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Study on alternative, non-tomato host plants of *Pepino mosaic virus*

Pepino mosaic virus (PepMV) is a *Potexvirus* that causes disease in greenhouse tomato crops worldwide. Outbreaks of PepMV in other crops than tomato have not been reported, and according to literature the virus has a rather narrow host range that is thought to be largely restricted to species of the *Solanaceae* family (Hanssen & Thomma, 2010). However, a study performed in Spain suggests that the virus can survive in a large spectrum of weeds and that weeds could thus be an important reservoir for the virus (Córdoba et al., 2004). Therefore, an additional study on alternative host plants of PepMV was performed in the Belgian tomato production area. This study was performed in the framework of a research project on PepMV in tomato crops in Belgium, with funding from the Flemish government (IWT project number 60669) and conducted by a consortium of three research institutes (Scientia Terrae, Proefstation voor de Groenteelt and Proefcentrum Hoogstraten).

Part 1. Sampling of weeds, ornamental plants and other food crops in and around greenhouses with PepMV (CH2 strain) infected tomato crops

During the tomato growing season of 2008, samples from different plant species (weeds, crops, ornamentals) growing in close vicinity to tomato plants infected with Pepino mosaic virus (PepMV), strain CH2, were collected and analysed to assess the risk of PepMV survival in, and transmission through, non-tomato host plants. Three different types of plant samples were collected:

1. Weeds growing around greenhouses with commercial tomato crops infected with the CH2 strain of PepMV (Table 1).

2. Ornamental plants growing in entrances, packaging halls or barns of greenhouses with commercial tomato crops infected with the CH2 strain of PepMV (Table 2).

3. Horticultural crops (non-tomato) growing in greenhouses of research stations with PepMV (CH2 strain) infected tomato crops (Table 3).

All samples were analysed for the presence of PepMV. After phenol-chloroform based RNA extraction, PepMV detection was performed using DAS-ELISA (Agdia Inc., Elkhart, USA) and RT-PCR with PepMV specific primers [redacted] DAS-ELISA is a moderately sensitive, robust method that detects the viral coat protein, while the RT-PCR assay is a highly sensitive method that detects the viral RNA after exponential amplification of the viral genome.

Samples that tested positive by DAS-ELISA and/or RT-PCR were analysed to verify the viability of the virus particles using a bioassay, i.e. inoculation of tomato test plants and subsequent ELISA analysis. An overview of the samples and the obtained results is provided in tables 1, 2 and 3.

Table 1: PepMV analyses on weeds sampled around commercial, PepMV (CH2) infected glasshouse tomato crops

Greenhouse location	Weed	Sampling location	ELISA	RT-PCR
Sint-Katelijne-Waver	*Agropyron repens*	next to composting pile	neg	pos
Duffel	*Capsella bursa-pastoris*	close to greenhouse frontage	neg	neg
Duffel	*Chenopodium album*	close to greenhouse frontage	neg	neg
Duffel	*Chenopodium album*	next to composting pile	neg	neg
Duffel	*Chenopodium bonus-henricus*	next to composting pile	neg	neg
Duffel	*Circium arvense*	next to composting pile	neg	pos
Sint-Katelijne-Waver	*Circium arvense*	close to greenhouse front	neg	neg
Sint-Katelijne-Waver	*Echinochloa crus-galli*	close to greenhouse front	neg	neg
Duffel	*Epilobium palustre*	no details available	neg	neg
Sint-Katelijne-Waver	*Epilobium palustre*	next to composting pile	neg	neg
Sint-Katelijne-Waver	*Epilobium palustre*	greenhouse frontage	neg	neg
Sint-Katelijne-Waver	*Equisetum arvense*	greenhouse frontage	neg	neg
Duffel	*Erigeron canadensis*	close to greenhouse frontage	neg	pos
Sint-Katelijne-Waver	*Erigeron canadensis*	close to greenhouse frontage	neg	neg
Duffel	*Galinsoga parviflora*	close to greenhouse frontage	neg	neg
Sint-Katelijne-Waver	*Galinsoga parviflora*	close to greenhouse frontage	neg	neg
Duffel	*Gallium aparine*	next to composting pile	neg	neg
Sint-Katelijne-Waver	*Gallium aparine*	next to composting pile	neg	pos
Sint-Katelijne-Waver	*Gallium aparine*	greenhouse frontage	neg	pos
Sint-Katelijne-Waver	*Heracleum sphondylium*	next to composting pile	neg	pos
Sint-Katelijne-Waver	*Lamium purpureum*	greenhouse frontage	neg	neg
Duffel	*Lotus corniculatus*	no details available	neg	neg
Sint-Katelijne-Waver	*Lysimachia* sp.	greenhouse frontage	neg	neg
Duffel	*Matricaria recutita*	next to composting pile	neg	neg
Duffel	*Matricaria recutita*	next to composting pile	neg	neg
Duffel	*Medicago lupulina*	next to composting pile	neg	pos
Sint-Katelijne-Waver	*Medicago lupulina*	greenhouse frontage	neg	pos
Duffel	*Gramineae* - mixture	next to composting pile	neg	pos
Sint-Katelijne-Waver	*Oxalis europaea*	greenhouse frontage	pos	neg
Duffel	*Plantago lanceolata*	greenhouse frontage	neg	neg
Sint-Katelijne-Waver	*Plantago lanceolata*	next to composting pile	neg	pos
Duffel	*Plantago major*	next to composting pile	neg	pos
Sint-Katelijne-Waver	*Plantago major*	greenhouse frontage	neg	neg
Duffel	*Polygonum aviculare*	close to greenhouse frontage	neg	pos
Sint-Katelijne-Waver	*Polygonum aviculare*	next to composting pile	neg	neg
Sint-Katelijne-Waver	*Polygonum mite*	greenhouse frontage	neg	neg
Duffel	*Polygonum persicaria*	next to composting pile	neg	neg
Duffel	*Polygonum persicaria*	next to composting pile	neg	neg
Sint-Katelijne-Waver	*Polygonum persicaria*	greenhouse frontage	neg	neg
Sint-Katelijne-Waver	*Pteridium aquilinum*	next to composting pile	neg	neg

