European Commission

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Pepino Mosaic Virus, EU strain, mild isolate Abp1
Pepino Mosaic Virus, CH2 strain, mild isolate Abp2
Active organism data

Volume 3 – Annex B.2 Biological properties

Rapporteur Member State: Spain

July 2019

Version History

When	What
	Completeness check report of the dossier submitted by the notifier
March 2019	DAR submitted to the Notifier. Reception of comments
July 2019	DAR revised

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B.2. BIOLOGICAL PROPERTIES OF THE MICROORGANISM

B.2.1. History of the microorganism and its uses, natural occurrence and geographical distribution. Historical background

Origin of the isolate

PepMV, EU strain, mild isolate Abp1 originates from a natural wild type PepMV, isolated from samples taken in a commercial tomato crop in Murcia (Spain) in 2001.

PepMV, CH2 strain, mild isolate Abp2 originates from a natural wild type PepMV, isolated from samples taken in a commercial tomato crop in Murcia (Spain) in 2007.

Method of isolation

Young leaves from the sprouts of tomato plants of commercial tomato greenhouses were taken, and kept at 4 ºC up to 4 days before further processing. Samples were divided in several 0.1g aliquots; one of those aliquots was processed by ELISA (Enzyme Linked Immunosorbent Assay) using a commercial antiserum specific for PepMV. The other aliquots of the same sample were frozen in liquid N_2 and kept at – 80 ºC for further analysis.

The samples with a positive result for PepMV in ELISA (antiserum used to detect presence of PepMV was Bioreba PepMV AgriStrip) were further characterized. From another frozen aliquot, the plant material was homogenized in phosphate buffer pH 8.0 and used to manually inoculate a systemic host such as *Nicotiana benthamiana* and tomato plants for further characterization. At 15 days post inoculation (dpi), *N. benthamiana* leaves showing PepMV symptoms were harvested, separated in several aliquots and kept lyophilized at room temperature in a dry and fresh ambient (this is the original microorganism seed stock of each MPCA).

PepMV, EU strain, mild isolate Abp1 induced clear symptoms in *N. benthamiana*, including mark mosaic, chlorosis and leaves distortion, while it did not induce any symptoms in tomato plants, (see figure B2.1.1-01 below and Table C.2.1.1-02 in Vol. 4).

PepMV, CH2 strain, mild isolate Abp2 induced symptoms in both *N. benthamiana* and tomato plants. In *N. benthamiana*, symptoms were marked mosaic, chlorosis and leaves distortion, while in tomato, symptoms were faint mosaic in the leaves and no symptoms on the fruit, (see figure B2.1.1-02 below and Table B2.1.1-01 in Vol. 4).

RMS comments:

- Further confidential information concerning the origin of PepMV Abp1 and Abp2 isolates were presented in Volume 4 (see ref. C.1.3.3/2 and figure C2.1.1-01).
- The Phenotipic characterization of PepMV symptoms was evaluated according to Hassens *et al.* 2009 (Table B.2.1.1-02), figure B2.1.1-01 for Abp1 and figure B2.1.1-02 for Abp2.

History of the organism and its uses

PepMV, EU strain, mild isolate Abp1 was characterized by sequencing in 2001. It was the first PepMV isolate whose genome was completely sequenced. This characterization showed that PepMV genome consists of a single stranded RNA of approximately 6.4 kb containing five open reading frames, including a replicate gene (RdRp) comprising methyltransferase (MET), helicase (HEL) and polymerase (POL) motifs, a triple gene block (TGB) encoding TGB1, TGB2 and TGB3, involved in viral movement and silencing suppression, and a coat protein (CP) which has a structural role, it is necessary for viral movement and it also functions as a silencing suppressor, Figure C2.1.1-01 (See Confidential Vol 4).

Studies on the natural populations of PepMV in commercial tomato greenhouses in Murcia region (Southeast Spain) were further conducted. From 2005-2008 a collection of 334 samples from potentially PepMV-infected tomato plants were obtained to study the variability and genetic structure of the PepMV natural populations. Those studies showed that after a likely introduction in 2003-2004, PepMV isolates of the CH2 strain spread to become prevalent in the region, although they did not displace the isolates from the EU strain, Table C2.1.1-01, (see Confidential Vol 4).

Several isolates were obtained from both above studies, including PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, which were used in further characterizations (see Vol4, table C2.1.1-1).

PepMV, CH2 strain, mild isolate Abp2, was first characterized by sequencing a 2,223 nt fragment comprising the complete tgb and cp genes and shown to belong to the CH2 strain.

These studies revealed that PepMV, isolate Abp1 belongs to the EU strain of PepMV and that isolate Abp2 belongs to the CH2 strain, they have a nucleotide sequence identity of 78.74 %. Both are mild isolates of PepMV (see Confidential Vol 4, ref. C.2).

PepMV symptomatology is highly variable in tomato plants, ranging from asymptomatic infections due to mild isolates to very severe symptoms due to aggressive isolates. Attenuated PepMV isolates were successfully used for protection against virulent isolates of the virus, using both natural mild isolates as well as artificially attenuated by mutation of capsid protein (Schenk *et al.*, 2010; Chewachong *et al.*, 2014).

RMS comments:

- Further information in confidential documents Volume 4.

Symptomatology in tomato and other host plants of Abp1 and Abp2

PepMV, EU strain, mild isolate Abp1 induced clear symptoms in *N. benthamiana*, including mark mosaic, chlorosis and leaves distortion, while it did not induce any symptoms in tomato plants, Table B2.1.1-01 , Table C.2.1.1-02 in Vol 4, and figure B2.1.1-01.

PepMV, CH2 strain, mild isolate Abp2 induced symptoms in both *N. benthamiana* and tomato plants. In *N. benthamiana*, symptoms were marked mosaic, chlorosis and leaves distortion, while in tomato, symptoms were faint mosaic in the leaves and no symptoms on the fruit, Table B2.1.1-01, Table C.2.1.1-02 in Vol 4 and figure B2.1.1-02.

Plant part	Symptom type	Score	Description	
Head[a]	Nettle-head	1	Absent	
		2	Leaves are somewhat pointed and upright with a slightly reduced surface	
		3	Leaves are pointed, upright or curled, with a reduced surface	
		4	Leaves resemble nettle leaves, with a serrated leaf margin and a reduced surface	
	Leaf bubbling	1	Absent	
		2	One bubbled leaf[b]	
		3	Two to four bubbled leaves[b]	
		4	All leaves are bubbled[b]	
Foliage[c]	Premature leaf senescence	1	Absent	
		2	Scorching-leaflet margins	
		3	Scorching-entire leaflets of min. one leaf[b]	
		4	Scorching-more than one leaf[b]	
Fruit	Marbling	1	Absent	
		2	One marbled fruit[b]	
		3	Two marbled fruits	
		4	More than two marbled fruits[b]	
	Flaming	1	Absent	
		2	One flamed fruit[b]	
		3	Two flamed fruits[b]	
		4	More than two flamed fruits[b]	
	Open fruit	1	Absent	
		2	One open fruit[b]	
		3	Two open fruits[b]	
		4	More than two open fruits[b]	
	Necrosis of the sepals	1	Absent	
		2	One fruit with sepal necrosis[b]	
		3	Two fruits with sepal necrosis[b]	
		4	More than two fruits with sepal necrosis[b]	

[a]Upper youngest leaves (plant top).
[b]Per plant.
[c]Lower leaves.

Table B2.1.1-01. Included by the RMS: PepMV symptom rating scale for tomato. With regard to fruit quality, scores were given for fruit marbling, fruit flaming or blotchy ripening, incidence of scars and open fruits, and necrosis or browning of the sepals (Hanssen *et al.*, 2009).

Figure B2.1.1-01 shows symptoms comparison in the leaves of tomato plants inoculated with PepMV, EU strain, mild isolate Abp1 and tomato plants inoculated with an aggressive isolate of PepMV EU strain, (isolated from a commercial greenhouse tomato crop in Alicante, Spain, September 2015). Figure B2.1.1-02 shows symptoms comparison in the leaves of tomato plants inoculated with PepMV, CH2 strain, mild isolate Abp2 and tomato plants inoculated with an aggressive isolate of PepMV, CH2 strain, (isolated from a commercial greenhouse tomato crop in Granada, Spain, March 2014).

A. B.

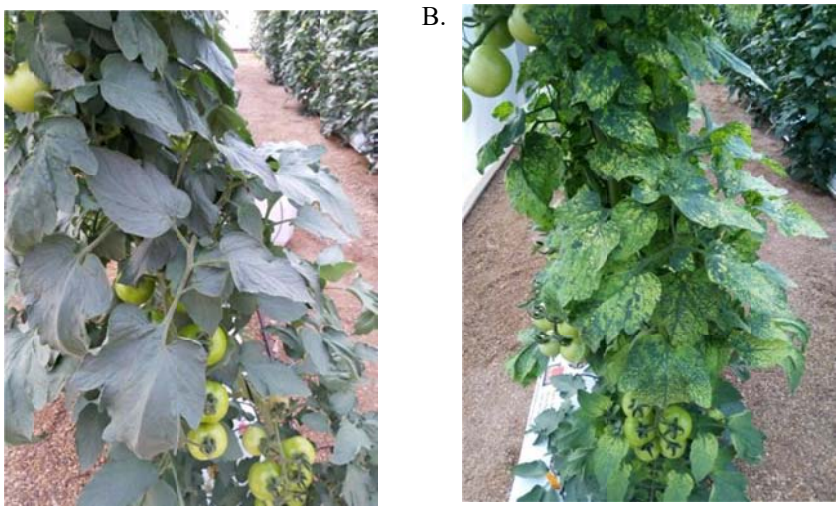

Figure B2.1.1-01. Tomato plants inoculated with PepMV, EU strain, mild isolate Abp1 (A) and tomato plants inoculated with PepMV, EU strain, aggressive EU isolate (B) (photographs are from Instituto de Formación Agraria y Pesquera, IFAPA, Centro La Mojonera, Almería).

A. B.

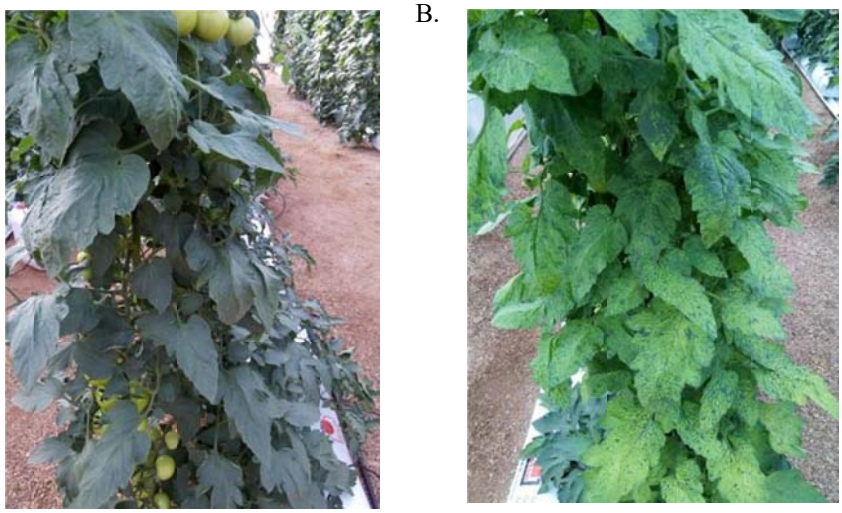

Figure B2.1.1-02. Tomato plants inoculated with PepMV, CH2 strain, mild isolate Abp2 (A) and tomato plants inoculated with PepMV, CH2 strain, aggressive CH2 isolate (B) (photographs are from Instituto de Formación Agraria y Pesquera, IFAPA, Centro La Mojonera, Almería).

RMS comment:

- Hernández-Llopis *et al.*, 2014 evaluated the potential physiological changes causes by systemic infections of two different isolates of PepMV CH2 strain, with differential symptoms (one aggressive and another asymptomatic). Results suggested there is a difference in the infective process between the symptomatic and asymptomatic isolates, but in both cases virus inoculation produce significant physiological change in the plant.
- The measures were made in two different physiological plant period, vegetative and fruit development: photosynthesis, pigment and carbohydrate (sugars and starch) content in leafs and in the plant growth as biomass and foliar surface. The plants showed very different characteristic (using PepMV symptom rating scale for tomato, figure B2.1.1-2) depending of the inoculated isolate. Since plants inoculate with aggressive isolate had yellow mosaic in the leaflet few days after inoculation, in the plants inoculate with attenuate isolate, the symptoms appear later on and mainly affecting plant general growth rate. All

plants inoculated with any of the strain (symptomatic or asymptomatic) have a significant lower photosynthesis and stomata rate compare with un-inoculated plants. Pigment contents were significant lower in plants inoculated with aggressive isolate, and significant higher sugar and starch contents. Plants inoculated with mild isolate have carbohydrate contents significant lower during reproductive period. Hasiów-Jaroszewska *et al.*, 2013 also have obtained efficient cross-protection results using mild PepMV-P22 against aggressive challenge isolates PepMV-P5-IY (yellowing) and PepMV-P19 (necrotic) PepMV-P5-IY (Figure B2.2.2-2.).

- RMS recommend to include a reference tomato plant no inoculated in order to compare symptoms against inoculated with PepMV asymptomatic, symptomatic and un-inoculated plants. It would be also convenient to have symptoms at the end of the production process, before harvest. There is no information about the effect of Abp1 and Abp2 on growth rate or production.

History of use of closely related strains or species
See Confidential Vol 4

B.2.1.2. Origin and natural occurrence

PepMV was first isolated in 1974 in Peru from pepino (*Solanum muricatum Ait.*) plants showing symptoms of yellow mosaic (Jones *et al.*, 1980). It was not reported as a pathogen of tomato (*Solanum lycopersicum L.*) until 1999 (van der Vlugt *et al.*, 2000), in greenhouses in The Netherlands, but has since spread rapidly in Europe (see ref C.1 in Vol 4; Cotillion *et al.*, 2002; ref C.6 in Vol 4; Mumford and Metcalfe, 2001; Pagan *et al.,* 2006; Pospieszny *et al.*, 2008; Roggero *et al.*, 2001) and beyond (French *et al.*, 2001; Ling 2007; Ling *et al.*, 2008; Maroon-Lago *et al.*, 2005; Soler et al. 2002). PepMV presence has been described in 19 countries in Europe (Table B2.1.2-1) and is included in European and Mediterranean Plant Protection Organization (EPPO) A2 list of pests recommended for regulation as quarantine pest (EPPO, 2016)[1].
Table B2.1.2-01 summarizes the geographical distribution of PepMV in Europe from EPPO Global database webpage (EPPO, 2017)[2].

