IFAPA Centro La Mojonera Tel 950 15 64 13 | 04745 Almeria, Spain | plants per plot) 48 plots | | plants) Hand sprayer | BBCH 13-15 | stage: 52 BBCH. Selected plots were treated manually with aggressive PepMV challenge | - PepMV symptoms in crop (bright yellow mosaic in leaves) - PepMV symptoms in fruits (discoloration, open necrotic fruits) - Production (total and marketable, number of fruits and kg) |
| ACEX1277/AB 2016 | Commercial greenhouse Tel 954 29 66 31 | El Albuñón, 30330 Cartagena Murcia, Spain | 4 m ² (2 m x 2 m) (1 row per plot, 5 plants per plot) 60 plots | Boludo | Foliar (entire plants) Hand sprayer | Planting time BBCH 13-15 | 21 days after treatment. Crop stage: 17-18 BBCH. Selected plots were treated manually with aggressive PepMV challenge | Assessments of phytotoxicity*: - PepMV symptoms in crop (bright yellow mosaic in leaves), - PepMV symptoms in fruits (discoloration, open necrotic fruits) - Production (total and marketable, number of fruits and kg) |
| ACEX1296/AB 2017 | Commercial greenhouse Tel 954 29 66 31 | Guía de Isora, 38680 Tenerife, Spain | 3 m ² (1,5 m x 2 m) (1 row per plot, 5 plants per plot) 48 plots | Naty | Foliar (entire plants) Hand sprayer | Planting time BBCH 13-15 | 20 days after treatment. Crop stage: 51 BBCH. Selected plots were treated manually with aggressive PepMV challenge | Assessments of phytotoxicity*: - PepMV symptoms in crop (bright yellow mosaic in leaves), - PepMV symptoms in fruits (discoloration, open necrotic fruits) - Production (total and marketable, number of fruits and kg) |

Notes: (1) Indicate the test report number including the year of establishing the trial (*e.g.* PM 96/1)

(2) Indicate the name, address and telephone number of the test unit

(3) Indicate the precise location of the trial and the country in which it was conducted (*e.g.* Rheims, France)

(4) Indicate the plot size

(5) Indicate the sample size per plot

(6) Indicate the method of application

(7) Indicate the type of equipment used

(8) Indicate the growth stage (s) (GS) of the crop and where relevant pests, in accordance with the BBCH Monograph, Growth Stages of Plants, 1997, Blackwell, ISBN 3-8263-3152-4), at each application and the corresponding severity of incidence of harmful organism

*Phytotoxicity was assessed evaluating bright yellow mosaic symptoms according to the following scale: 1: No symptoms, 2: Mild symptoms, 3: Moderate symptoms (yellow spots; interveinal yellowing of the tips), 4: Severe symptoms (complete yellowing of at the least 2/3 leaves), 5: Very severe symptoms (complete yellowing of at the least 4/5 leaves).

Material and Methods

Sites

Sites were selected on the bases of being representative of different greenhouse tomato cropping systems in Europe in general, and in Spain in particular.

Experimental details

Trials were carried out to evaluate the efficacy and crop safety of AbioProtect® and its components, PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, as a cross-protection treatment against aggressive isolates of PepMV present in Southeast Spain, when applied on tomato seedlings (BBCH 13-15) before transplanting on to the commercial tomato greenhouse. Trial plots size ranged from 300 m² to 800 m².

Formulation applied and application rates

Details of the formulations (vaccines) tested, rates and timings are indicated in Table MP 6.2/02.

Table MP 6.2/02 Formulations tested and challenge treatments applied

| Product | Active Ingredient (ai) | Concentration | Rate | Timing | Form. type |
|---------------------------------------|---------------------------------------|---|--------|-------------------------------------|--|
| PPA1 (Plant Protection Agent 1) | PepMV-Abp1 mild EU isolate | $\geq 2.5 \times 10^{11}$ viral genome copies/L | 5 L/ha | 13 BBCH | Tomato watery leaves extract containing PepMV, EU strain, mild isolate Abp1 (SC) |
| PPA2 (Plant Protection Agent 2) | PepMV-Abp2 mild CH2 isolate | $\geq 2.5 \times 10^{11}$ viral genome copies/L | 5 L/ha | 13 BBCH | Tomato watery leaves extract containing PepMV, CH2 strain, mild isolate Abp2 (SC) |
| AbioProtect® (PPP, Vaccine) | PepMV-Abp1 + PepMV-Abp2 (PPA1 + PPA2) | $\geq 5 \times 10^{11}$ viral genome copies/L | 5 L/ha | 13 BBCH | Tomato watery leaves extract containing PepMV, EU and CH2 strains, mild isolates Abp1 and Abp2(SC) |
| PepMV isolate (Inoculum, Challenge 1) | PepMV aggressive EU isolate | - | - | 17-52 BBCH (~3 weeks after vaccine) | Inoculum containing PepMV, EU strain, aggressive isolate |
| PepMV isolate (Inoculum, Challenge 2) | PepMV aggressive CH2 isolate | - | - | 17-52 BBCH (~3 weeks after vaccine) | Inoculum containing PepMV, CH2 strain, aggressive isolate |

Application method

Due to the number of different treatments to be assessed and to avoid miss handling them; the application was done manually in most of the trials, in spite that the treatments are applied by airbrush with a pressure of 75 psi in commercial applications.