Duffel	*Ranunculus acris*	next to composting pile	neg	neg
Sint-Katelijne-Waver	*Ranunculus repens*	greenhouse frontage	neg	neg
Duffel	*Rorippa sylvestris*	close to greenhouse frontage	neg	neg
Sint-Katelijne-Waver	*Rorippa sylvestris*	greenhouse frontage	neg	neg
Duffel	*Rumex obtusifolius*	next to composting pile	neg	neg
Sint-Katelijne-Waver	*Rumex obtusifolius*	greenhouse frontage	neg	neg
Duffel	*Senecio vulgaris*	no details available	neg	neg
Sint-Katelijne-Waver	*Senecio vulgaris*	greenhouse frontage	neg	pos
Sint-Katelijne-Waver	*Solanum nigrum*	next to composting pile	neg	pos
Sint-Katelijne-Waver	*Sonchus oleraceus*	greenhouse frontage	neg	neg
Duffel	*Sonchus oleratius*	next to composting pile	neg	neg
Sint-Katelijne-Waver	*Stellaria media*	greenhouse frontage	neg	neg
Duffel	*Symphytum officinale*	no details available	neg	neg
Duffel	*Taraxacum* sp.	next to composting pile	neg	neg
Sint-Katelijne-Waver	*Taraxacum* sp.	greenhouse frontage	neg	neg
Duffel	*Trifolium repens*	next to composting pile	neg	pos
Duffel	*Urtica dioica*	next to composting pile	neg	neg
Sint-Katelijne-Waver	*Urtica dioica*	next to composting pile	neg	pos
Sint-Katelijne-Waver	*Urtica dioica*	composting pile	neg	pos
Sint-Katelijne-Waver	*Verbasicum sp.*	greenhouse frontage	neg	pos
Duffel	*Vicia cracca*	close to greenhouse frontage	neg	neg
Sint-Katelijne-Waver	***Vicia cracca***	close to greenhouse frontage	**pos**	**pos**
Duffel	*Zea mais*	next to composting pile	neg	neg
Sint-Katelijne-Waver	*Convolvulus* sp.	In the greenhouse	neg	neg

From a total of 54 samples, 18 tested positive by the highly sensitive RT-PCR method but not by ELISA, and only one (*Vicia cracca*) was positive by both ELISA and RT-PCR (Table 1). Unfortunately there was not enough material from the positive *Vicia cracca* sample to perform a bioassay, so it was not possible to check the viability of detected virus particles. Attempts to collect additional infected *Vicia cracca* leaf material failed because new samples all tested negative. It could thus not be verified whether *Vicia cracca* can function as a PepMV reservoir or not. The 18 samples that tested positive by RT-PCR but not by ELISA probably contained a very low concentration of PepMV particles. RT-PCR is highly sensitive and can detect down to a few viral particles, while the sensitivity of ELISA is 100 to 1000 times lower. The viral particles that were detected by RT-PCR could have been present as an external contamination on the analysed leaf tissue, without actual replication in the plant cell, or they could have been present inside the plant cells with a very limited replication. Therefore, it is highly unlikely that these weeds are suitable hosts for PepMV and by consequence it is unlikely that they would serve as a reservoir for PepMV dissemination.