Country	Current status (2017)
Austria	Present, few occurrences
Belgium	Present, restricted distribution
Bulgaria	Present, few occurrences
Cyprus	Present, restricted distribution
Denmark	Present, few occurrences
France	Present, few occurrences
Germany	Present, few occurrences
Greece	Present, restricted distribution
Hungary	Present, few occurrences
Ireland	Present, few occurrences
Italy	Present, few occurrences
Lithuania	Present, few occurrences
Netherlands	Present, restricted distribution
Poland	Present, few occurrences
Spain	Present, widespread
Switzerland	Present, restricted distribution
Turkey	Present, few occurrences
Ukraine	Present, no details
United Kingdom	Present, few occurrences

Table B2.1.2-01. Geographical distribution of PepMV in Europe.

[1] EPPO. (2016) A1 and A2 lists of pests recommended for regulation as quarantine pests, EPPO standard PM 1/2(25). EPPO standards: general phytosanitary measures. Paris: EPPO.
[2] EPPO. (2017) Pepino mosaic virus (PepMV): Overview, distribution and Host plants, EPPO Global Database, https://gd.eppo.int/taxon/PEPMV0.

This table shows that PepMV is widespread in Europe, introduction of PepMV, EU strain, mild isolate Abp1 and/or PepMV, CH2 strain, mild isolate Abp2 in (protected) tomato crops is therefore not expected to affect the level of natural occurrence of the virus.

Four main PepMV genotypes can be distinguished, the original Peruvian genotype (LP), the European (tomato) genotype (EU), the American genotype (US1), and the Chilean genotype (CH2), with an intergenotype RNA sequence identity ranging from 78% to 95% (see ref. C.5 in Vol 4). More recently, Moreno-Pérez *et al.* 2014 reported the occurrence in wild tomatoes of isolates belonging to a new PepMV genotype, not yet reported in domestic tomato and named the South Peruvian genotype (PES). The EU genotype was predominant in North America (Ling *et al.*, 2008), though a recent shift toward the CH2 genotype has been described (Ling *et al.*, 2013). Figure B2.1.2-01 shows a phylogenetic tree of PepMV isolates belonging to the five recognized strains, and Figure B2.1.2-02 phylogenetic tree with ancestral state reconstruction of PepMV isolates grouping in the four main genotypes and shows strains distribution by countries worldwide. The PepMV EU genotype was the first to appear in Europe, although the CH2 genotype is currently the most frequent (see ref. C.2 in Vol 4, see ref. C.5 in Vol 4), while isolates of the EU genotype are persisting both in single and mixed infections (see ref. C.2 in Vol 4).

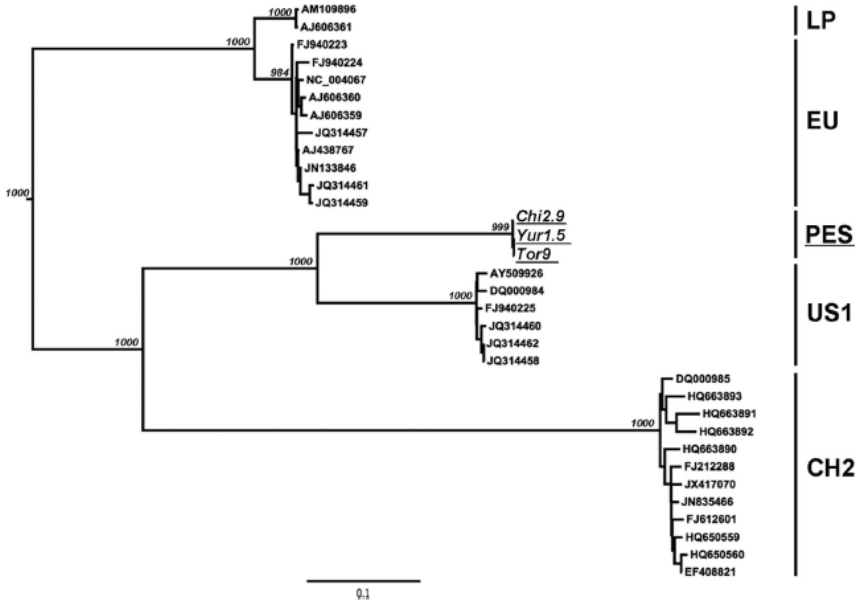

Figure B2.1.2-01. Maximum-likelihood phylogenetic tree of PepMV isolates showing five recognized strains EU, CH2, LP, PES and US1, (Moreno-Pérez *et al.*, 2014).

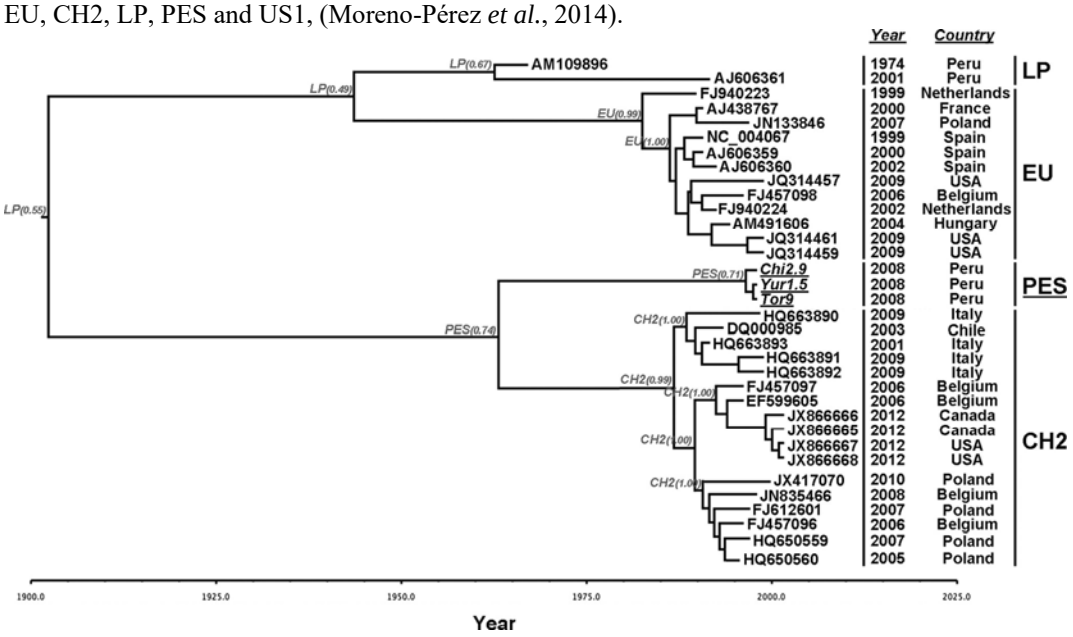

Figure B2.1.2-02. Included by the RMS: Phylogenetic tree with ancestral state reconstruction of PepMV isolates. Branch tip times reflect the times of viral sampling. The tree is automatically rooted through the use of a relaxed molecular clock, and the total depth of the tree is the time to the most recent common ancestor. Tip names indicate the GenBank accession number. The two columns on the right indicate the year of isolation and country of origin of the PepMV isolate that originated each sequence. (Moreno-Pérez *et al.*, 2014).

PepMV, mild isolate Abp1 belongs to the EU genotype, and PepMV, mild isolate Abp2 belongs to the CH2 genotype. Mild and aggressive isolates are known from both the EU and the CH2 strain (see **Figure C2.1.2-01 in Vol 4)**.

The RMS has compile the summary above incorporating the abstracts and relevant excerpts from the study Pagan *et al.*, 2006 from applicant reference.

Reference:	Pagán *et al.*, 2006. Phytopathology, 96:274-279. Virology Genetic Structure of the Population of PepMVvirus Infecting Tomato Crops in Spain.
Report No.:	
Guideline:	Not applicable
GLP:	Not applicable
Abstract	The population structure of PepMV, which has caused severe epidemics in tomato in Spain since 2000, was analyzed. Isolates were characterized by the nucleotide sequence of the triple gene block and coat protein gene and, for a subset of isolates, a part of the RNA-dependent RNA polymerase gene. The full-length sequence of the genomic RNA of a *Solanum muricatum* isolate from Peru also was determined. In spite of high symptom diversity, the Spanish population of PepMV mostly comprised highly similar isolates belonging to the strain reported in Europe (European tomato strain), which has been the most prevalent genotype in Spain. The Spanish PepMV population was not structured spatially or temporally. Also, isolates highly similar to those from non-tomato hosts from Peru (Peruvian strain) or to isolate US2 from the United States (US2 strain) were detected at lower frequency relative to the European strain. These two strains were detected in peninsular Spain only in 2004, but the Peruvian strain has been detected in the Canary Islands since 2000. These results suggest that PepMV was introduced into Spain more than once. Isolates from the Peruvian and US2 strains always were found in mixed infections with the European tomato strain, and inter-strain recombinants were detected. The presence of different strains of the virus, and of recombinant isolates, should be considered for the development of control strategies based on genetic resistance.

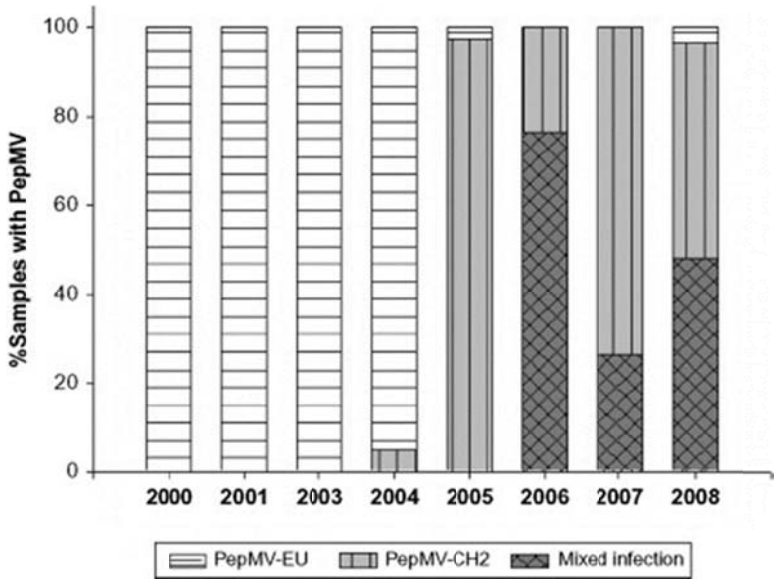

Figure B2.1.2-03. Included by the RMS: Detection of European strain (PepMV-EU) isolates and Chilean strain (PepMV-CH2) isolates from infected tomato samples in commercial fields in Murcia region, Spain during 2000 and 2004 (Pagan *et al.*, 2006).

RMS comment:
The study indicated that EU strain isolates and CH2 strain isolates are common component of the plant microbiota of tomato plants and has been isolated from numerous localities in Spain. The study by Pagan *et al.* 2006 was also mentioned in Volume 3, B.9 effect on non-target. The study also indicated that levels of CH2 and EU strains ranged from between 10^3 – 10^5 genome copies/g dry plant in five sites in Spain. These virus concentration levels were used as a natural occurring concentration, therefore, this study was also presented in Volume 3, B.9: Effect on non-target. See this section for more details.

Overall RMS comment B.2.1:
Phylogenetic analysis supports the cocirculation of isolates.
All the studies were considered relevant for the RMS. But the applicant has provided little conclusions under this section. In order to give more background to the studies, the RMS has provided the abstracts in Pagan *et al.*, 2006 showing the distribution of strains PepMV-EU and PepMV-CH2 in commercial tomato green house in South Spain (Figure B2.1.2-03) and phylogenetic tree of PepMV isolates (Figure B2.1.2-02), showing strains distribution by European countries, Moreno-Pérez *et al.*, 2014.

B.2.2 Information on target organism(s)

B.2.2.1 Description of target organism(s)

PepMV has recently become a major limiting factor regarding tomato production. PepMV symptomatology in tomato plants is highly variable, ranging from asymptomatic infections to very severe symptoms (Hanssen *et al.*, 2009). The virus affects the ripening process of tomatoes, leading to fruit discoloration symptoms, such as marbling, blotchy ripening and flaming (Hanssen *et al.*, 2009). Symptoms on the vegetative plant parts comprise nettleheads, leaf bubbling, mild chlorosis, small yellow spots on the leaves and, in some cases, marked leaf mosaics, bright yellow leaf mosaics and leaf or stem necrosis (Hasiów-Jaroszewska *et al.*, 2013). Figure B2.2.1-1., shows diversity of symptoms of aggressive strains of PepMV in tomato plants. Figure C2.1.1-2 in Vol 4 and B2.2.1-1 shows the diversity of production rate in a cross-protection trial tomato yield with PepMV mild isolates and aggressive isolates.

Symptom induction by PepMV is highly dependent on environmental conditions, including plant growth stage at the time of infection, light intensity, temperature fluctuations and nutrition, and it also depends on the tomato cultivar (Pagan *et al.* 2006; Sempere *et al.*, 2016). Some authors found that minor genetic differences between isolates may result in large differences in the nature and severity of symptoms (see ref. C.5 in Vol 4; Hasiów-Jaroszewska *et al.*, 2011; Hasiów-Jaroszewska *et al.*, 2013). However, symptoms do not depend only on the genetics of the virus but they are determined by a set of factors, such as environment and host plant cultivar. The triple interaction environment/cultivar/virus genotype is what determines the symptomatology showed by infected plants (Sempere *et al.*, 2016). In this regard, PepMV does not significantly differ from other plant viruses for which similar phenomena have been described (Hull, 2014). Schenk *et al.* (2010) demonstrated that infection of tomato plants with attenuated isolates alone did neither affect bulk yield, nor quality of the harvested tomato fruits.

Contaminated hands, clothing or tools transmit PepMV very efficiently. Direct contact between healthy and infected plants during routine crop handling also suffices to spread PepMV infection (Ferguson, 2001; Van der Vlugt, 2009). The incidence of PepMV on tomato is very high in some tomato cultivation areas, where the virus may affect up to 90% of the greenhouses (Soler-Aleixandre *et al.*, 2005).

Preventing infections and limiting the spread of PepMV in greenhouses requires strict hygiene measures. However due to year-round cultivation, hygienic measures are not sufficient anymore as greenhouses contain tomato plants during the whole year. Therefore, PepMV could remain in the greenhouses constantly, and there is a need for other means of PepMV control.