Assessment methods-crop yield

Plots were harvested by hand picking the fruit. Assessments of number and kg of fruits per plot were conducted, differentiating between total production (number of fruits/m² and kg/m²) and marketable production (number of fruits/m², % of marketable fruits and kg/m²).

Assessment method symptoms

Crop safety in protected tomato has been considered in all effectiveness trials by assessing PepMV symptoms. Such assessment in the crop was conducted by giving individual scores to each plant according to an appropriate severity scale. Incidence data were also obtained. Assessments interval was modified depending on the evolution of the symptoms observed.

Bright yellow mosaic was evaluated by evaluating yellowing symptoms following this severity scale:

- 1: No symptoms
- 2: Mild symptoms
- 3: Moderate symptoms (yellow spots; interveinal yellowing of the tips)
- 4: Severe symptoms (complete yellowing of at the least 2/3 leaves)
- 5: Very severe symptoms (complete yellowing of at the least 4/5 leaves).

Symptoms were assessed in leaves and in fruit as shown in Figures MP 6.4/01 and MP 6.5/01. Further details of individual efficacy trials are included in the individual trial reports (complete details could be found in Documents K-MP 6.2/01/02/03, /04, /05 and /06 (Céspedes, 2015b; Prats, 2017a, 2017b and 217d).

Statistical analysis

Assessment data were analysed using a two-way analysis of variance (ANOVA) on untransformed and transformed data at a 95% confidence limit.

LSD multiple comparison test was then applied to separate any treatment differences that may be implied by the ANOVA TEST at a 95% confidence level.

Analysis details included in the result tables of the individual trial reports are: co-efficient of variation (CV), least significant difference (LSD), F probability for treatments (p(F)), and data type (indicates transformation type if appropriate). Where a transformation has been carried out this is indicated in the table as follows: Detransf. (Arcsi): Arcsine square root percent - $\text{ARCSIN}(\text{SQR}(X/100))$; Detransf. (Sqr): Square root - $\text{SQR}(X + .5)$; Detransf (Log): Log - $\text{LOG}(X + 1)$.

The tabulated data presented in this document (Tables MP 6.4/01, MP 6.4/02, MP 6.5/01 and MP 6.5/02 and Figures MP 6.4/01 and MP 6.4/02 below) only represents the means of selected treatments, within an assessment. Tables of data comprising all treatment means are presented in the individual trial reports. Also plot mean data, raw data and analysis details of untransformed data are included in Appendix of each individual trial report.

B.3.11 Information on the development of resistance

As the mode of action is based on cross-protection in the tomato crop against aggressive isolates of PepMV the possibility of development of resistance is not relevant. Please refer to Volume 3 Annex B.2 data point B.2.7 Genetic stability and factors affecting it and Volume 3 Annex B.3 (Data on application) data point B.3.5 Information on the occurrence or possible occurrence of the development of resistance of the target organism(s) for further information.

B.3.12 Adverse effects on treated crops

B.3.12.1 Effects on the quality of plants or plant products

The quality of the plants was evaluated by assessing the presence of symptoms in the leaves and in the fruits; production was differentiated in total production and marketable production in the efficacy trials conducted.

Treatment of tomato plants with the Plant Protection Product AbioProtect® does not produce any taint or odor in the fruits of the plants treated, or in any other aspects related with the quality of the plants or of the fruits. It should be noted that it is a treatment conceived for production of premium quality fresh tomato, in which symptoms in fruits is a decrease of quality and value of the product.

According to the data obtained from the different efficacy trials it could be concluded that the formulation AbioProtect® and its components achieve a high efficacy against PepMV, showing no symptoms in fruits during

the trials with similar data to the un-inoculated control and with clear significant differences with the challenged inoculated controls.

Table MP 6.4/01 and Figure MP 6.4/01 represent means of PepMV symptoms and other damage observed in fruits from a selected trial of those indicated on section MP 6.2. Tables of data comprising all treatment means for each trial are presented in the individual trial reports.