Table 2: PepMV analyses on ornamental plants sampled from entrance halls/barns of diverse PepMV (CH2) infected glasshouse tomato crops

Ornamentals/weeds	Sampling location	ELISA	RT-PCR	Bioassay
Ficus sp.	greenhouse entrance	neg	neg	n.d.
Calla sp.	greenhouse entrance	neg	neg	n.d.
Musa sp.	greenhouse entrance	neg	neg	n.d.
Non-determined fern type	greenhouse entrance	neg	neg	n.d.
Nerium oleander	greenhouse entrance	neg	neg	n.d.
Amaryllis sp.	greenhouse entrance	neg	pos	neg

All six samples collected from ornamental plants tested negative by ELISA and only one sample, from *Amaryllis*, tested positive by RT-PCR (Table 2). That sample was used in a bioassay to inoculate tomato plants , but those plants remained negative, indicating that the detected virus particles were not viable anymore or that the concentration was too low for transmission. Probably this was an external contamination of PepMV particals on the leaf surface of the *Amaryllis* plant. Based on these results it seems very unlikely that non-solanaceous ornamentals in vicinity to PepMV infected tomato crops would serve as a reservoir for PepMV dissemination.

Table 3: PepMV analyses on horticultural crops (non-tomato) growing in greenhouses of research stations with PepMV (CH2 strain) infected tomato crops

Greenhouse location	Crop	Sampling location	ELISA	RT-PCR	Bioassay
Hoogstraten	Pepper - blocky type	In the greenhouse	neg	pos	neg
Sint-Katelijne-Waver	Pepper - blocky type	In the greenhouse	neg	neg	neg
Sint-Katelijne-Waver	Pepper - conical type	In the greenhouse	neg	pos	neg
Sint-Katelijne-Waver	**Pepper – chilli type**	In the greenhouse	**pos**	**pos**	**pos**
Sint-Katelijne-Waver	**Eggplant**	In the greenhouse	**pos**	**pos**	**pos**
Sint-Katelijne-Waver	Cucumber	In the greenhouse	neg	neg	n.d.
Sint-Katelijne-Waver	Lettuce	In the greenhouse	neg	neg	n.d.
Sint-Katelijne-Waver	Squash	In the greenhouse	neg	neg	n.d.
Hoogstraten	Strawberry	In the greenhouse	neg	neg	n.d.
Sint-Katelijne-Waver	Beans	Open field around greenhouse	neg	neg	n.d.

From the 10 samples obtained from other horticultural crops growing in close vicinity to a PepMV (CH2) infected tomato crop, three tested positive by RT-PCR, two of which were also positive by ELISA: chili pepper and eggplant, two solanaceous crops. Both samples tested also positive in the bioassay, indicating that the samples contained a sufficiently high amount of viable virus particles to cause infection in test plants. Interestingly, RT-PCR results obtained for pepper samples varied with the type of pepper: only the chilli type could be identified as a host for PepMV, even though the plants didn't show any viral symptoms. The blocky pepper types were mostly negative and the conical type tested positive by RT-PCR but not by ELISA or bio-assay. The eggplant sample contained

a high concentration of virus (ELISA value close to saturation), but the plants didn't display any viral symptoms. These results confirm that only solanaceous crops can be suitable hosts for PepMV, and that even within this family big differences in susceptibility exist depending on the plant species and on the genetic background of the variety. None of the tested crops, not even the positive eggplant and pepper plants, showed any virus symptoms.

CONCLUSIONS

Altogether these results confirm that the natural host range of PepMV is largely restricted to solanaceous plants. In glasshouse production in Belgian conditions, the risk for dissimination of PepMV through weeds appears to be very limited. The discrepancy with the results obtained in Spain could be linked to difference in the tomato cropping system, where Spanish growers often have open field production and greenhouse production of tomato on one site. The risk for PepMV transmission through non-solanaceous ornamentals growing close to PepMV infected crops was also shown to be limited or even absent, although the amount of ornamentals tested was rather low. It was not possible to sample more ornamentals, as most tomato growers avoid the presence of ornamentals in packaging areas and entrance halls/barns because of the risk for viroid transmission. Also therefore, the risk for transmission through ornamentals is limited. Non-solanaceous vegetable crops grown in close vicinity to tomato plants with PepMV were negative and could thus be excluded as natural hosts for PepMV. Only eggplant contained a high concentration of PepMV particles, which could be detected by ELISA and RT-PCR and confirmed by a bioassay. Eggplant can thus be considered as an efficient but asymptomatic host for PepMV. The results obtained for pepper were somewhat less clear, with most varieties testing negative and only one variety, a chili pepper type, being positive by both RT-PCR and bioassays but not by ELISA .