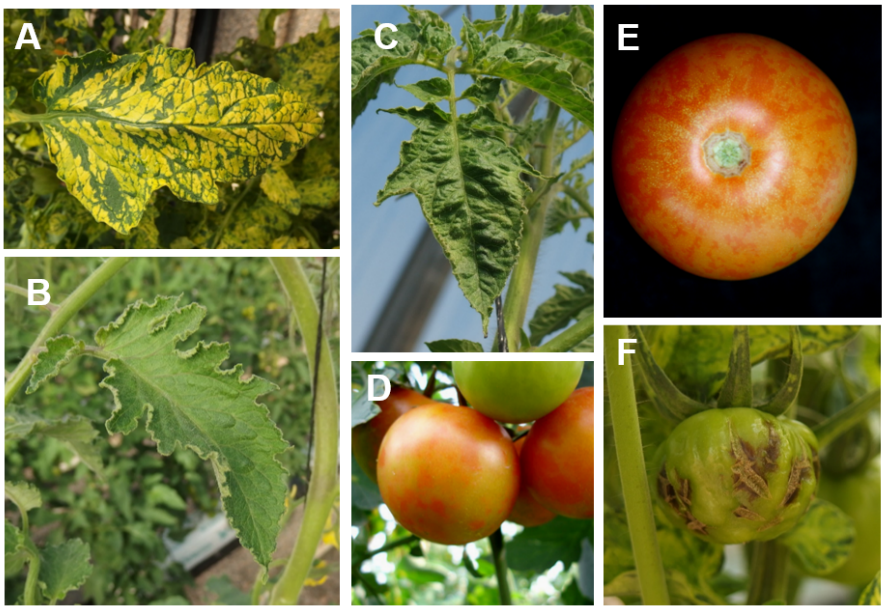

Figure B2.2.1-1. Diversity of symptoms of aggressive strains of PepMV in tomato plants: A, bright yellow mosaics. B, leaf lamina distortion. C, leaf bubbling. D, fruit discoloration. E, fruit marbling. F, scars on the fruit surface. Poster presentation AbioProtect: Cross protection against Pepino mosaic virus (PepMV), presented by Agüero, J., Garcia-Villalva, J., Sempere, R.N., Gómez, P., Casado, A., Méndez Colmenero, A., Hernando, Y. and Aranda, M.A. at the V international symposium on tomato diseases celebrated in Malaga, Spain, in June 13-16, 2016.

RMS comment:
Affected plants are often stunted. However, the main impact of PepMV is on fruit quality (Fig. B2.2.1-1), although it does not appear to affect the yield (see ref. C.17 in Vol 4; Pagan *et al.*, 2006). Fruit symptoms can arise with or without symptoms in the rest of the plant, and symptom expression is dependent on the cultivar, lighting and/or temperature within glasshouses), and on the PepMV isolate (Hanssen *et al.*, 2009).

B.2.2.2 Mode of action

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, 2012). 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 phenomenon was first reported with Tobacco mosaic virus (TMV) in 1929 (McKinney, 1929), and the first demonstrations of virus-disease control by mild strains were done with Citrus tristeza virus (CTV: Genus *Closterovirus*) (Grant and Costa, 1951). 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 leaf roll virus (PLRV), other RNA viruses, DNA viruses, and viroids (Gal-On and Shiboleth 2006; Pennazio *et al.*, 2001; Tatineni and French, 2016) Cross-protection seemed to be a general phenomenon with viruses for which distinct strains could be found, figure B2.2.2-2, (Hasiów-Jaroszewska *et al.*, 2014).

Cross-protection using attenuated viruses offers a promising strategy for biological control of plant viral diseases. Viral cross-protection in plants is a phenomenon, where a mild virus isolate can protect plants against damage caused by a severe challenge isolate of the same virus. It has been used on a large scale in cases where no resistant plants are available.

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, several 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 Shiboleth, 2006), though alternative models have been proposed recently (Zhang *et al.*, 2017). In Ref. C.3 in Vol 4 results suggest that the interaction between PepMV isolates largely depends on RNA sequence homology and that post-transcriptional gene silencing plays an important role in cross-protection.

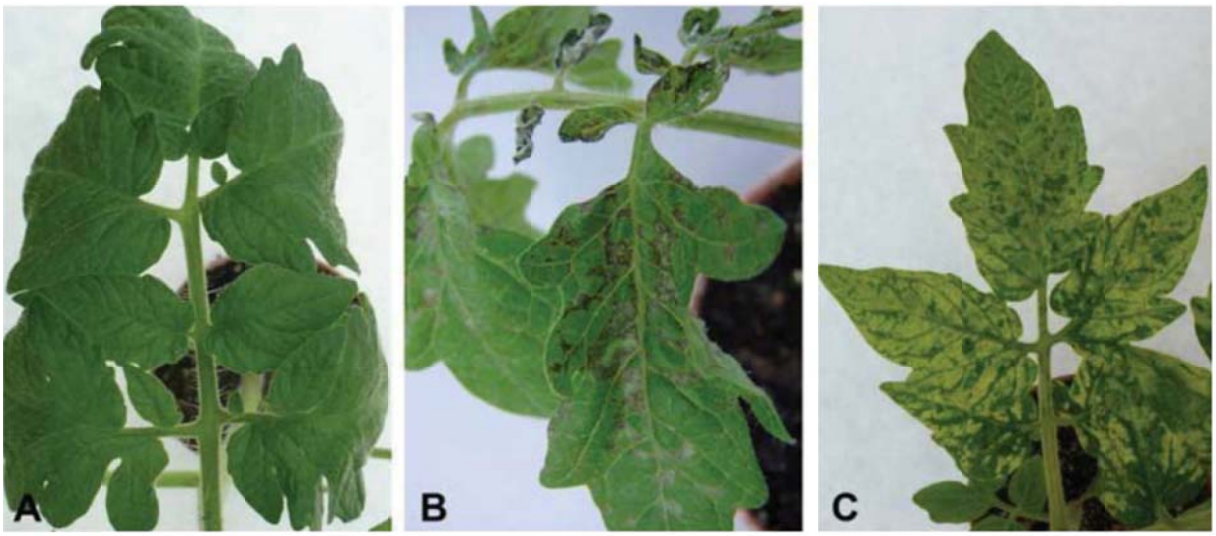

Figure B2.2.2-2. Included by the RMS: Tomato plants infected with asymptomatic PepMV-P22 mild isolate, necrotic PepMV-P19 aggressive isolate, and yellowing PepMV-P5-IY aggressive isolates, respectively (Hasiów-Jaroszewska *et al.*, 2014).

Isolates of the EU strain and the CH2 strain of PepMV are the most common in Europe. Isolates of the same strain of PepMV share a sequence identity varying between 95 and 100% among them, while isolates from different strains have a sequence identity varying from 78 to 94%. The CH2 and EU are the most divergent strains of PepMV isolates, and both have a sequence identity around 78.2 to 78.8 %. PepMV, EU strain mild isolate Abp1 have a sequence identity with PepMV, CH2 strain, Abp2 of 78.4 %. Inoculation of tomato plants simultaneously with both isolates Abp1 and Abp2 provides wide spectrum cross-protection against aggressive isolates from both the EU and the CH2 strain of PepMV, as well as from other strains (more detailed information on protection against aggressive isolates of PepMV could be found in Document M-MP section 6 of this dossier).

Differences in cross-protection between pathotypes of PepMV representing CH2 and EU genotypes have been examined. The potential of mild PepMV-Abp1 and PepMV-Abp2 isolates to protect tomato against aggressive challenge isolates from CH2 and EU strains causing yellowing and necrotic symptoms was established.

Infection of a tomato plant with PepMV, EU strain, mild isolate Abp1 and/or PepMV, CH2 strain, mild isolate Abp2 does not influence 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.

PepMV, EU strain, mild isolate Abp1 is recommended for use as a treatment in tomatoes to prevent infection from aggressive isolates of PepMV EU genotype. PepMV, CH2 strain, mild isolate Abp2 is recommended for use as a treatment in tomatoes to prevent infection from aggressive isolates of PepMV CH2 genotype. Both isolates together are recommended for use as a treatment in tomatoes to prevent infection from a wide spectrum

of aggressive isolates of PepMV. Application should be done so that the mild isolates enter the tomato plants before aggressive isolates occur. Reference need to be included. RMS have moved this paragraph to mode of action.

Consolidated data on cross-protection achieved by Abp1 and Abp2 can be found in Agüero, J., Gómez-Aix, C., Sempere, R.N., Garcia-Villalva, J., García-Núñez, J., Hernando, Y. and Aranda, M.A. (2018). Stable and Broad spectrum cross-protection against Pepino mosaic virus attained by mixed infection Front. Plant Sci. 9:1810. doi: 10.3389/fpls.2018.01810.

Overall RMS comment B.2.2:
Applicant has provided well documented data of the mode of action and efficacy of several mild isolates of PepMV CH2 genotype and EU genotype.

B.2.3 HOST SPECIFICITY RANGE AND EFFECT ON SPECIES OTHER THAN THE TARGET HARMFUL ORGANISM

PepMV is widespread in Europe, as described in B.2.1.2. The virus does not infect only tomato crops, it can also be found in several families of wild plants (Table B2.1.2-01, Table B2.3-01 and table B2.5-1). The application of PepMV Abp1 and Abp2 mild isolate inside a high technology g, weed-free protected greenhouse reduced spread to other host than tomato plants. Even there is many weed species that can be infected by PeMV in Europe, weed reservoirs of PepMV, would be considered low.

Introduction of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 in the environment is therefore not expected to alter the natural occurrence of the virus.

Natural hosts:
Tomato (*S. lycopersicum*) is the most important natural host of PepMV. It was observed for the first time in tomato crops in the Netherlands in 1999, and is currently a major disease of glasshouse tomato crops worldwide (see ref. C.5 in Vol 4).

Pepino (*S. muricatum*) is a PepMV host in Peru (Jones *et al.*, 1980; Soler *et al.*, 2002). Efforts have been made to grow pepino as a fruiting crop at a commercial scale under greenhouse conditions in the Mediterranean area of the EU but this has not yet been successful (Prohens *et al.*, 2005). The most recent information is that there is no commercial production of pepino in Spain (Werkman *and* Sansford, 2010).

In Spain, symptomless infections of PepMV were found in weed species (*Amaranthus sp., Malva parviflora, Nicotiana glauca, Solanum nigrum and Sonchus oleraceus*) near to greenhouses with PepMV infected tomato plants (Table B2.3-01 and Jordá *et al.*, 2001).

In a later publication, the weed species *Bassia scoparia, Claystegia sepium, Chenopodium murale*, *Convolvulus althaeoides, Convolvulus arvensis, Conyza albida, Coronopus sp., Diplotaxis erucoides, Echium creticum, Echium humile, Heliotropium europaeum, Moricandia arvensis, Onopordum sp., Piptatherum multiflorum, Plantago afra, Rumex sp., Sisymbrium irio, Sonchus tenerrimus* and *Taraxicum vulgare,* which were growing in or around tomato fields in Murcia and Almeria provinces of Spain, tested positive for PepMV (Córdoba *et al.*, 2004). No artificial inoculation studies have been performed to determine the nature of these infections and therefore the exact role of these weed species in the epidemiology of PepMV is not known (Werkman and Sansford, 2010). Papayiannis *et al.*, 2012 also described *Calendula arvensis* and *Chrysantemum segetum* as natural hosts of PepMV in surveys made in Cyprus.

In surveys in Peru, PepMV has been found to be naturally present in wild Solanum species (*S. chilense, S. chmielewskii, S. parviflorum and S. peruvianum*) (Soler *et al*., 2002). These species do not occur naturally in Europe (Peralta *and* Spooner, 2000). Only one out of five plants of *S. peruvianum* infected with PepMV had symptoms (Soler *et al.*, 2002). PepMV was also detected in tomato and pepino in the same surveys (Werkman and Sansford, 2010).
PepMV has also been detected in potato (*Solanum tuberosum* cv. 'Yungay') in the field in the Andes in Peru. In addition, 14% of tested accessions in the potato germplasm collection at the Centro Internacional de la Papa (CIP) in Peru have been found susceptible to PepMV. Under experimental conditions potato could be infected by different strains of PepMV by mechanical inoculation but with a very low success rate, and rarely local or

systemic symptoms were observed. Only on one occasion could the virus be detected in plants grown from tubers harvested from an inoculated potato plant (Werkman *and* Sansford, 2010).

Blystad *et al.*, 2015 showed that potato could be infected by the most common strains of PepMV occurring in Europe, although local and systemic symptoms seldom develop. Jones *et al.* (1980) also recorded symptomless infection in four potato cultivars, Martin and Mousserion, 2002 recorded infection and symptom development in four out of seven inoculated potato cultivars, and Fakhro *et al.*, 2011 recorded symptomless infection with an EU strain isolate in a single cultivar.

Recently, basil (*Ocimum basilicum*) was reported to be a natural host of PepMV in greenhouse-grown plants in Sicily, Italy. Infected, symptomatic plants were detected in July 2008 in greenhouses in an area where tomato plants were found to be infected by PepMV 3 years earlier (Davino *et al.*, 2009). Subsequent to this report investigations were undertaken to determine whether this would be an epidemiologically significant new host. However, the original isolate that was obtained was not infectious and attempts to confirm infection were therefore unsuccessful. Moreover, attempts to inoculate other isolates of PepMV onto basil plants did not result in infection. Therefore, the status of basil as a natural host for PepMV is doubtful (Werkman and Sansford, 2010).

	% of plants infected or symptomatic with isolate (strain)[a]:							
	Chi2.9 (PES)		Tor9 (PES)		Mu07-20 (EU)		Al08-66 (CH2)	
Host species	Infection	Symptoms	Infection	Symptoms	Infection	Symptoms	Infection	Symptoms
Solanum lycopersicum	100	100 C	100	100 C	100	25 AS, 75 LC	100	25 AS, 75 C/LC
Solanum peruvianum	50	50 AS	50	50 AS	100	100 AS	25	25 AS
Solanum pimpilellifolium	100	100 C/N	100	100 C/N	100	100 C	100	100 C
Solanum chilense	0		50	50 AS	0		25	25 AS
Solanum habrochaites	25	25 AS	50	50 AS	25	25 AS	0	
Solanum muricatum	100	50 C/W, 50 C	75	25 LC/W, 50 C	75	50 C, 25 N	100	50 C, 50 C/LC
Solanum melongena	100	100 N	100	50 AS, 50 W	100	75 AS, 25 C	100	100 AS
Nicotiana benthamiana	25	25 C/LC	0		75	75 C/LC	50	50 AS, 50 LC
Nicotiana clevelandii	100	25 C, 75 LC	100	100 C/LC	100	100 C/LC	75	75 C/LC
Nicotiana occidentalis	100	100 LC/N	100	100 C/LC	100	100 LC	100	100 C/N
Nicotiana tabacum	50	50 AS	0		25	25 AS	0	
Capsicum annuum	0		0		0		0	
Datura stramonium	100	25 C/LC, 25 C/W, 25 C, 25 C/LC/W	100	100 C/LC/W	100	100 C/LC	100	100 C/N
Physalis floridiana	0		0		0		0	

[a] Numbers are the percentage of infected or symptomatic plants over four plants inoculated. Symptoms: AS, asymptomatic; C, chlorosis; LC, leaf curl; N, necrosis; W, wilting.