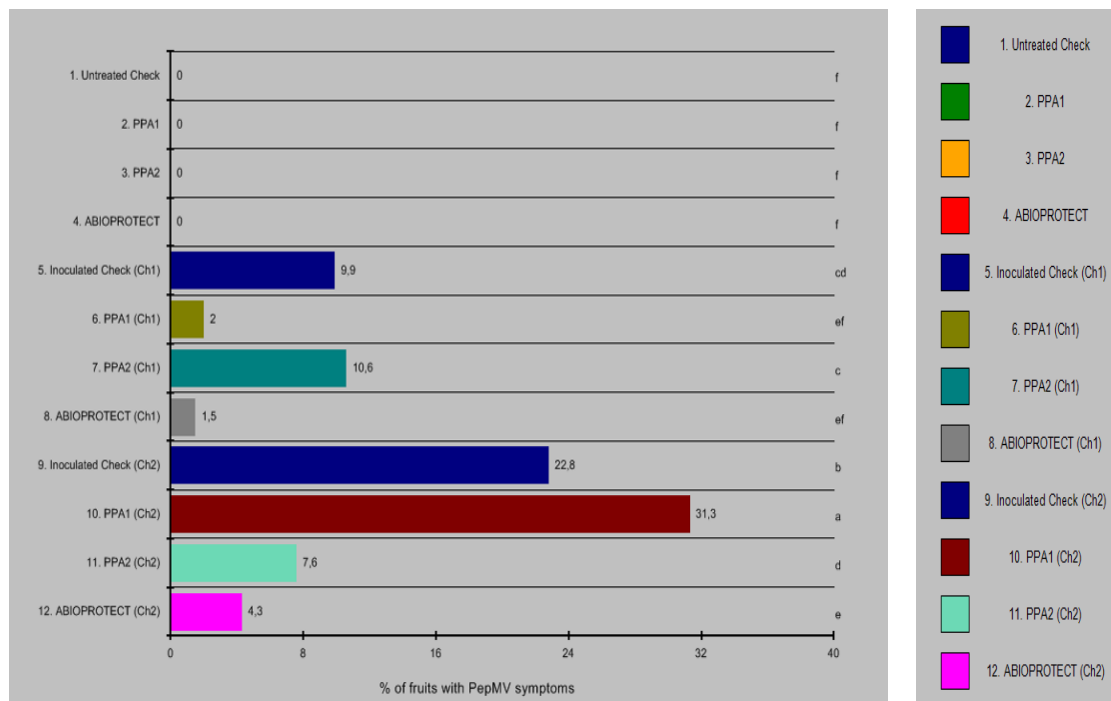
Table MP 6.4/01 Mean of PepMV symptoms and other damage observed in fruits per treatment. Treatment means with no letters in common are significantly different, LSD test ($P \leq 0.05$)

| Trt. No. | Product | App rate (L fp/ha) ¹ | PepMV symptoms | | Other damage | |
|------------------|----------------------------|---------------------------------|----------------|-------------|---------------|-------------|
| | | | No. of fruits | % of fruits | No. of fruits | % of fruits |
| 1 | CONTROL (untr.) | - | 0.0 e | 0.0 f | 4.5 a | 1.6 a |
| 2 | PepMV-Abp1 | 5 | 0.0 e | 0.0 f | 1.8 a | 0.7 a |
| 3 | PepMV-Abp2 | 5 | 0.0 e | 0.0 f | 2.3 a | 0.8 a |
| 4 | AbioProtect® | 5 | 0.0 e | 0.0 f | 2.3 a | 0.8 a |
| 5 | CONTROL (Ch1) ² | - | 28.5 c | 9.9 cd | 5.0 a | 1.7 a |
| 6 | PepMV-Abp1 (Ch1) | 5 | 6.0 de | 2.0 ef | 3.3 a | 1.1 a |
| 7 | PepMV-Abp2 (Ch1) | 5 | 30.3 c | 10.6 c | 5.0 a | 1.8 a |
| 8 | AbioProtect® (Ch1) | 5 | 4.5 de | 1.5 ef | 2.3 a | 0.7 a |
| 9 | CONTROL (Ch2) ³ | - | 62.8 b | 22.8 b | 6.3 a | 2.2 a |
| 10 | PepMV-Abp1 (Ch2) | 5 | 93.5 a | 31.3 a | 1.3 a | 0.4 a |
| 11 | PepMV-Abp2 (Ch2) | 5 | 22.3 c | 7.6 d | 6.3 a | 2.2 a |
| 12 | AbioProtect® (Ch2) | 5 | 12.0 d | 4.3 e | 4.0 a | 1.4 a |
| Date type | | | No transf. | No transf. | No transf. | No transf. |
| LSD | | | 9.57 | 2.88 | 3.95 | 1.35 |
| CV | | | 30.61 | 26.59 | 74.66 | 72.56 |
| p(F), treatments | | | 0.0001 | 0.0001 | 0.1383 | 0.1237 |

¹L fp/ha: liter of formulated product per hectare.