It can be concluded that the risk for weeds, ornamentals or non-tomato (and even non-solanaceous) crops to suffer damage from PepMV infection, or to function as reservoir for PepMV transmission, is extremely limited.

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Part 2. Artificial inoculations of different varieties of pepper and potato

As the results obtained in Part 1 confirm that the host range of PepMV is mainly restricted to solanaceous crops, the susceptibility of two economically important solaneceous crops for PepMV was studied in more detail through artificial inoculations: pepper and potato. Eggplant was not included in this study since the results above already show that eggplant is a good but asymptomatic host of PepMV.

Six different varieties of pepper, covering the most important pepper types, were sown and grown in climate chambers. In the seedling stage, they were artificially inoculated with an aggressive CH2 isolate of PepMV using carborundum powder to damage the leaf surface. Twelve days after inoculation, root and leaf samples from all six varieties were analysed for PepMV using ELISA and RT-PCR (Table 4).

Table 4: different pepper varieties analysed for PepMV by RT-qPCR after artificial inoculation with the CH2 strain (aggressive isolate) strain of PepMV

Pepper type	Variety	Root analysis RT-PCR	Leaf analysis RT-PCR
Blocky	Roxy	neg	neg
Blocky	Derby	pos	neg
Conical	Gepetto	neg	neg
Conical	Palermo	pos	neg
Conical	Danubia	neg	neg
Chili	Fireflame	pos	neg

ELISA analyses were negative on all samples; RT-PCR results are shown in Table 4. All leaf samples tested negative by RT-PCR, so none of the tested pepper varieties was systemically infected with the CH2 isolate, although three varieties displayed low concentrations of virus in the roots (Table 4). None of the pepper varieties showed any viral symptoms.

It can be concluded that pepper, in general, is not a host for PepMV, although some varieties may (locally) contain low amounts of viral particles. As none of the varieties displayed any viral symptoms, the risk for damage of PepMV infection in pepper crops is extremely limited.

Twelve different varieties of potato were grown in experimental greenhouses in Louvain-La-Neuve, Belgium. Five plants per variety were artificially inoculated with an aggressive CH2 isolate of PepMV by (1) gentle rubbing and (2) rubbing with carborundum powder. Three weeks after inoculation, leaf samples from individual plants from all twelve varieties were analysed for PepMV using ELISA. In total 120 individual potato plants were analysed. Results are presented in Table 5. This study was performed [redacted] in the framework of the research project PRAVEG, with funding from the Belgian federal government.

Table 5: different potato varieties analysed for PepMV by ELISA three weeks after artificial inoculation with the CH2 strain of PepMV (aggressive isolate) – tests performed [redacted]

Variety	Inoculation through rubbing	Inoculation with carborundum powder
Charlotte	pos (2/5)	neg
Victoria	neg	pos (2/5)
Agria	neg	neg
Fontane	neg	neg
Astérix	neg	neg
Ramos	pos (1/5)	neg
Bintje	neg	neg
Franceline	neg	neg
Nicola	neg	neg
Désirée	neg	neg
Aviance	neg	neg
Prologue	neg	neg

On a total of 120 potato plants, only 5 tested positive for PepMV by ELISA three weeks after inoculation. None of these plants showed any viral symptoms. Two varieties gave two positive plants out of 10, and one variety gave one positive plant out of 10. These results show that the susceptibility of potato to PepMV is very low and that it is highly unlikely that PepMV would cause damage in potato crops.

CONCLUSIONS

Artificial inoculation of different pepper and potato varieties showed that nor pepper nor potato is a suitable host for PepMV. Some varieties may (locally) contain a low amount of viral particles, but no viral symptoms could be observed and none of the tested varieties tested positive for PepMV in all the analysed plants or plant parts. **Therefore, none of the tested pepper or potato varieties is expected to become systemically infected by PepMV under natural conditions.**

REFERENCES

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[redacted] on the 26th of June 2012.

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