Table B2.3-01. Host range and symptomatology of PepMV isolates, (Moreno-Pérez *et al.,* 2014).

RMS comments:
Even the PepMV host range is mainly restricted to tomato plant and others species from the *Solanaceae* family, PepMV could have involved spread from cultivated plant species to wild and host adaptation, Table B2.3-01 (Moreno-Pérez *et al.*, 2014).

As PepMV is widespread in Europe, as described in 2.1.2, introduction of mild PepMV isolates Abp1 (EU genotype) and Abp2 (CH2 genotype) in protected tomato crops, is not expected to affect level of natural occurrence of the virus and is not expected to increase a possible risk to other *Solanaceae* plant crops as potatoes or spread to weeds outside the protected green houses.

According to Jorda *et al.* 2001: PepMV was detected in 35% of the samples. Like PepMV, the virus infected (as confirmed by ELISA) greenhouse-grown *Datura stramonium*, *Nicandra physalodes*, *Nicotiana benthamiana*, *N. clevelandii, Solanum tuberosum*, and *Vigna sinensis* and did not infect *Capsicum annuum, Cucumis sativus, Chenopodium amaranticolor, C. quinoa*, Petunia × hybrida, *Phaseolus vulgaris, Physalis floridana, N. glutinosa, N. rustica,* or *N. tabacum*. The virus did infect *Gomphrena globosa*, which normally is not infected by PepMV

Experimental hosts:
Several species have been found to be experimentally susceptible to infection by PepMV following artificial inoculation. These are known as experimental hosts. These species belong to the *Solanaceae* family. Two plant species that are grown as economically important crops are considered to be experimental host in this case:
Eggplant or Aubergine: Eggplant (*S. melongena*) was found to be infected by PepMV by mechanical inoculation (Verhoeven *et al.*, 2003). The virus could be detected in inoculated plants in high virus titers and sometimes

severe local and systemic symptoms were observed. In the PEPEIRA project, where pest risk analysis for PepMV was carried out, eggplant has been tested and found to be infected in greenhouses where eggplants were grown next to a PepMV-infected tomato crop (Werkman and Sansford, 2010).

In order to investigate alternative hosts Blystad *et al.,* 2015 compared the infectivity and symptom development of three, PepMV strains, EU-tom, CH2 and US1, by inoculating PepMV strains on tomato, possible alternative host plants in the family *Solanaceae* and selected test plants. They showed that eggplant is an alternative host of PepMV.
In pepper (*Capsicum annuum*) no natural infections are known (table B2.3-01). Leaves can be infected by mechanical inoculation by different strains of PepMV, but with a low success rate, and systemic infection does not occur (Werkman and Sansford, 2010). Sweet pepper is not an important host of PepMV according to Blystad *et al*, 2015.
Other species of the *Solanaceae* family, non-crop species (Table C2.1.1-02 in Vol 4 and Table B2.3-2.), are used for diagnostic and propagation purposes. PepMV can infect systemically *Datura metel, D. stramonium, Nicotiana debneyi, N. benthamiana* (Jones *et al.*, 1980; Verhoeven *et al.*, 2003). Some PepMV isolates can infect *N. glutinosa* and *N. tabacum* (Verhoeven *et al.*, 2003). Interestingly, a co-inoculation of a particular EU and a particular CH2 isolate resulted in an infection in *N. glutinosa* and *N. tabacum* while the single inoculations of the particular isolates were not infectious in the same hosts (see ref. C.2 in Vol 4). Symptoms that are expressed in the experimental hosts can be yellow mosaic on leaves, necrotic and chlorotic spots and flecking. *Nicotiana occidentalis 37B* was identity as a useful indicator plant for PepMV studies, since it reacts with a different symptomatology to each one of the PepMV strains, Blystad *et al.,* 2015.

The EPPO Global database webpage (EPPO, 2017)[3] lists the following hosts for PepMV:

Major host	Common name
Solanum lycopersicum	Tomato
Solanum muricatum	Pepino
Minor host	
Ocimum basilicum	Basil
Solanum melongena	Aubergine
Wild host /weeds	
Amaranthus graecizans	Tumbleweed, pigweed
Amaranthus retroflexus	Reedrot amaranth, redroot pigweed, comon amaranth
Amaranthus viridis	Slender amaranth, green amaranth
Calendula arvensis	Field maringold
Chenopodiastrum murale	nettle-leaved goosefoot, Australian-spinach, salt-green, sowbane.
Convolvulus arvensis	Field bindweed
Convolvulus humilis	
Glebionis segetum	corn marigold, corn daisy
Malva neglecta	common mallow, buttonweed, cheeseplant, cheeseweed, dwarf mallow,
Malva nicaeensis	French mallow, bull mallow
Malva parviflora	least mallow, cheeseweed, cheeseweed mallow, small-whorl mallow
Malva sylvestris	common mallow, high mallow
Plantago lagopus	
Plantago major	greater plantain, common plantain
Solanum nigrum	European black nightshade, black nightshade, duscle, garden nightshade, hound's berry, petty morel, wonder berry, small-fruited black nightshade,
Sonchus asper	sharp-fringed sow thistle, prickly sow thistle, spiny sow thistle, spiny-leaved
Sonchus oleraceus	common sowthistle, sow thistle, smooth sow thistle, annual sow thistle, hare's colwort, hare's thistle, milky tassel, swinies
Sonchus tenerrimus	slender sow thistle

Table B2.3-2. EPPO Global database webpage (EPPO, 2017)[4] hosts for PepMV.

The RMS has compile the summary above incorporating the abstracts and relevant excerpts from the study Córdoba et al., 2004 regarding natural host plant of PepMV in Spain.

[3] EPPO. (2017) Pepino mosaic virus (PepMV): Overview, distribution and Host plants, EPPO Global Database, https://gd.eppo.int/taxon/PEPMV0.
[4] EPPO. (2017) Pepino mosaic virus (PepMV): Overview, distribution and Host plants, EPPO Global Database, https://gd.eppo.int/taxon/PEPMV0.

Reference:	Córdoba *et al.*, 2004. New Natural Hosts of PepMV virus in Spain. Plant Disease, 88-8.
Report No.:	
Guideline:	Not applicable
GLP:	Not applicable

Abstract PepMVvirus (PepMV) was first detected in Spain in 2000. The virus infects tomato (*Lycopersicon esculentum* Mill.) crops and causes a variety of symptoms including leaf distortion, chlorosis, mosaic, blistering of the leaf surface, green striations on the stem and sepals, and fruit discoloration. PepMV is present along the southern and eastern regions of Spain (provinces of Granada, Almeria, Murcia, Alicante, Valencia, and Barcelona), Balearic, and the Canary Islands. In the summer and autumn of 2001 and 2002, virus-like symptoms were observed in native plants growing in or around tomato fields in Murcia and Almeria provinces. To study the alternate hosts that may serve as virus reservoirs, 62 samples of 42 common weed species, including asymptomatic plants, were collected and analyzed for PepMV using double-antibody sandwich enzyme-linked immunosorbent assay with a commercial antiserum (DSMZ As-0554; Biologische Bundesantstal, Braunschweig, Germany). The following weed hosts tested positive for PepMV: Bassia scoparia (L.) Voss., *Calystegia sepium (L.) R.Br., Chenopodium murale L., Convolvulus althaeoides L., Convolvulus arvensis L., Conyza albida Willd. ex Spreng., Coronopus sp., Diplotaxis erucoides (L.) DC, Echium creticum L., E. humile Desf., Heliotropium europaeum L., Moricandia arvensis (L.) DC., Onopordum sp., Piptatherum multiflorum (Cav.) Beauv., Plantago afra L., Rumex sp., Sisymbrium irio L., Sonchus tenerrimus L., and Taraxacum vulgare (Lam.) Schrank*. The presence of PepMV in these weed species was confirmed using reverse transcription-polymerase chain reaction with primers specific for PepMV. Although the number of samples examined may be insufficient to assess precisely the role of weed reservoirs in outbreaks of PepMV, these findings reveal potential virus sources and contribute to further understanding of PepMV epidemiology in Spain.

A survey of alternative and potential non-tomato host plants of PepMV was conducted in a representative area of tomato cultivation in Spain (Mazarrón, Murcia, southeast Spain). Samples from weeds in the surroundings of two tomato greenhouses, one treated with AbioProtect® in July 2016 (Vaccinated, V) and the other not treated (non-vaccinated, NV), were taken. Twelve different weeds, belonging to 8 different families, were sampled from location V, and ten, belonging to 8 different families, were sampled from location NV. Only one sample, corresponding to the species Solanum nigrum, taken in the surroundings of the non-vaccinated greenhouse, showed presence of PepMV; no weed sampled in the surroundings of the vaccinated greenhouse resulted in presence of PepMV. In this study vaccination of a tomato greenhouse with PepMV does not appear to affect the level of natural occurrence of the virus (details in Document K-MA 7.1/01, Agüero, 2017b).

PepMV as other plant viruses is not related with any animal or human pathogen because it only reproduces in living plant cells. Detail explanation on the absence of pathogenicity to animals and humans is included in data point 2.6 relationships to known plant, animal or human pathogens below.

Overall RMS comment B.2.3:

- Reference need to be included to confirm the effect on *Solanaceae* plant family. Suggested references: According to Jones *et al.*, 1980 PepMV was transmitted by inoculation of sap to 32 species from three families out of 47 species from nine plant families tested. It caused a yellow mosaic in young leaves of pepino and either a mild mosaic or symptomless infection in 12 wild potato species, five potato cultivars and potato clone USDA 41956 but *S. stoloniferum* and potato cultivars Merpata and Revolucion reacted with severe systemic necrotic symptoms. The virus was transmitted by plant. It was best propagated and assayed in *Nicotiana glutinous*. In Rodriguez *et al.*, 2014 study, samples from 320 native perennial plant species, belonging to 20 botanical families were analyzed by enzyme-linked immunosorbent assay (ELISA) for the presence of PepMV, confirmed that Mediterranean native flora could act as plant virus reservoirs, thus posing a risk for neighbouring crops.

- Abp1 is an EU strain and Abp2 is a CH2 strain, and would probably share same host range of the rest of EU and CH2 isolates. Therefore, there is no reference or documents to confirm above statements about specific host range of Abp1 and Abp2.

B.2.4 DEVELOPMENT STAGES/LIFE CYCLE OF THE MICROORGANISM

Plant viruses differ from animal viruses in that they have mostly non-enveloped particles. Furthermore, viral cell-to-cell transport occurs through intercellular channels (plasmodesmata) rather than via plasma membrane budding.

Since plant viruses are obligate, biotrophic parasites, their life cycle starts with penetration of the virion and is as follows:

- **Penetration** of the virion into the cell: Plant viruses are unable to penetrate the plant cuticle and cell wall. It is believed that the virion enters the cytoplasm of the cell passively through wounds caused by mechanical damage to the cuticle and cell wall, or through the stomata.

- **Removal of the coat protein shell of the virion**: after penetration, the coat protein shell of the virion is removed (partially or completely) in the cytoplasm.

- **Expression of the viral genome mediated by the plant cell translation** apparatus. Translation of viral RNA in the cytoplasm produces viral proteins that are required for completion of the virus life cycle. All viruses must direct the formation of at least four types of proteins: replication proteins that are essential for nucleic acid production, at least a silencing suppressor protein necessary for RNAi suppression, structural proteins that form the protein shell and other minor components contained in the virions, and movement proteins that mediate virus transport within and between plant cells. Viral replication proteins combine with cellular proteins to produce a complex of proteins that manufactures multiple copies of the virus genome. These newly-made genomes interact with the structural proteins to form new virions.

- **Movement into neighboring cells**: Plant viruses rely on the availability of connections between cells and the vascular system and utilize the resources of endogenous host trafficking systems, such as cytoskeleton, endoplasmic reticulum (ER) and Golgi, to facilitate movement in a susceptible host and establish a successful infection. For mechanically transmitted viruses, such as Potexviruses, infection initiates in epidermal cells, spreads by cell-to-cell movement via mesophyll and bundles sheath cells to the phloem parenchyma and companion cells. Depending on the virus, the viral genomes or the virions are transported into neighbouring cells through small channels called plasmodesmata that form connections between cells. Plant viruses are unique because they live exclusively in the symplast of their host. This lifestyle requires that plant viruses move between cells to re-initiate infections in order to accumulate in sufficient levels and tissues to guarantee their survival. Many plant viruses produce movement proteins that modify the plasmodesmata channels and facilitate viral movement into neighboring cells. The process of cell-to-cell movement is relatively slow: it takes from one to a few hours for a virus to multiply in a cell and move to the next cell.

- **Long-distance movement:** To successfully colonise an entire plant, a virus needs to enter the vascular system of the plant. The process of systemic, or long-distance transport normally proceeds through the phloem sieve elements where viruses move passively with the flow of photosynthesis. After quite rapid systemic spread of the virus (centimeters per hour) in the phloem, the virus moves from the phloem into surrounding cells where it reproduces and spreads by cell-to-cell movement. The time between initial infection of one or a few cells and systemic infection of the plant varies from a few days to a few weeks depending on the virus, host plant, and environmental conditions.

Transmission of the virus from one plant to another completes the virus life cycle (Gergerich and Dolja, 2006).

Due to the nature of plant viruses, the generation time is not well established, it could be considered either the process of cell to cell movement which is relatively slow: it takes from one to a few hours for a virus to multiply in a cell and move to the next cell; or the time between initial infection of one or a few cells and systemic infection of the plant that which varies from a few days to a few weeks depending on the virus, host plant, and environmental conditions.