²Ch1: challenge 1, EU aggressive; ³Ch2: Challenge 2, CH2 aggressive.

Figure MP 6.4/01 Mean of % of fruits with PepMV (PEPMVO) symptoms observed per treatment. Treatment means with no letters in common are significantly different, LSD test ($P \leq 0.05$)



PPA1: PepMV-Abp1; PPA2: PepMV-Abp2, Ch1: challenge 1: EU aggressive; Ch2: Challenge 2 CH2 aggressive.
SCALE: 1: No symptoms. 2: Mild symptoms. 3: Moderate symptoms. 4: Severe symptoms. 5: Very severe symptoms.

B.3.12.2 Effects on the transformation process

Not relevant as the formulation is for use in commercial production of premium quality tomatoes for the fresh market to be consumed without any transformation process. It should be noted that in southeast Spain as well as in most countries in Europe tomato greenhouse production is a high input production only profitable when the product is marketed in fresh. Nonetheless treatment with AbioProtect® will not have any interference on transformation processes as viruses have no metabolism of their own, it does not produce residues and neither leaves residues at harvest.

B.3.12.3 Effects on the yield of treated plants or plant products

As already indicated, the formulation AbioProtect® and its components achieve a high efficacy against PepMV, showing no symptoms during the trials with similar data and total production (yield) to the un-inoculated control and with clear significant differences with the challenged inoculated controls.

Table MP 6.4/02 Represents mean total production and marketable production of a selected trial of those indicated in section MP 6.2. Tables of data comprising all treatment means for each trial are presented in the individual trial reports.

Table MP 6.4/02 Mean total production (fruits/m² and kg/m²) and marketable production (fruits/m², % of marketable fruit and kg/m²) per treatment. Treatment means with no letters in common are significantly different, LSD test ($P \leq 0.05$)

| Trt. No. | Product | App (L fp/ha) ¹ | Total production | | Marketable production | | |
|----------|-----------------|----------------------------|-----------------------|-------------------|-----------------------|-----------------------|-------------------|
| | | | Fruits/m ² | kg/m ² | Fruits/m ² | % of marketable fruit | kg/m ² |
| 1 | CONTROL (untr.) | - | 57.9 a | 6.66 a | 57.0 ab | 98.4 a | 6.63 ab |

| Trt. No. | Product | App (L fp/ha) ¹ | Total production | | Marketable production | | |
|------------------|----------------------------|----------------------------|-----------------------|-------------------|-----------------------|-----------------------|-------------------|
| | | | Fruits/m ² | kg/m ² | Fruits/m ² | % of marketable fruit | kg/m ² |
| 2 | PepMV-Abp1 | 5 | 54.1 a | 5.90 a | 53.8 abc | 99.3 a | 5.88 bcd |
| 3 | PepMV-Abp2 | 5 | 54.0 a | 6.03 a | 53.6 abc | 99.2 a | 6.01 abc |
| 4 | AbioProtect® | 5 | 53.7 a | 6.13 a | 53.2 abc | 99.2 a | 6.11 abc |
| 5 | CONTROL (Ch1) ² | - | 57.1 a | 5.98 a | 50.4 c | 88.3 c | 5.25 de |
| 6 | PepMV-Abp1 (Ch1) | 5 | 59.1 a | 6.52 a | 57.2 ab | 96.8 ab | 6.35 abc |
| 7 | PepMV-Abp2 (Ch1) | 5 | 57.1 a | 6.33 a | 50.0 c | 87.7 c | 5.60 cd |
| 8 | AbioProtect® (Ch1) | 5 | 59.6 a | 6.80 a | 58.3 a | 97.7 ab | 6.68 a |
| 9 | CONTROL (Ch2) ³ | - | 55.2 a | 5.76 a | 41.4 d | 75.1 d | 4.53 ef |
| 10 | PepMV-Abp1 (Ch2) | 5 | 59.8 a | 6.37 a | 40.9 d | 68.3 e | 4.43 f |
| 11 | PepMV-Abp2 (Ch2) | 5 | 57.4 a | 6.56 a | 51.7 bc | 90.2 c | 6.01 a-d |
| 12 | AbioProtect® (Ch2) | 5 | 57.3 a | 6.22 a | 54.1 abc | 94.3 b | 5.92 a-d |
| Date type | | | No transf. | No transf. | No | No transf. | No transf. |
| LSD | | | 6.72 | 0.787 | 6.21 | 3.47 | 0.758 |
| CV | | | 8.19 | 8.69 | 8.31 | 2.63 | 9.08 |
| p(F), treatments | | | 0.5826 | 0.2027 | 0.0001 | 0.0001 | 0.0001 |

¹L fp/ha: liter of formulated product per hectare

²Ch1: challenge 1, EU aggressive; ³Ch2: Challenge 2, CH2 aggressive.

B.3.13 Phytotoxicity to target plants (including different cultivars), or to target plant products

Phytotoxicity and crop safety in protected tomato has been considered in all effectiveness trials. Treatment with AbioProtect® and its components resulted in no symptoms or some mild symptoms of PepMV infection. In cases where some mild symptoms appear those were generally transient and, in most cases, did not affect quality of the fruits. Moreover, yield was not affected.

Table MP 6.5/01, MP 6.5/02 and Figure MP 6.5/01 Represent mean of % incidence of plants affected and mean of severity of leaf symptoms of a selected trial. Tables of data comprising all treatment means for each trial are presented in the individual trial reports.

Table MP 6.5/01 Mean of % incidence in tomato plants (LYPES) per treatment. Treatment means with no letters in common are significantly different, LSD test ($P \leq 0.05$)

| Tr t. N o. | Product | Appl rate (L fp/ha) ¹ | A4 0 DBIn ⁴ | A5 14 DAIn ⁵ | A6 28 DAIn | A7 42 DAIn | A8 56 DAIn | A9 70 DAIn | A 17 158 DAIn |
|---------------------|----------------------------|---|------------------------------|-------------------------------|------------------|------------------|------------------|------------------|---------------------|
| | | | Yellowi ng | Yellowi ng | Yellowi ng | Yellowi ng | Yellowi ng | Yellowi ng | Yellowi ng |
| 1 | CONTROL (untr.) | - | 0.0 a | 0.0 c | 0.0 c | 0.0 c | 0.0 c | 0.0 c | 0.0 c |
| 2 | PepMV-Abp1 | 5 | 0.0 a | 5.0 c | 0.0 c | 0.0 c | 0.0 c | 0.0 c | 0.0 c |
| 3 | PepMV-Abp2 | 5 | 0.0 a | 0.0 c | 0.0 c | 0.0 c | 0.0 c | 0.0 c | 0.0 c |
| 4 | AbioProtect® | 5 | 0.0 a | 0.0 c | 0.0 c | 0.0 c | 0.0 c | 0.0 c | 0.0 c |
| 5 | CONTROL (Ch1) ² | - | 0.0 a | 100.0 a | 100.0 a | 100.0 a | 100.0 a | 100.0 a | 100.0 a |
| 6 | PepMV-Abp1 (Ch1) 2 | 5 | 0.0 a | 0.0 c | 0.0 c | 0.0 c | 0.0 c | 0.0 c | 0.0 c |
| 7 | PepMV-Abp2 (Ch1) 2 | 5 | 0.0 a | 55.0 b | 65.0 b | 95.0 b | 95.0 b | 95.0 a | 100.0 a |
| 8 | AbioProtect® (Ch1) 2 | 5 | 0.0 a | 0.0 c | 0.0 c | 0.0 c | 0.0 c | 0.0 c | 0.0 c |
| 9 | CONTROL (Ch2) ³ | - | 0.0 a | 100.0 a | 100.0 a | 100.0 a | 100.0 a | 100.0 a | 100.0 a |
| 10 | PepMV-Abp1 (Ch2) 3 | 5 | 0.0 a | 85.0 a | 100.0 a | 100.0 a | 100.0 a | 100.0 a | 100.0 a |
| 11 | PepMV-Abp2 (Ch2) 3 | 5 | 0.0 a | 0.0 c | 0.0 c | 0.0 c | 0.0 c | 20.0 b | 20.0 b |
| 12 | AbioProtect® (Ch2) 3 | 5 | 0.0 a | 0.0 c | 0.0 c | 0.0 c | 0.0 c | 0.0 c | 0.0 c |
| Date type | | | No | No | No | No | No | No | No |
| LSD | | | 0.00 | 16.81 | 14.24 | 4.17 | 4.17 | 10.69 | 9.63 |
| CV | | | 0.0 | 40.5 | 32.42 | 8.77 | 8.77 | 21.41 | 19.05 |
| p(F), treatments | | | 1.0000 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |

¹L fp/ha: liter of formulated product per hectare.

²Ch1: challenge 1, EU aggressive; ³Ch2: Challenge 2, CH2 aggressive.

⁴DBIn: Days before inoculation, ⁵DAIn: Days after inoculation.