The type of reproduction is not applicable for plant viruses. Their multiplication takes place by translation of the viral genome mediated by the plant cell translation apparatus. Translation of viral RNA in the cytoplasm produces viral proteins that are required for completion of the virus life cycle.
Plant viruses do not have resting stages and do not survive long outside the plant cell. PepMV is thought to remain viable in dry plant material for as long as 3 months, where, at 18°C to 21°C, it can remain infective for more than 90 days. In moist organic debris held at 10°C, the virus remains stable and considered capable of infection for a relatively long period (Ferguson, 2001).

Virulence is not applicable to PepMV, EU strain, mild isolate Abp1 and to PepMV, CH2 strain, mild isolate Abp2, as these attenuated isolates only prevent the other aggressive isolates from entering into the crop.

PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, do not have metabolism of their own and therefore are not able to produce metabolites (including toxins) in their different development stages.

Overall RMS comment B.2.4:
In the case of animal viruses, their genomes (+RNA, -RNA, or DNA) enter cell as a part of viral particle, which may be relatively large and contain a variety of proteins facilitating transport within the cell, including nucleus. Plant viruses invade cell mainly by transporting genomic RNA via plasmodesmata, cytoplasmic channels connecting cytoplasms of neighbouring cells. Although such transport require virus-encoded “movement proteins”, often no formation of virus particles is required, and viral genome entering new cells is less protected, and, in general, not all viral proteins could be easily transported through plasmodesmata.

B.2.5. INFECTIVENESS, DISPERSAL AND COLONIZATION ABILITY

The infective/toxic dose is not applicable to PepMV, EU strain, mild isolate Abp1 and to PepMV, CH2 strain, mild isolate Abp2, as these attenuated isolates only prevent the other aggressive isolates from entering into the crop.

PepMV transmission:
PepMV systemically infects tomato plants and rapidly colonizes the entire tomato crop through mechanical transmission. PepMV is very efficiently transmitted mechanically in tomato by standard crop handling through contaminated tools, hands and clothing and by direct plant-to-plant contact (Jones *et al.*, 1980; Spence *et al.*, 2006; Wright and Mumford, 1999). Mechanical transmission by workers and visitors is assumed to be the most common way of PepMV transmission.

Others transmission route (RMS does not agree with “less important transmission routes”)

- **Seed transmission**: Seed transmission has been demonstrated with rates up to 2% depending on time of harvest, tomato variety and seed cleaning or disinfection (Córdoba-Sellés *et al.*, 2007; Hanssen *et al.*, 2010b; Krinkels, 2001; Ling, 2008, Van der Vlugt, 2009). When seed is infected, the virus can be found externally on the seed coat and not in the embryo or endosperm (Krinkels, 2001; Hanssen *et al.*, 2010b) showed that the overall level of seed transmission obtained under ‘worst case scenario’ conditions is only 0.026 %. A high virus concentration is found on the seed coat but transmission to the seedling occurs only rarely. The virus could not be found inside the seeds, although some results suggest that the risk of transmission increases with the length of the time interval between infection of the mother plants and seed harvest (Hanssen *et al.*, 2010b).

- **Tomato plants transmission**: most seedlings produced in a country are used in that country, in Spain tomato seedlings are generally produced in professional nurseries and provided with the corresponding phytosanitary passport guaranteeing that are free from PepMV among other pathogens, therefore transmission by tomato plants coming from the nurseries is unlikely.

- **Mechanical transmission:** PepMV is 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).

- **Bumble bees transmission**: Bumblebees can transmit the virus mechanically (Lacasa *et al.*, 2003; Shipp *et al.*, 2008; Stobbs *et al.*, 2009), however the main risk from bumble bees is associated with spread within an infected greenhouse or within a dense tomato production area, not with large-distance spread.
- Bumblebees used by growers to pollinate tomatoes can move freely in and out of the green houses, and bees carrying virus inoculum from infected greenhouse tomatoes could establish and spread PepMV. Greenhouse trials demonstrated the ability of bumblebees) to transmit PepMVvirus (PepMV) from infected tomato plants to perennial other solanaceae plants as Solanum dulcamara L.

The RMS has compile the summary above incorporating the abstracts and relevant excerpts from the study Stobbs and Greig, 2014 regarding bumblebee transmission of PepMV between tomato plants.

Reference:	Stobbs and Greig, 2014. First report of bumblebee (*Bombus impatiensCresson*) transmission of PepMV virus between tomato (*Solanum lycopersicum L.*) and perennial climbing nightshade (*Solanum dulcamaraL.*) Canadian Journal of Plant Pathology, Volume 36-4, 529-533
Report No.:	
Guideline:	Not applicable
GLP:	Not applicable
Abstract	Greenhouse trials demonstrated the ability of bumblebees (*Bombus impatiens Cresson*) to transmit PepMVvirus (PepMV) from infected tomato plants to perennial climbing nightshade (*Solanum dulcamara* L) in 2 of 3 trials (5.1% and 5.6% frequency, respectively). The efficiency of transmission was lower than that between tomato plants in previous studies (80%). Low rates of transmission were also seen in bee transmission from nightshade plants back to tomato (6.3%, 3.7% and 2.8%), and between nightshade plants (8.3% and 2.8%). Nightshade was easily infected by mechanical inoculation in controls. Bumblebees used by growers to pollinate tomatoes can move freely in and out of the production houses, and bees carrying virus inoculum from infected greenhouse tomatoes could establish and spread PepMV in nearby climbing nightshade populations. This overwintering reservoir could allow for ongoing virus introduction from the field through pollinating bees back into tomato production houses seasonally. The virus could also spread from infected climbing nightshade into tomato field plantings through similar bee activity.

- **Whitefly transmission**: (included by the RMS): Transmission of PepMv by whitefly seems to be low according to Noël *et al.*, 2014. Two experiments were conducted to investigate the transmission of the PepMV and the greenhouse whitefly (*Trialeurodes vaporariorum*) from tomato to tomato. The results confirmed the low transmission role of flywhite, showed that the number of PepMV particles carried on whitefly bodies was low, with an average occurrence of 1.33 on the 55 whiteflies tested after the insects were in contact with infected plants for 5 days. This low occurrence was confirmed by observation under microscope, which showed an absence of PepMV-contaminated tomato sap on the insect bodies, suggesting that PepMV transmission by whiteflies could occur when they feed on the plant.

- **Fungi transmission:** The root-infecting parasitic fungus *Olpidium virulentus* can facilitate PepMV transmission (Alfaro-Fernández *et al.*, 2010). These transmission assays demonstrated the possibility of PepMV transmission by *O. virulentus* collected from tomato crops. PepMV was only transmitted to plants irrigated with the drainage water collected from PepMV-infected plants whose roots contained the fungal culture from tomato with a transmission rate of 8%. No infection was detected in plants irrigated with the drainage water collected from plots with only a fungus or virus infection. Both the virus and fungus were detected in water samples collected from the drainage water of the acquisition–source plants of the assay.

Persistance:

Persistence in water: PepMV has been found to be spread by recirculating water from plant to plant (Schwarz *et al.*, 2010), and to be able to survive and be transmitted in water (Mehle *et al.*, 2014). Recently other authors

have shown that PepMV dispersal could be prevented using a sensor based disinfectant (Bandte *et al.*, 2016). Although recirculating water of greenhouses must be disinfected before discharge in the environment, emission to surface water cannot be excluded as the water can be drained in emergencies. It was described that recirculating water can also spread the virus (Schwarz *et al.*, 2010) and that PepMV can survive and be transmitted in water Mehle *et al.* (2014). Transmission through crop handling practices is expected to go faster.

A GEP trial on the persistence of PepMV in water from tomato crops treated with PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, found that PepMV was not persistent in the leachate from the tomato plants treated and therefore there is no risk of infection from this leachate (Document K-MP 6.2/04, Prats, 2017a).

The RMS has provided data below on water persistence according to K-MA 6.2/04 applicant document:

Reference	**K-MA 6.2/04** Field study to evaluate the crop safety and the efficacy of the Plant Protection Products Peprotect (PPP) and its components or agents (ppa1 and ppa2) the components or agents of AbioProtect® (PPA1 and PPA2), for the control of PepMV in tomato crop (Southeast Spain, 2016). (Unpublished report). Study code: ACX/1274/AB
Guideline	No OECD for water persistence.
GLP	The study was conducted according to GLP principles/regulations. Certified laboratory.
Objectives	The objective of this study was to determine the persistency of the products Abp1 and Abp2 in water.
Material and methods	The persistency of AbioProtect® in water was assessed by studying the presence of PepMV in samples of leachate taken from Tr.4 (AbioProtect®). Number of assays 1.
Test material	AbioProtect®, active ingredients (a.i.): PepMV-Abp1 + PepMV-Abp2 (Abp1 + Abp2) $\geq 5 \times 10^5$ viral genomecopies x μl^{-1} Tomato watery leaves extract containing PepMV, EU and CH2 strain, mild isolates, as a suspension concentrate (SC).
Number of test samples	5 tomato plants (BBCH: 18) (3 manual inoculation + 2 watering inoculation).
Treatments	5L leachate taken from Tr.4 (AbioProtect®). The persistency of AbioProtect® in water was assessed by studying the presence of PepMV in treated plants. Control plants were inoculated with aggressive isolates CH2 and EU.
Duration	Six months (06/09/2016-02/03/2017)
Test conditions	T=20ºC 21, 19 and 12 days after each inoculation)
Evaluation	Assessment and presence of PepMV by molecular hybridization with generic probe for PepMV in: - Tomato plants inoculated with trt. 4. Infected PepMv leachate. - Tomato plants watering with infected PepMv leachate.
Deviations from guideline	No guideline was followed.

RMS conclusion:

- The persistency of AbioProtect® in water was assessed by studying the presence of PepMV in samples of leachate taken from Tr.4 (AbioProtect®). In the tomato cultures in greenhouse the release of PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 to the environment through air is thought to be negligible. The virus does not produce spores or other airborne structures that can be spread through air movement. The virus only multiplies in living plant cells and is deactivated by UV light.
- None of the inoculated tomato plants showed symptoms associated with a PepMV infection.
- The analysis did not detect the presence of PepMV in the plants inoculated with the leachate of Treatment 4.
- In the case of the tomato plants used as controls, the analysis detected the presence of PepMV. According to the results obtained in the study conducted in this trial to evaluate the persistency of AbioProtect® in water, it can be concluded that the Plant Protection Product AbioProtect® has no persistency in the leachate from tomato plants treated with AbioProtect®. Therefore, there is no risk of PepMV infection with this leachate.

- The plants were evaluated 21, 19 and 12 days after each inoculation, at this time the developments of PepMV doesn't occur yet according to Hernández-Yopis 2014.
- There is no negative control of leachate.

Persistence in soil: A GEP trial on the persistent of PepMV in soil recovered from tomato crops treated with PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2, found that PepMV was not persistent in the soil from the tomato plants treated and therefore there is no risk of infection from this soil or to subsequent crops (Document K-MP 6.2/05, Prats, 2017b).

RMS has provided data below on soil persistence according to K-MA 6.2/05 applicant document:

Reference	**K-MA 6.2/05** Field study to evaluate the crop safety and the efficacy of the Plant Protection Products Peprotect®(PPP1) and AbioProtect® (PPP2), and of the components or agents of AbioProtect® (PPA1 and PPA2), for the control of PepMV in tomato crop (Southeast Spain, 2016). (Unpublished report). Study code: ACX/1277/AB
Guideline	No OECD for soil persistence.
GLP	The study was conducted according to GLP principles/regulations. Certified laboratory.
Objectives	The objective of this study was to determine the persistency of the products Abp1 and Abp2 in soil. Number of assays 1. 3 soil samples per treatments- two soil depths.
Material and methods	
Test material	AbioProtect®, active ingredients (a.i.): PepMV-Abp1 + PepMV-Abp2 (Abp1 + Abp2) ≥ 5 x 10^5 viral genomecopies x μl^{-1} Tomato watery leaves extract containing PepMV, EU and CH2 strain, mild isolates, as a suspension concentrate (SC).
Number of test samples	.3 replicates per treatment/2 depths (5 and 35cm).
Treatments	The persistency of AbioProtect® in soil was assessed by studying the presence of PepMV in samples of soil taken from Tr.4 (AbioProtect®) and from Tr.1 (untreated control) for comparison (control).
Duration	Six months (21/11/2016-26/05/2017)
Test conditions	Tº= 26ºC; Hr= 40.00%; Wettness of foliage=dry; wetness of soil (2-5cm)= wet.
Evaluation	Presence of PepMV in the samples. - Direct analysis of extracts from the soil samples. Samples extracts were analysed with AgriStrip PepMV Kit (Bioreba). - Analysis of plants inoculated with extracts from the soil samples. Molecular hybridization with generic probe for PepMV.
Deviations from guideline	No guideline was followed.

RMS conclusion:
Results of the samples from Tr. 4 (AbioProtect®) were negative; the analyses did not detect the presence of PepMV in the soil samples taken from Tr. 4 or in the plants inoculated with extracts from the soil samples taken from Tr.4. According to the results obtained in the study conducted in this trial to evaluate the persistency of AbioProtect® in soil, it can be concluded that the Plant Protection Product AbioProtect® has no persistency in soil. Therefore, there is no risk of PepMV infection in a soil with plants treated with AbioProtect® or in a soil where there were plants treated with AbioProtect®.

Persistence on crop residue: The virus is thought to remain viable in dry plant material for as long as 3 months, where, at 18°C to 21°C, it can remain infective for more than 90 days. In moist organic debris held at 10°C, the virus remains stable and considered capable of infection for a relatively long period (Ferguson, 2001; Mayne and O'Neil 2017) found PepMV in roots recovered from soil immediately after infected plant removal, but no viable virus was detected in sap transmission tests on roots recovered at two, four or six weeks after plant removal and tomato seedling growing on this soil proved negative for PepMV, indicating that the risk of PepMV remaining in fine roots or soil after crop removal at levels sufficient to result in PepMV infection is low to negligible.