Table MP 6.5/02 Mean of severity of leaf symptoms (yellowing) per treatment according to the severity scale indicated in point MP 6.2 above. Treatment means with no letters in common are significantly different, LSD test ($P \leq 0.05$)

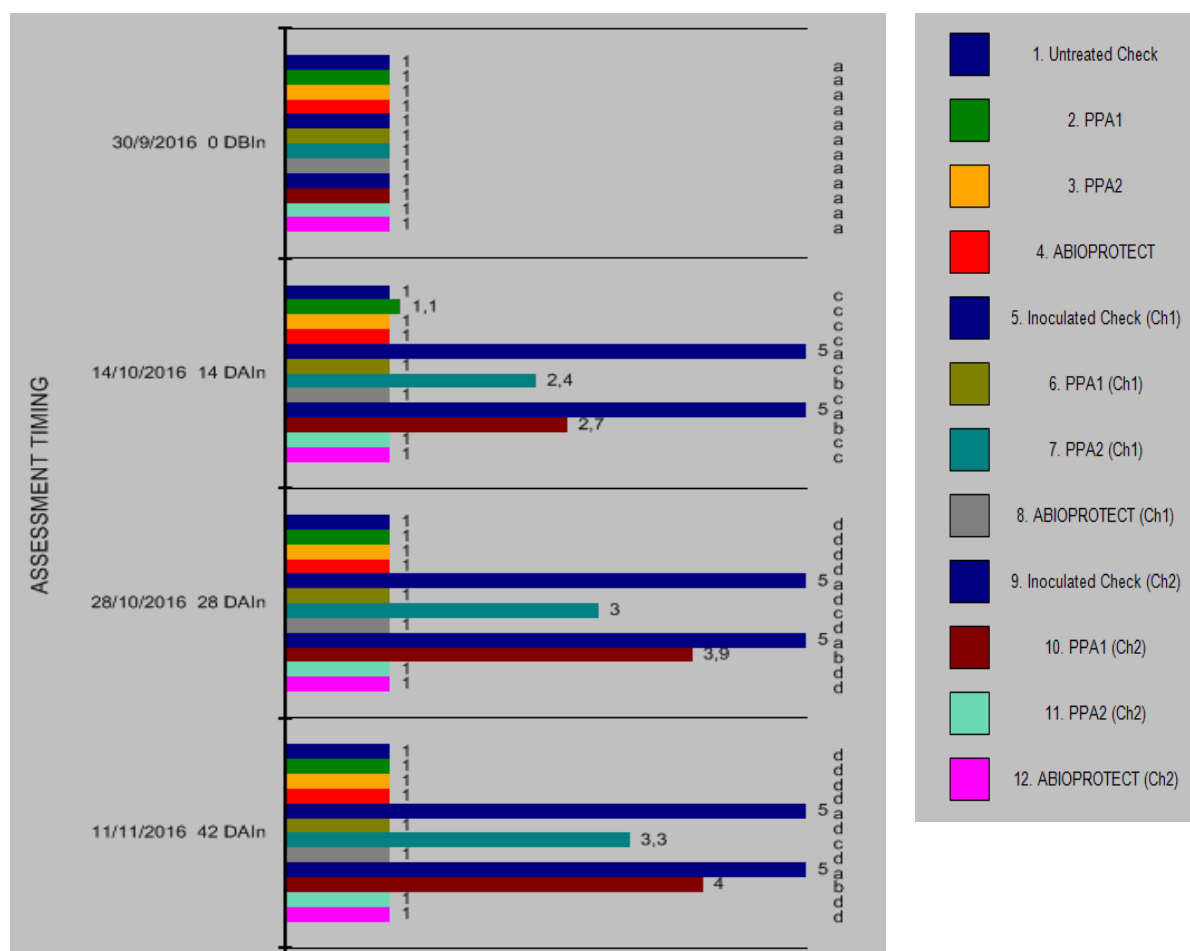
| Tr t. No . | Product | App rate (L fp/ha) 1 | A4 0 DBIn ⁴ | A5 14 DAIn ⁵ | A6 28 DAIIn | A7 42 DAIIn | A8 56 DAIIn | A9 70 DAIn | A17 158 DAIn |
|---------------------|----------------------------|----------------------------------|---------------------------|-------------------------------|----------------|----------------|----------------|------------------|--------------------|
| | | | Yellowi ng | Yellowi ng | Yellowi ng | Yellowi ng | Yellowi ng | Yellowi ng | Yellowi ng |
| 1 | CONTROL (untr.) | - | 1.0 a | 1.0 c | 1.0 d | 1.0 d | 1.0 d | 1.0 e | 1.0 d |
| 2 | PepMV-Abp1 | 5 | 1.0 a | 1.1 c | 1.0 d | 1.0 d | 1.0 d | 1.0 e | 1.0 d |
| 3 | PPA2 | 5 | 1.0 a | 1.0 c | 1.0 d | 1.0 d | 1.0 d | 1.0 e | 1.0 d |
| 4 | AbioProtect | 5 | 1.0 a | 1.0 c | 1.0 d | 1.0 d | 1.0 d | 1.0 e | 1.0 d |
| 5 | CONTROL (Ch1) ² | - | 1.0 a | 5.0 a | 5.0 a | 5.0 a | 5.0 a | 5.0 a | 5.0 a |
| 6 | PepMV-Abp1 (Ch1) | 5 | 1.0 a | 1.0 c | 1.0 d | 1.0 d | 1.0 d | 1.0 e | 1.0 d |
| 7 | PPA2 (Ch1) | 5 | 1.0 a | 2.4 b | 3.0 c | 3.3 c | 3.3 c | 3.3 c | 3.8 b |
| 8 | AbioProtect (Ch1) | 5 | 1.0 a | 1.0 c | 1.0 d | 1.0 d | 1.0 d | 1.0 e | 1.0 d |
| 9 | CONTROL (Ch2) ³ | - | 1.0 a | 5.0 a | 5.0 a | 5.0 a | 5.0 a | 5.0 a | 5.0 a |
| 10 | PepMV-Abp1 (Ch2) | 5 | 1.0 a | 2.7 b | 3.9 b | 4.0 b | 4.0 b | 4.0 b | 4.0 b |
| 11 | PPA2 (Ch2) | 5 | 1.0 a | 1.0 c | 1.0 d | 1.0 d | 1.0 d | 1.4 d | 1.4 c |
| 12 | AbioProtect (Ch2) | 5 | 1.0 a | 1.0 c | 1.0 d | 1.0 d | 1.0 d | 1.0 e | 1.0 d |
| Date type | | | No | No | No | No | No | No | No |
| LSD | | | 0.00 | 0.36 | 0.44 | 0.28 | 0.28 | 0.35 | 0.22 |
| CV | | | 0.0 | 13.08 | 14.63 | 9.35 | 9.35 | 11.19 | 6.83 |
| p(F), treatments | | | 1.0000 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 | 0.0001 |

1L fp/ha: liter of formulated product per hectare.

2Ch1: challenge 1: EU aggressive; 3Ch2: Challenge 2 CH2 aggressive.

4DBIn: Days before inoculation, 5DAIn: Days after inoculation.

Figure MP 6.5/0.1 Mean of severity of leaf symptoms (yellowing) according to the severity scale indicated in point MP 6.2 above. Treatment means with no letters in common are significantly different, LSD test ($P \leq 0.05$).



PPA1: PepMV-Abp1; PPA2: PepMV-Abp2, Ch1: challenge 1: EU aggressive; Ch2: Challenge 2 CH2 aggressive.

SCALE: 1: No symptoms. 2: Mild symptoms. 3: Moderate symptoms. 4: Severe symptoms. 5: Very severe symptoms.

B.3.14 Observations on undesirable or unintended side-effects, e.g. on beneficial and other non-target organisms, on succeeding crops, other plants or plants used for propagating purposes (e.g. seeds, cuttings, runners)

Viruses can only reproduce inside their host cells, plant viruses can only reproduce in plant living cells and PepMV can only reproduce inside its host plants. Multiplication in soil, water or air is therefore of little relevance and no undesirable or unintended side-effects have been observed.

PepMV is very efficiently mechanically transmitted; in fact, contaminated hands, clothing or tools facilitate PepMV transmission. Crop workers can transmit the virus simply by brushing against affected plants and during crop nursing activities such as pruning and harvesting (Ferguson, 2001; Van der Vlugt, 2009).

Please refer to MA 2.2 information on the target organism, and to MA 2.3 host specificity range and effect on species other than the target harmful organism (Document M-MA) for further information.

The main PepMV transmission route is mechanically. Although, some reports have analyzed the potential PepMV transmission by vectors. As this is the case of several reports studying transmission of PepMV by bumblebees (Lacasa *et al.* 2003; Shipp *et al.*, 2008; Stobbs *et al.*, 2009; Stobbs and Greig, 2014), these authors found that bumblebees could disperse PepMV, however, a specific PepMV-bumblebee vector relation does not appear to exist. Also, the possibility that PepMV could be transmitted by the soil fungus *Olpidium virulentus* has been studied at laboratory level (Alfaro-Fernández *et al.*, 2010), however the extent of such transmission in the field remains unclear.

B.3.14.1 Impact on succeeding crops

Impact on succeeding crops was not tested in the efficacy trials. However, as the persistence in water GEP study concluded that the Plant Protection Product AbioProtect® (formulated with equivalent amounts of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2) has no persistency in the leachate from tomato plants treated with AbioProtect® (see Document K-MP 6.2/04, Prats, 2017a), it could be concluded that there is no risk of PepMV infection with this leachate to succeeding crops.