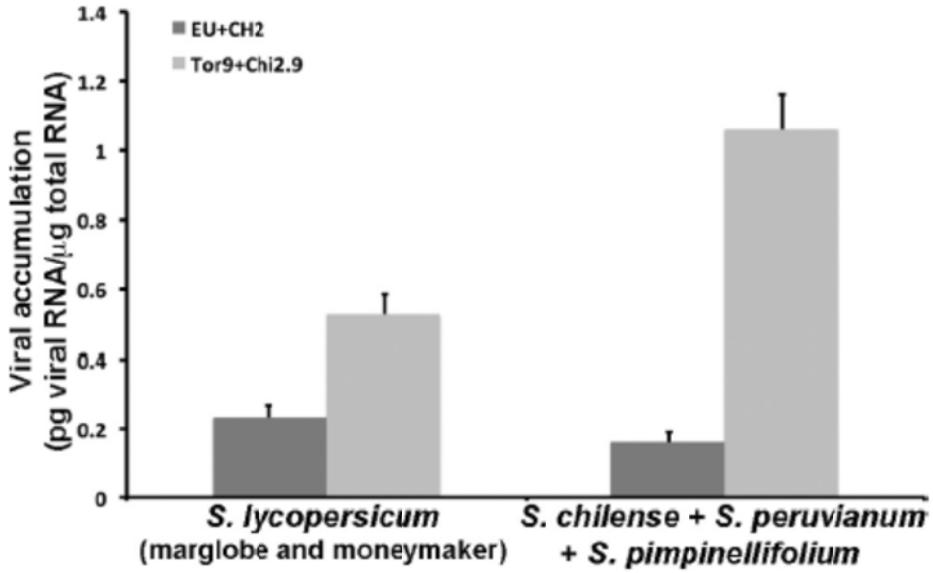

Figure B2.5.-1. Accumulation of PepMVvirus isolates in different host plant species. (Moreno-Pérez *et al.*, 2014).

The accumulation of isolates from tomato crops in Spain (dark gray bars) or from wild *Solanum* spp. in Peru (light gray bars) was compared in two different host types: tomato (*S. lycopersicum cv. Marglobe* and cv. *Moneymaker)* and wild tomato (*S. peruvianum, S. pimpinellifolium,* and *S. chilense*). Data are means and standard errors from at least 5 infected plants. (Moreno-Pérez *et al.*, 2014).

	µg viral RNA/g total RNA for isolate[a]:			
Solanum species and cultivar	Mu07-20 (EU)	Al08-66 (CH2)	Chi2.9	Tor9
S. lycopersicum				
Marglobe	0.269 ± 0.076	0.049 ± 0.011	0.509 ± 0.046	0.316 ± 0.058
Moneymaker	0.415 ± 0.056	0.168 ± 0.052	0.615 ± 0.117	0.613 ± 0.127
S. pimpinellifolium	0.284 ± 0.064	0.103 ± 0.029	1.592 ± 0.276	1.121 ± 0.149
S. chilense	0.117 ± 0.026	0.016 ± 0.006	1.402 ± 0.272	0.337 ± 0.124
S. peruvianum	0.455 ± 0.075	0.018 ± 0.005	0.954 ± 0.149	0.849 ± 0.146

[a] Data are means ± standard errors for at least 5 plants.

Table B2.5-1. Accumulation of PepMV isolates in different Solanum species (Moreno-Pérez *et al.*, 2014).

Virus accumulation significantly depended on the host species, on the virus isolate, and on the species-per-isolate interaction. (Figure B2.5.-1) Accumulation in *Solamun lycopersicum* of EU isolates range between 0.269 -0.415µg viral RNA/g total RNA and in CH2 strain 0.049-0.168 µg viral RNA/g total RNA (table B2.5-1).

Persistence on host plants (added by RMS): Analyses of the data presented in Figure B2.5.-1: showed that accumulation of PepMV isolates from wild hosts was higher in wild than in domestic tomatoes, while tomato isolates showed a nonsignificant trend toward higher accumulation in cultivated than in wild Solanum species. Also, accumulation of PepMV isolates from domestic tomato was lower than accumulation of PepMV isolates from wild tomatoes in both and domestic tomatoes. These analyses show strong evidence of host adaptation for PepMV isolates from wild hosts, and they suggest adaptation to tomato of tomato isolates. Also, they suggest that there is a trade-off between virus fitness in wild tomato species and in domestic tomato. The more efficient multiplication in wild than in cultivated hosts of PepMV-PER isolates supports a scenario of adaptation to their wild hosts. Conversely, tomato isolates of PepMV strains EU and CH2 show a trend toward more efficient multiplication in tomato than in wild Solanum species, which is also suggestive of a process of host adaptation.

Environmental requirements

After PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2 have entered the host cell they are protected against environmental conditions. Therefore, the humidity, temperature or pH outside the host plant is not considered environmental requirements for viability and replication of the virus in the plant. However, at very high temperatures in the greenhouse (>45 ºC) the virus titer can decrease and the symptoms can disappear, but this is a transient effect. The virus starts to degrade at temperatures above 60 ºC. The ideal pH for survival and replication is the pH on the cytoplasm of the host plant.

Solar radiation: Symptoms caused by PepMV are more readily seen during the fall and winter months when light levels are lower. During the brighter months, plants may harbor the virus but may not show any symptoms (Ferguson, 2001).

Temperature:
The survival of PepMV in dried sap depends on temperature, with longer persistence at cooler temperatures. At 5ºC, the virus survived and was infective after 4 weeks but not 5 weeks. At 15ºC, the virus survived and was infective after 2 weeks but not 3 weeks. At 25ºC, the virus survived and was infective after 4 days but not 7 days (O'Neill *et al.*, 2003).

RMS has provided data below on the storage stability at different conditions according to K-MA 2.2/01 applicant document:

Reference	**K-MA 2.2/01** (Agüero, 2017c). Study to evaluate the storage stability and shelf life of the Microbial Pest Control Product AbioProtect® and its components PepMVvirus (PepMV), EU strain, mild isolate App1 and PepMV, CH2 strain, mild isolate Abp2 (Unpublished report). Study code: ABP04/2017
Guideline	There are no validated guidelines for the performance of this type of studies. It has NOT been conducted in conformity of the requirements stated under national GEP regulation (RD 2163/1994 and OM11 Dec 1995).
GLP	The study was conducted according to GLP principles/regulations. Certified laboratory.
Objectives	Aassessment of the storage stability and shelf life of: *Objective 1)* Long term storage of the MPCAs Abp1 and bp2 at -18ºC. *Objective 2)* Final product AbioProtect® stored at different temperatures -18, 4 and 20ºC for up to 35 days.
Material and methods	Stability was tested by inoculation of tomato seedlings with test material after storage at the established temperature.
Test material	*Objective 1)* -PepMV, EU strain, mild isolate App1. Batches (L-2.8-240616-Abp1-C and L-2.9-250616-Abp1-C).Control Batch (L-8-151116-Abp1-C). - PepMV, CH2 strain, mild isolate Abp2. Batches (L-2.8-240616-Abp2-C; L-2.9-250616-Abp2-C). Control Batch (L-8-151116-Abp2-C). - MPCP (AbioProtect®). Batches (L-AB04-240217; L-AB05-240217). Control Batch (AB03-240217). *Objective 2)* - PepMV, EU strain, mild isolate App1. Batches Batch (L-8-151116-Abp2-C). - PepMV, CH2 strain, mild isolate Abp2. Batch (L-8-151116-Abp1-C). - MPCP (AbioProtect®). Batch (AB03-240217).
Target plant	Tomato seedlings Cv Moneymaker (BBCH13-15).
Number of test samples	*Objective 1)* 5 aliquots per treatment batch. 18 plants/tray from 3 rows randomly selected. *Objective 2)* 5 aliquots per batch stored at 3 temperatures. Total of 15 aliquots. 3 seedlings/aliquot.
Treatments	*Objective 1)* 2 test products batches of MPCP (AbioProtect®) and a control batch at -18ºC. *Objective 2)* 1 batch of the final product AbioProtect® AB03-240217 stored at -18, 4 and 20ºC.

Duration	*Objective 1)* - 9 months storage (January 20th -March 24th, 2017) of test material. - Evaluation of plant material 20 days post inoculation (dpi). *Objective 2)* -35 days storage of test material. - Evaluation of plant material 13 dpi.
Test conditions	*Objective 1)* -Storage test material temperature T^a= -18 ±2ºC. - Seedling growing conditions (16-h photoperiod, 24-26°C day, 16-18ºC night). *Objective 2)* - Storage test material temperatures T^o=-18, 4 and 20ºC.. -Seedling growing conditions (16-h photoperiod, 24-26°C day, 16-18ºC night).
Evaluation	
	Objective 1) - Storage stability after 9 months of the MPCAs Abp1 and Abp2. - Self-live tested 20 dpi in tomato plant. *Objective 2)* - Storage stability after 1, 7, 14, 21, and 35days of the Abioprotect product. - Self-live tested 13 dpi in tomato plant. Method of detection: Molecular hybridization with digoxigenin (DIG)-labelled RNA specific probes (Más and Pallás, 1995) for PepMV.
guideline	No guideline was followed.

RMS conclusion:
Objetive 1) Assessment of the storage stability and shelf life of AbioProtect® components, PepMV, EU strain, mild isolate App1 and PepMV, CH2 strain, mild isolate Abp2, stored at -18ºC for 9 months before formulating AbioProtect® was also conducted.

- Batches L-2.8-240616-Abp1-C and L-2.9-250616-Abp1-C of PepMV, EU strain, mild isolate App1, and L-2.8-240616-Abp2-C and L-2.9-250616-Abp2-C of PepMV, CH2 strain, mild isolate Abp2, were defrosted slowly after storage at -18 ºC for 9 months and used to formulate two AbioProtect® batches (L-AB04-240217 and L-AB05-240217).
- Abp1 and Abp2 were detected in the plants inoculated with AbioProtect® batches (L-AB04-240217 and L-AB05-240217) formulated with each MPCAs previously stored at -18ºC for at least 9 months.
- Therefore, according to the results of the present study PepMV, EU strain, mild isolate App1 and PepMV, CH2 strain, mild isolate Abp2 have a storage stability and shelf life of at least 9 months when stored at -18 ºC and need to be defrosted slowly previous to formulation of AbioProtect® and subsequent dilution at the application dose.

Objetive 2) Assessment of the storage stability and shelf life of AbioProtect® stored at -18, 4 and 20ºC for up to 35 days was conducted. AbioProtect® stability was tested by inoculation of tomato seedlings after storage at the established temperature for 1, 7, 14, 21 and 35 days.

- Abp1 and Abp2 were detected in the plants inoculated with AbioProtect® stored at -18 and 4ºC at all periods of time assayed (1, 7, 14, 21 and 35 days).
- Abp1 was detected only in plants inoculated with AbioProtect® previously stored at 20ºC for 1 day and Abp2 was detected in plants inoculated with AbioProtect® previously stored at 20ºC for 1, 7, 14 and 35 days, but not at 21 days.
- Therefore, the results of the present study indicate that the stability and shelf life of AbioProtect® and its components could be guaranteed for 35 days upon storage at -18 and 4 ºC, and for 1 day upon storage at 20ºC.

No information regarding the technical properties after storage up to 35 days at 4ºC or -18ºC (shelf life claimed by the applicant) has been reported. The RMS has considered these data are not necessary due to the specific application and used of Abiopep according to the applicant:
MPCP is only applied by qualified Abiopep personnel, it is always formulated on demand after slowly defrosting the MPCAs at 4 ±2ºC, kept refrigerated at 4-7ºC until use on the same day. The technical properties have been tested in the MPCP thus formulated showing that no particular problems are to be expected when the product is used as recommended.

According to regulation 283/2013, B 3.7. Recommended methods and precautions concerning handling, storage, transport or fire.

Recommended methods and precautions for handling, storage, transport or fire:

Handling and storage: The MPCAs have a shelf life of 9 months at <-18ºC following production. MPCA should be defrosted slowly at 4ºC, before formulating the MPCP. Once formulated to achieve maximum efficiency the product must be stored refrigerated in a dry area until use in the same day, in the original packaging and out of the reach of children. Keep it also away from food, drink and animal feed stuff.
Transport: is not regulated. Not considered a hazard product according to national and international transport regulations.
Fire: since the product is a water base plant extract, it is not flammable and the risk of fire is extremely low.

According to different studies regarding PepMV persistence at different environment conditions:

- PepMV, EU and CH2 strains, from macerated infected leaves remained infectious in water at 20 °C for up to 3 weeks (Mehle et al. 2014).
- No viable PepMV was detected in sap transmission tests on roots recovered at two, four or six weeks after PepMV-infected plant removal (Mayne and AO'Neill, 2017)
- PepMV was confirmed in tomato roots to at least 30-cm depth. Virus at transmissible levels was detected in roots 31 days after plants were cut-off at soil level, but not after 57 days (O'Neill et al., 2003).
- Under greenhouse conditions, PepMV can survive and remain infectious for several weeks in plant debris and on contaminated surfaces or tools (Van der Vlugt, 2009). Water-mediated transmission of PepMV has been shown for PepMV strain EU (Alfaro-Fernández et al., 2010; Schwarz et al., 2010).

Overall RMS comment B.2.5:

The applicant have provided four specific complite GEP studies on persistence, stability and dispersion AFTER application in tomato plants for Abp1 and Abp2 strains to support information above (KMP6.1/04,Prats 2017a; KMP6.2/05,Prats 2017b; KMP6.2.5/01, Prats 2017c, and Prats 2017d, KMP6.2/06).

No viable PepMV was found on roots from plants infected with mild isolate Abp1 and mild isolate Abp2 30 days after removal of the crop on hydroponic grow bags (please refer to Document K-MA 7.1.1/02, Céspedes, 2015a).The study on the persistence of PepMV in hydroponic grow bags, failed to detect any viable PepMV on roots from plants infected with mild isolate Abp1 and mild isolate Abp2, 30 days after removal of the crop indicating that PepMV is not persistent in the substrate from tomatoes treated with PepMV, EU strain, mild isolate Abp1 and PepMV, CH2 strain, mild isolate Abp2.

An English summary of Document K-MA 7.1.1-02 Céspedes 2015a containing the main results and conclusions is provided (Document K-MA 7.1.1-02/s Céspedes 2015a) and an extract is included below:

Evaluation of different disinfectants with and without solarisation for disinfection of coconut fibre bags substrates of a tomato cultivar inoculated with PepMV

A trial was conducted to evaluate the disinfection of coconut fibre substrates in which three tomato cultivars inoculated with different PepMV virus isolates had been grown. The trial was carried out in a representative area for greenhouse tomato cultivation in Spain, located in El Ejido, Almería, in a greenhouse of the Experimental Station Cajamar Las Palmerillas.