Besides, the GEP studies on persistence in soil or substrate showed that PepMV is not persistence in the soil or substrate of the tomato plants treated with AbioProtect® and its components (see Documents K-MP 6.2/05, Prats, 2017b; K-MA 7.1.1/02, Céspedes, 2015a), and therefore there is no risk of PepMV infection of succeeding crops from the soil or the substrate of the plants treated.

Please refer to MA 2.3 host specificity range and effect on species other than the target harmful organism (Document M-MA) for further information.

B.3.14.2 Impact on other plants, including adjacent crops

Impact on other plants including adjacent crops was not tested as there are no indications that the plant protection product could affect adjacent crops via vapor drift. Furthermore as indicated in MP 6.6.1 based on the nature of AbioProtect® and in the results of the persistence in water GEP study, the persistence in soil and substrate GEP studies (Documents K-MP 6.2/05, Prats, 2017b; K-MA 7.1.1/02, Céspedes, 2015a; K-MP 6.2/04, Prats, 2017a) as well as the study on the presence of PepMV in weeds and plants on the vicinity of tomato greenhouses where the formulation has been applied (Document K-MA 7.1/01, Agüero, 2017b), it could be concluded that there is no risk of PepMV infection to other plants including adjacent crops.

Please refer to MA 2.3 Host specificity range and effect on species other than the target harmful organism (Document M-MA section 2) for further information.

B.3.14.3 Impact on treated plants or plant products to be used for propagation

Not relevant. The formulation AbioProtect® is not for used in plants or plants product to be use for propagation, more specifically it is not intended to be use in the production of seeds, cuttings or runners for propagation.

B.3.14.4 Effects on beneficial and other non-target organisms

PepMV is ubiquitous in nature and no impact of the formulation AbioProtect® or its ingredients (PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2) are expected on beneficial and other non-target organisms. In the different tests and studies conducted no effects on the incident of other non-target organisms or environmental effects have been observed. Please refer to Document M-MA section 8 and Document M-MP section 10 for further information.

B.3.15 Summary and evaluation of efficacy data (3.9-3.13)

Co-infection of tomato seedling with 2 mild isolates of PepMV (EU strain, isolate Abp1 and CH2 strain, isolate Abp2) showed to be effective against aggressive isolates of PepMV in preliminary research tests. A GEP dose trial showed that the formulation AbioProtect® containing $>5 \times 10^{11}$ genome copies of PepMV/L, ($>2.5 \times 10^{11}$ genome copies of PepMV, EU strain, mild isolate Abp1/L, and $>2.5 \times 10^{11}$ genome copies of PepMV, CH2 strain, mild isolate Abp2/L) applied at 8 L/ha present a better efficiency and velocity of infection than when applied at 5 L/ha or 3 L/ha. Considering this data together with previous experience the application dose of AbioProtect® is set up at 5 L/ha (10,000 plants in general practice in greenhouse tomato cultivation). Only in tomato production areas with a record of high incidence of aggressive PepMV infections it could be increased up to 8 L/ha.

The efficacy of AbioProtect® and its components was tested in six greenhouse GEP certified trials in different locations in Spain from 2014-2017. The trials demonstrated the effectiveness and appropriate crop safety of AbioProtect® against infection by PepMV aggressive isolates from both the EU and the CH2 strains, in different tomato cultivars and at a dose of 5 L/ha in only one application to the tomato seedling (BBCH stage 13-15), right

before transplanting to the commercial greenhouse tomato plot. The protection conferred with AbioProtect® treatment lasted until the end of the crop. The data where obtained in greenhouse trials and therefore are representative for the entire EU. As the mode of action is based on cross-protection the possibility of development of resistance is not relevant.

Treatment with AbioProtect® and its components resulted in no symptoms or some mild symptoms of PepMV infection. In cases where some mild symptoms appear those were generally transient and, in most cases, did not affect quality of the fruits. Moreover, yield was not affected. The treatment does not pose any risk to or impact on succeeding or adjacent crops or plants, is not for use in plants to be used for propagation and has no effect on beneficial or other non-target organisms.

In conclusion AbioProtect® has proved a good performance in practice providing wide spectrum protection against infection induced by PepMV (PEPMVO) aggressive isolates in tomato (LYPES), under the agricultural, plant health and environmental conditions of tomato greenhouse cultivation in EU. The dose of 5 L/ha, applied once on tomato seedlings (13-15 BBCH) gives a adequate protection against PepMV aggressive isolates of both the EU and the CH2 strain.

B.3.16 References relied on

- Agüero J. (2017b) Report of the project ABP03/2017 "Study of the presence of *Pepino mosaic virus* (PepMV) on alternative and potential non-tomato host plants". Abiopep S.L., Spain.
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