The substrates with plant material infected with PepMV came from the trial conducted at the Experimental Station Cajamar Las Palmerillas called "Vaccination strategy to control Pepino mosaic virus (PepMV) in tomato", coinciding with the end of the trial. The products to be evaluated were 10000 ppm bleach, calcium hypochlorite, ozone, TERRA DIS (chlorine dioxide), HUGA-SAN-50 (hydrogen peroxide + silver chloride), Metam sodium, Agrocelhone (dichloropropene + chloropicrin).

A treatment was established for each product to be tested, in addition to a control treatment, and evaluated in 2 conditions, covering with plastic and without cover. In addition, a treatment with new bags, free of infected plant material was also established.

Roosts were sampled from the bags to be studied before treatment applications to determine the presence and infectivity of PepMV by a bioassay. Bags were covered with plastic for solarisation on 14/07/2015. Products application and solarisation was performed between 21/07/2015 and 28/08/2015. The same day roots were

sampled to evaluate by a bioassay the presence and infectivity of PepMV, and 6 seedlings of Guanche cultivar per bag were transplanted to evaluate substrate infectivity after treatment.
The first bioassay, before treatment, confirmed PepMV presence on the roots but was negative for the infectivity test, indicating that the experiment started with a PepMV previously inactivated.
In the conditions of the trial PepMV was inactive in the coconut fibre bags.

The first bioassay, before treatment, confirmed PepMV presence on the roots but was negative for the infectivity test, indicating that the experiment started with a PepMV previously inactivated. The second bioassay confirmed the presence of PepMV by molecular techniques after the disinfections, but remained inactive when evaluating its capacity to infect healthy plants from inoculum prepared from the roots.

Plants grown in the test bags resulted negative for PepMV by DAS-ELISA and molecular hybridization after 45 days.

In the conditions of the trial PepMV was inactive in the coconut fibre bags, probably due to inactivation during the month elapsed from cutting the plants and taking the samples for the first bioassay.

The infective/toxic dose is not applicable to PepMV, EU strain, mild isolate Abp1 and to PepMV, CH2 strain, mild isolate Abp2, as these attenuated isolates only prevent the other aggressive isolates from entering into the crop. Nevertheless, PepMV, EU strain, mild isolate Abp1 and to PepMV, CH2 strain, mild isolate Abp2, infect the tomato plant in order to prevent against aggressive isolates from entering into the crop. Abp1 and Abp2 microorganisms must enter the body of the tomato host, and need to be able to reproduce to form new infective units to protect the plant. The infectivy of Abp1 and Abp2 was evaluated as the characteristic that allow the virus to infect the tomato plants and alter plant cell in order to activate plant immune defense system. Nevertheless, the pathogenicity of Abp1 and Abp2 is none, as they act as immune plant protector organisms.

B.2.6. RELATIONSHIP TO KNOWN PLANT OR ANIMAL OR HUMAN PATHOGENS

PepMV belongs to the order Tymovirales that include plant viruses only. All closely related species are plant pathogens. PepMV is closely related to Narcisus mosaic virus (NMV), Scallion virus X (SVX), Cymbidium mosaic virus (CymMV) and Potato aucuba mosaic virus (PAMV) (Cotillion *et al.*, 2002). The highest overall nucleotide identities are with NMV and CymMV, the best available means to distinguish them is by nucleotide sequencing (see ref C.1 in Vol 4).

Plant viruses are not related with any animal or human pathogen because they only reproduce in living plant cells. They cannot replicate in humans or other animals, largely due to the lack of specific receptors for recognition and entry into host cells. There are no documented cases of plant viruses causing diseases in humans.

Viruses such as PepMV are transmitted among plants by mechanical means and do not enter cells via specific receptors, as do animal viruses. Animal viruses enter host cells by a process called endocytosis. Plant viruses, by contrast, enter through wounds in the cell's outer coverings - e.g., through abrasions made by wind or through punctures made by insects. Plant viruses like PepMV are ubiquitous in plants and fruits and therefore humans are continuously exposed to them.

Tomato fruits represent an important part of human diet and possess many health-related compounds. A certain percentage of the population cannot consume this vegetable because they suffer from local and systemic allergic reactions.

A study was conducted to analyze the potential effect of PepMV infection in the expression of allergens leading to a higher allergenic potential of tomato fruits (Welter *et al.*, 2013). This study showed that PepMV infection of tomato plants can lead to long-lasting up-regulation of particular allergens in fruits, but the hypothesis that this results in higher allergic potential of the fruits was proved invalid.

The RMS has compile the summary above incorporating the abstracts from the study Welter *et al.*, 2013.

Reference: **Welter *et al.*, 2013. PepMVvirus infection of tomato affects allergen expression, but not the allergenic potential of fruits.**

Report No.:	KMA 2.6-2
Reference:	PloS one 8:e65116.
Guideline:	None
GLP:	No

Abstract: The plant pathogen PepMVvirus (PepMV) is a major disease of greenhouse tomato crops worldwide. Plant pathogens can induce expression of defence- or pathogenesis-related proteins, including identified allergens. Therefore we hypothesised that PepMV infection results in the expression of allergens leading to a higher allergenic potential of tomato fruits. Transcript level analyses showed differential expression of 17 known and putative tomato fruit allergen encoding genes at early and late time points after PepMV inoculation, but no general induction was detected. Immunoblot analyses were conducted and IgEs from a serum pool of tomato allergic subjects reacted with 20 proteins, of which ten have not yet been described. In parallel, skin prick tests with a group of tomato allergic subjects did not show a general difference between PepMV infected and non-infected tomato fruits and basophil activation tests confirmed these results. In summary, PepMV infection of tomato plants can lead to long-lasting up-regulation of particular allergens in fruits, but the hypothesis that this results in a higher allergenic potential of the fruits proved invalid.

RMS comment:
This study aimed to answer the question of whether infection of tomato plants with PepMV increase systemic allergic reactions on certain percentage of the population. To detect new putative allergens that might arise in tomato fruits infected with PepMV, immunoblot analyses with a serum pool of nine tomato allergic subjects were conducted. Nine of the putative allergens occurred in protein extracts from both infected and non-infected fruits. This study also shows that allergen transcript levels vary after viral pathogen attack in different tomato plant organs (leaves and fruits) weeks after inoculation with PepMV, which should be generally considered regarding the defence response of a plant at the RNA accumulation level. Additionally, clinical allergy tests showed high inter- individual variation to PepMV infected and non-infected tomato fruits. These inter-individual differences, and the fact that plants grown under commercial greenhouse conditions might differ regardless of the PepMV infection, make it difficult to formulate a final statement about the allergenicity of PepMV infected tomato fruits. That for, RMS does not entirely agree with final conclussion of the applicant.

The study ref C.1 in Vol 4, indicated that PepMv isolates Ab1 and Abp2 were not related with human or animal pathogenetic viruses.

B.2.7. GENETIC STABILITY AND FACTORS AFFECTING IT

See confidential Vol 4.

B.2.8 INFORMATION ON THE PRODUCTION OF METABOLITES (ESPECIALLY TOXINS)

Viruses have no metabolism of their own and are therefore not able to produce secondary metabolites.

For PepMV the complete viral genome sequence is known and the five typical Potexvirus encoded proteins are well understood. None of these proteins show any homology to known human or animal toxins. It can therefore be stated with certainty that PepMV does not produce toxins, not even after infecting the plant host cell.

B.2.9 ANTIBIOTICS AND OTHER ANTIMICROBIAL AGENTS.

Not applicable to viruses: viruses are not metabolically active and therefore cannot produce antimicrobial substances; they are not sensitive to antibiotics and therefore cannot become resistant to these substances or spread resistance.

B.2.10 REFERENCES RELIED ON

The applicant has provided summaries and results of the scientific peer-review open literature, on the active substance and its relevant metabolites dealing with side-effects on health, the environment and non-target species and published within the last 10 years before the date of submission of the dossier. There is no information whether this literature search was performed in accordance to the provisions of the EFSA Guidance "Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) 1107/2009".

The literature search provided was conducted in accordance to the guidelines set up in document European Food Safety Authority; Submission of scientific peer-reviewed open literature for the approval of pesticide active substances under Regulation (EC) No 1107/2009 (OJ L 309, 24.11.2009, p.1-50), (EFSA Journal 2011; 9(2):2092. [49pp.]. doi:10.2903/j.efsa.2011.209)2. Full details and justification of how the literature search was performed could be found in Document K-MA 5.2.5 Hernando 2017.

Data point	**Author(s)**	**Year**	**Title** **Company Report No** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study** **Y/N**	**Data Protec-tion Claimed** **Y/N**	**Justification if data protection is claimed**	**Owner***
B.2.1.1/01	Chewachong G.M., Miller S.A., Blakeslee J.J., Francis D.M., Morris T.J., Qu F.	2014	Generation of an attenuated, cross-protective *Pepino mosaic virus* variant through alignment-guided mutagenesis of the viral capsid protein. Phytopathology 105:126-134. DOI: 10.1094/PHYTO-01-14-0018-R No GLP Published	N	N		LIT
B.2.1.1/02 B.2.2.1/01	Schenk M.F., Hamelink R., van der Vlugt R.A.A., Vermunt A.M.W., Kaarsenmaker R.C., Stijger I.C.C.M.M	2010	The use of attenuated isolates of Pepino mosaic virus for cross-protection. European Journal of Plant Pathology 127:249-261. DOI: 10.1007/s10658-010-9590-4 No GLP Published	N	N		LIT
B.2.1.1/03 B.2.2.1/02	Sempere R.N., Gómez-Aix C., Ruiz-Ramon F., Gómez P., Hasiów-Jaroszewska B., Sánchez-Pina M.A., Aranda M.A.	2016	*Pepino mosaic virus* RNA-dependent RNA polymerase POL domain is a hypersensitive response-like elicitor shared by necrotic and mild isolates. Phytopathology 106. DOI: 10.1094/phyto-10-15-0277-r No GLP Published	N	N		LIT
B.2.1.1/04	Vermunt A.M.W., Kaarsemaker R.C.	2017	Multi-genotype cross-protection against *Pepino mosaic virus* in tomato. Crop Protection 96:116-122. DOI: 10.1016/j.cropro.2017.02.007 No GLP Published	N	N		LIT
B.2.1.2/01 B.2.6/01	Cotillion A.C., Girard M., Ducouret S.	2002	Complete nucleotide sequence of the genomic RNA of a French isolate of *Pepino mosaic virus* (PepMV). Archives of Virology 147:2231-2238. DOI: 10.1007/s00705-002-0873-8 No GLP Published	N	N		LIT

Data point	**Author(s)**	**Year**	**Title** **Company Report No** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study** **Y/N**	**Data Protec-tion Claimed** **Y/N**	**Justification if data protection is claimed**	**Owner***
B.2.1.2/02	French C. J., Bouthillier M., Bernardy M., Ferguson G., Sabourin M., Johnson R.C., Masters C., Godkin S., Mumford R.	2001	First report of *Pepino mosaic virus* in Canada and the United States. Plant Disease 85:1121. DOI: 10.1094/PDIS.2001.85.10.1121B No GLP Published	N	N		LIT
B.2.1.2/03 B.2.3/01 B.2.5/01	Jones R.A.C., Koenig R., Lesemann D.	1980	Pepino mosaic virus, a new potexvirus from pepino (*Solanum muricatum*) Annals of Applied Biology 94:61-68 No GLP Published	N	N		LIT
B.2.1.2/04 B.2.5/02	Ling K.S.	2007	Molecular characterization of two *Pepino mosaic virus* variants from imported tomato seed reveals high levels of sequence identity between Chilean and US isolates. Virus Genes 34. DOI: 10.1007/s11262-006-0003-x. No GLP Published	N	N		LIT
B.2.1.2/05	Ling K.S., Wintermantel W.M., Bledsoe M	2008	Genetic composition of *Pepino mosaic virus* population in North American greenhouse tomatoes. Plant Disease 92:1683-1688. DOI: 10.1094/PDIS-92-12-1683. No GLP Published	N	N		LIT
B.2.1.2/06	Ling K.S., Li R., Bledsoe M.	2013	*Pepino mosaic virus* genotype shift in North America and development of a loop-mediated isothermal amplification for rapid genotype identification. Virology Journal 10. DOI: 10.1186/1743-422x-10-117 No GLP Published	N	N		LIT
B.2.1.2/08	Maroon-Lago C.J., Guaragna M.A., Jordan R.L., Hammond J., Bandla M., Marquardt S.K.	2005	Two unique US isolates of *Pepino mosaic virus* from a limited source of pooled tomato tissue are distinct from a third (European-like) US isolate. Archives of Virology 150:1187-1201. DOI: 10.1007/s00705-005-0495-z No GLP Published	N	N		LIT
B.2.1.2/09	Moreno-Pérez M.G., Pagán I., Aragón-Caballero L., Cáceres F., Fraile A., García-Arenal F.	2014	Ecological and genetic determinants of *Pepino mosaic virus* emergence. Journal of virology 88:3359-3368. No GLP Published	N	N		LIT

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B.2.1.2/10	Mumford R.A. and Metcalfe E.J.	2001	The partial sequencing of the genomic RNA of a UK isolate of *Pepino mosaic virus* and the comparison of the coat protein sequence with other isolates from Europe and Peru. Archives of Virology 146. DOI: 10.1007/s007050170015. No GLP Published	N	N		LIT
B.2.1.2/11 B.2.2.1/03	Pagan I., Córdoba-Sellés M.d.C., Martínez-Priego L., Fraile A., Malpica J.M., Jordá C., García-Arenal F.	2006	Genetic structure of the population of *Pepino mosaic virus* infecting tomato crops in Spain. Phytopathology 96:274-279 No GLP Published	N	N		LIT
B.2.1.2/12	Pospieszny H., Hasiów B., Borodynko N.	2008	Characterization of two distinct Polish isolates of *Pepino mosaic virus*. European Journal of Plant Pathology 122. DOI: 10.1007/s10658-008-9280-7 No GLP Published	N	N		LIT
B.2.1.2/13	Roggero P., Masenga V., Lenzi R., Coghe F., Ena S., Winter S.	2001	First report of *Pepino mosaic virus* in tomato in Italy. Plant Disease 3 No GLP Published	N	N		LIT
B.2.1.2/14 B.2.3/02	Soler S., Prohens J., Díez M.J., Nuez F.	2002	Natural occurrence of *Pepino mosaic virus* in *Lycopersicon* species in central and southern Peru. Journal of Phytopathology 150:49-53. DOI:10.1046/j.1439-0434.2002.00712.x. No GLP Published	N	N		LIT
B.2.1.2/15	van der Vlugt R.A.A., Stijger C.C.M.M., Verhoeven J.T.J., Lesemann D.E.	2000	First Report of *Pepino Mosaic Virus* on Tomato. Plant Disease 84:103. DOI: 10.1094/PDIS.2000.84.1.103C. No GLP Published	N	N		LIT
B.2.2.1/04 B.2.2.2/01 B.2.5/03	Ferguson G.	2001	Managment of *Pepino mosaic virus* in greenhouse tomatoes. Factsheet. Ministry of Agriculture, Food and Rural Affairs, Ontario. No GLP Published	N	N		LIT

Data point	**Author(s)**	**Year**	**Title** **Company Report No** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study** **Y/N**	**Data Protec-tion Claimed** **Y/N**	**Justification if data protection is claimed**	**Owner***
B.2.2.1/05	Hanssen I.M., Peter van Esse H., 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. DOI: 10.1104/pp.111.173906. No GLP Published	N	N		LIT
B.2.2.1/06	Hasiów-Jaroszewska B., Borodynko N., Jackowiak P., Figlerowicz M., Pospieszny H.	2011	Single mutation converts mild pathotype of the *Pepino mosaic virus* into necrotic one. Virus research 159:57-61 No GLP Published	N	N		LIT
B.2.2.1/07	Hasiów-Jaroszewska B., Paeleman A., Ortega-Parra N., Borodynko N., Minicka J., Czerwoniec A., Thomma B.P., Hanssen I.M.	2013	Ratio of mutated versus wild-type coat protein sequences in Pepino mosaic virus determines the nature and severity of yellowing symptoms on tomato plants. Molecular Plant Pathology 14:923-933. No GLP Published	N	N		LIT
B.2.2.1/08	Hull R.	2014	Plant Virology. Chapter 3, pp 63-91 Academic press, San Diego, CA No GLP Published	N	N		LIT
B.2.2.1/09	Soler-Aleixandre S., López C., Díez M.J., de Castro A.P., Nuez F.	2005	Association of *Pepino mosaic virus* with tomato collapse. Journal of Phytopathology 153:464-469. DOI: 10.1111/j.1439-0434.2005.01002.x No GLP Published	N	N		LIT
B.2.2.1/10 B.2.2.2/02 B.2.5/04	van der Vlugt R.	2009	*Pepino mosaic virus*. Hellenic Plant Protection Journal 2:47-56 No GLP Published	N	N		LIT
B.2.2.2/03	Gal-On A., Shiboleth Y.M.	2006	Cross-protection, in: G. Loebenstein and J. P. Carr (Eds.), Natural Resistance Mechanisms of Plants to Viruses. Springer Netherlands, Dordrecht. pp. 261-288 No GLP Published	N	N		LIT

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B.2.2.2/04	McKinney H.H.	1929	Mosaic diseases in the Canary Islands, West Africa and Gibraltar. Journal of Agricultural Research. 39:577-578. No GLP Published	N	N		LIT
B.2.2.2/05	Natsuaki T.	2012	Viral attenuation and cross protection to control plant viral diseases Food and Fertilizer Technology Center. No GLP Published	N	N		LIT
B.2.2.2/06	Pennazio S., Roggero P., Conti M.	2001	A history of plant virology. Cross-protection. The New Microbiologica 24:99-114. No GLP Published	N	N		LIT
B.2.2.2/07	Sherwood J.L.	1987	Mechanisms of cross-protection between plant virus strains. Plant resistance to viruses:136-150. No GLP Published	N	N		LIT
B.2.2.2/08	Tatineni S., French R.	2006	The coat protein and Nla protease of two *potyviridae* family members independently confer superinfection exclusion. Journal of Virology 90:10886-10905. DOI: 10.1128/jvi.01697-16 No GLP Published	N	N		LIT
B.2.2.2/09	Zhang X.F., Sun R., Guo Q., Zhang S., Meulia T., Halfmann R., Li D., Qu F.	2017	A self-perpetuating repressive state of a viral replication protein blocks superinfection by the same virus. PLOS Pathogens 13:e1006253. DOI: 10.1371/journal.ppat.1006253. No GLP Published	N	N		LIT
B.2.3/03	Agüero J.	2017b	Study of the presence of *Pepino mosaic virus* (PepMV) on alternative and potential non-tomato host plants. Abiopep S.L., Spain Report number ABP03/2017 No GLP Not published	N	N		Abiopep S.L.
B.2.3/04	Blystad D.R., Vlugt R., Alfaro-Fernandez A., Cordoba M.D., Bese G., Hristova D., Pospieszny H., Mehle N., Ravnikar M., Tomassoli L., Varveri C., Nielsen S.L.	2015	Host range and symptomatology of *Pepino mosaic virus* strains occurring in Europe. European Journal Plant Pathology 143. DOI: 10.1007/s10658-015-0664-1 No GLP Published	N	N		LIT

Data point	**Author(s)**	**Year**	**Title** **Company Report No** **Source (where different from company)** **GLP or GEP status** **Published or not**	**Verte-brate study** **Y/N**	**Data Protec-tion Claimed** **Y/N**	**Justification if data protection is claimed**	**Owner***
B.2.3/05	Córdoba M.C., Martínez-Priego L., Jordá C	2004	New natural hosts of *Pepino mosaic virus* in Spain. Plant Disease 88:906. DOI: 10.1094/PDIS.2004.88.8.906D No GLP Published	N	N		LIT
B.2.3/06	Davino S., Accotto G.P., Masenga V., Torta L., Davino M.	2009	Basil (*Ocimum basilicum*), a new host of *Pepino mosaic virus*. Plant Pathology 58:407. DOI: 10.1111/j.1365-3059.2009.02026.x No GLP Published	N	N		LIT
B.2.3/07	Fakhro A., von Bargen S., Bandte M., Büttner C., Franken P., Schwarz D.	2011	Susceptibility of different plant species and tomato cultivars to two isolates of *Pepino mosaic virus*. European Journal of Plant Pathology 129:579-590. DOI: 10.1007/s10658-010-9722-x. No GLP Published	N	N		LIT
B.2.3/08	Jordá C., Perez A.L., Martínez-Culebras P., Abad P., Lacasa A., Guerrero M.	2001	First report of *Pepino mosaic virus* on tomato in Spain. Plant Disease 85:1292 No GLP Published	N	N		LIT
B.2.3/09	Martin J., Mousserion C.	2002	Potato varieties which are sensitive to the tomato strain of *Pepino mosaic virus* (PepMV). La Défense des Végétaux (France), Phytoma. No GLP Published	N	N		LIT
B.2.3/10	Papayiannis L.C., Kokkinos C.D., Alfaro-Fernández A.	2012	Detection, characterization and host range studies of *Pepino mosaic virus* in Cyprus. European Journal of Plant Pathology 132:1-7. DOI: 10.1007/s10658-011-9854-7 No GLP Published	N	N		LIT
B.2.3/11	Peralta I., Spooner D.	2000	Classification of wild tomatoes: a review. Kurtziana 28:45-54 No GLP Published	N	N		LIT
B.2.3/12	Prohens J., Rodríguez-Burruezo A., Nuez F.	2005	Utilization of genetic resources for the introduction and adaptation of exotic vegetable crops: The case of pepino (*Solanum muricatum*). Euphytica 146:133-142. DOI: 10.1007/s10681-005-3882-3 No GLP Published	N	N		LIT

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B.2.3/13	Verhoeven J.T.J., van der Vlugt R.A.A., Roenhorst J.W.	2003	High similarity between tomato isolates of *Pepino mosaic virus* suggests a common origin. European Journal of Plant Pathology 109:419-425. DOI: 10.1023/a:1024261121468. No GLP Published	N	N		LIT
B.2.3/14	Werkman A., Sansford C.	2010	Pest Risk Analysis for *Pepino mosaic virus* for the EU. Deliverable Report 4.3. EU Sixth Framework Project Project PEPEIRA. No GLP Published	N	N		LIT
B.2.4	Gergerich R.C., Dolja V.V.	2006	Introduction to plant viruses, the invisible foe. The Plant Health Instructor. No GLP Published	N	N		LIT
B.2.5/05	Agüero J.	2017c	Study to evaluate the storage stability and shelf life of the Microbial Pest Control Product AbioProtect® and its components *Pepino mosaic virus* (PepMV), EU strain, mild isolate App1 and PepMV, CH2 strain, mild isolate Abp2. Abiopep S.L., Spain. Report number: ABP04/2017 No GLP Not published	N	N	Proprietary information	Abiopep S.L.
B.2.5/06	Alfaro-Fernández A., Del Carmen Córdoba-Sellés M., Herrera-Vásquez José Á., Cebrián M.d.C., Jordá C	2010	Transmission of *Pepino mosaic virus* by the fungal vector *Olpidium virulentus*. Journal of Phytopathology 158:217-226. DOI: 10.1111/j.1439-0434.2009.01605.x No GLP Published	N	N		LIT
B.2.5/07	Céspedes A.J.	2015a	Evaluación de diferentes desinfectantes con y sin solarización para la desinfección de sacos de sustrato de fibra de coco de un cultivo de tomate inoculado con PePMV. Estación Experimental Las Palmerillas (El Ejido, Almería), Spain. Report number: LPA/2015-23/S GEP Not published	N	N		LIT
B.2.5/08	Córdoba-Selles M.d.C., García-Rández A., Alfaro-Fernández A., Jordá-Gutiérrez C.	2007	Seed transmission of *Pepino mosaic virus* and efficacy of tomato seed disinfection treatments. Plant Disease 91:1250-1254. DOI: 10.1094/PDIS-91-10-1250 No GLP Published	N	N		LIT

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B.2.5/09	Hanssen I.M., Mumford R., Blystad D., Cortez I., Hasiów-Jaroszewska B., Hristova D., Pagán I., Pereira A., Peters J., Pospieszny H., Ravnikar M., Stijger I., Tomassoli L., Varveri C., van der Vlugt R., Nielsen S.L.	2010b	Seed transmission of *Pepino mosaci virus* in tomato Eur J Plant Pathol (2010) 126:145–152 No GLP Published	N	N		LIT
B.2.5/10	Krinkels M.	2001	PepMV causes sticky problem. Prophyta, May 2001:30-33 No GLP Published	N	N		LIT
B.2.5/11	Lacasa A., Guerrero Díaz M.M., Hita I., Martínez M.A., Jordá C., Bielza P., Contreras J., Alcázar A., Cano A.	2003	Implicaciones de los abejorros (*Bombus* spp.) en la dispersión del virus del mosaico del pepino dulce (*Pepino mosaic virus*) en cultivos de tomate. Boletín de sanidad vegetal. Plagas 29 No GLP Published	N	N		LIT
B.2.5/12	Mayne S., O'Neill T.	2017	*Pepino mosaic virus* of tomato – new results on strains, symptoms and persistence. Protected Edibles. ADAS, UK No GLP Published	N	N		LIT
B.2.5/13	Mehle N., Gutiérrez-Aguirre I., Prezelj N., Delić D., Vidic U., Ravnikar M.	2014	Survival and transmission of *Potato virus Y*, *Pepino mosaic virus*, and Potato Spindle Tuber Viroid in Water. Applied and Environmental Microbiology 80:1455-1462. DOI: 10.1128/aem.03349-13 No GLP Published	N	N		LIT
B.2.5/14	O'Neil T., Spence N., Mumford R., Skelton A.	2003	Final Report on project PC 181: Protected tomato: sources, survival and disinfection of *Pepino mosaic virus* (PepMV) ADAS/CSL, UK No GLP Published	N	N		LIT
B.2.5/15	Prats C.	2017a	Field study to evaluate the crop safety and the efficacy of the Plant Protection Product (PPP) AbioProtect, and its components or agents (PPA1 and PPA2), for the control of PepMV in tomato crop (Southern Spain, 2016). Agrocolor S.L., Spain Report Number ACEX/1274/AB GEP Not published	N	Y	Proprietary information	Abiopep S.L.

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B.2.5/16	Prats C.	2017b	Field study to evaluate the crop safety and the efficacy of the Plant Protection Product (PPP) AbioProtect, and its components or agents (PPA1 and PPA2), for the control of PepMV in tomato crop (Southeast Spain, 2016). Agrocolor S.L., Spain. Reprot number: ACEX/1277/AB GEP Not published	N	Y	Proprietary information	Abiopep S.L
B.2.5/17	Schwarz D., Beuch U., Bandte M., Fakhro A., Büttner C., Obermeier C.	2010	Spread and interaction of *Pepino mosaic virus* (PepMV) and *Pythium aphanidermatum* in a closed nutrient solution recirculation system: effects on tomato growth and yield. Plant Pathology 59:443-452. DOI: 10.1111/j.1365-3059.2009.02229.x No GLP Published	N	N		LIT
B.2.5/18	Shipp J.L., Buitenhuis R., Stobbs L., Wang K., Kim W.S., Ferguson G	2008	Vectoring of *Pepino mosaic virus* by bumble-bees in tomato greenhouses. Annals of Applied Biology 153:149-155. DOI: 10.1111/j.1744-7348.2008.00245.x No GLP Published	N	N		LIT
B.2.5/19	Spence N.J., Basham J., Mumford R.A., Hayman G., Edmondson R., Jones D.R.	2006	Effect of *Pepino mosaic virus* on the yield and quality of glasshouse-grown tomatoes in the UK. Plant Pathology 55:595-606. DOI: 10.1111/j.1365-3059.2006.01406.x. No GLP Published	N	N		LIT
B.2.5/20	Stobbs L., Greig N., Weaver S., Shipp L., Ferguson G	2009	The potential role of native weed species and bumble bees (*Bombus impatiens*) on the epidemiology of *Pepino mosaic virus*. Canadian Journal of Plant Pathology 31:254-261 No GLP Published	N	N		LIT
B.2.5/21	Wright D., Mumford R.	1999	*Pepino mosaic Potexvirus* (PepMV): first records in tomato in the United Kingdom. Central Science Laboratory. No GLP Published	N	N		LIT
B.2.6/02	Welter S., Dölle S., Lehmann K., Schwarz D., Weckwerth W., Worm M., Franken P.	2013	*Pepino mosaic virus* infection of tomato affects allergen expression, but not the allergenic potential of fruits. PloS one 8:e65116. No GLP Published	N	N		LIT

*LIT: LITERATURE

RMS LITERATURE ADDED

Hanssen, I. M., Paeleman, E., Vandewoestijne, L., Van Bergen, C., Bragard, B., Lievens, AC., Vanachter and Thomma, J. 2009. Pepino mosaic virus isolates and differential symptomatology in tomato. Plant Pathology. 58, 450–460.

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