PepMV (CH2 strain) persistence in soil

Content

Scientia Terrae *performs research based on the on this moment leading scientific views and knowledge. Since the PPP analysed in this study is a plant virus to be used for crossprotection or vaccination purposes, and no such products have previously been registered as PPP in Europe, no specific guidelines or reference protocols could be followed. Scientia Terrae used all its knowledge and expertise in the domain of Plant Virology to perform this study. The study was conducted with uttermost care, following internal quality standards. Scientia Terrae will not accept any responsibility for possible damage which is directly or indirectly the consequence of analyses, judgments or recommendations made in this report.*

1. STUDY DETAILS

PRODUCT:

TOMATO WATERY LEAF EXTRACT CONTAINING PepMV, CH2 STRAIN, ISOLATE 1906

10.1.c Wob juncto 63.2.d Vo 1107/2009

GOAL OF THE STUDY:

The goal of the presented study is to determine the persistence in soil of the PPP [redacted] PEPMV, CH2 STRAIN, ISOLATE 1906', a biological product containing a mild CH2 variant of *Pepino mosaic virus* (PepMV) to be used as a plant vaccine to protect tomato plants from infection with other variants of PepMV, CH2 strain. The PPP is sprayed at high pressure to infect tomato plants with this mild PepMV isolate. Contact of the virus particles with the soil is very limited in case of soilless cultures, which constitutes the majority of the greenhouse tomato crops in Central and North Europe, but is expected to be more relevant in Southern Europe were cultivation of tomato in the soil is more common. Therefore, in this study, the persistence and viability of the virus particles in the soil is investigated. Laboratory experiments were set-up in Scientia Terrae Research Institute and a greenhouse trial was performed at the commercial, biological tomato production company [redacted] Two different greenhouses at the same site, both [redacted] were included in the studies. In both greenhouses, tomatoes (variety Capricia, Rijkzwaan) were grown for approximately 11 months, following commercial production standards. Although the PPP application was performed within the 120 days authorisation period of PMV-01 (PPP [redacted] PEPMV, CH2 STRAIN, ISOLATE 1906') for the 2012-2013 [redacted] the trial was done with a trial permit because the submitted dossier focuses on substrate cultures.

10.1.c Wob juncto 63.2.a Vo 1107/2009 juncto 39.2.a Vo 178/2002

BATCH SPECIFICATIONS:

Laboratory experiments:

Production date: [redacted]

Batch number: [redacted]

Commercial greenhouse (bio) experiment:

Production date [redacted]

Batch number: [redacted]

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APPLICATION:

For the laboratory experiments, the PPP was applied into soil (potting mix) in a ratio of 1 mL product per 10 g soil.

For the commercial greenhouse (bio) experiment, the PPP was sprayed on young tomato plants following the indications provided on the label of the commercial product. As the plants were cultivated in soil, part of the product fell onto the soil around the treated plants. Two different greenhouses at the same production site (both owned by [redacted] were included in the study. The greenhouses were treated on the 26th December 2012.

STORAGE CONDITIONS:

For the laboratory experiments, after homogenization, the soil containing the above mentioned amount of PPP was stored at room temperature (app. 20°C) and at 4°C.

For the commercial greenhouse (bio) experiment, the temperature in the greenhouse was controlled following commercial tomato production standards throughout the season.

TIME POINTS

For the laboratory experiments: 3 samples for each storage temperature were taken at 1, 3, 7, 14, 31, 38 and 52 days after the time of application.

For the commercial greenhouse experiment: 3 samples were taken at two different depths (0-5 cm and 15-20 cm) in each greenhouse at 8 months after the time of application. Sampling date: August 29th, 2013. Samples taken by the study director.

2. PART A: Persistence of PepMV in soil in laboratory experiments

2.1. ANALYSES

- **Determination of virus concentration using a TaqMan RT-qPCR assay (Gutierrez-Aguilar et al., 2009)**
- **Bioassays (inoculation of test plants) to check the infectivity and viability of the viral particles in the PPP that was applied into the soil, using a sample of the soil as inoculum.**

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Per time point, 2 tomato plants [redacted] were inoculated. Two methods were used to prepare the inoculum: i) the soil was suspended in phosphate buffer in a ratio 1:1 (g/mL) and the mixture was used directly for inoculation; ii) the soil was suspended in phosphate buffer in a ratio 1:2 (g/mL), this mixture was centrifuged subsequently, the supernatant was used for inoculation. A mixed sample from each set of two plants was taken at 7 days after inoculation. All samples were analysed for presence and concentration of virus particles using the above mentioned TaqMan RT-qPCR assay.

A TaqMan RT-qPCR assay results in Ct values (threshold detection cycles). Note that the Ct value is inversely correlated with the virus concentration: the higher the Ct-value, the lower the virus concentration. [redacted] **The standard curve and the details of the method are provided in the technical dossier (Tier II, Document M-MCPA, IMM 1.4.1).**

Reference

Gutiérrez-Aguirre I. et al., 2009. Real-time quantitative PCR based sensitive detection and genotype discrimination of Pepino mosaic virus. Journal of Virological Methods 162 : 46-55.

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2.2. RESULTS

2.2.1. Determination of PepMV concentration in the soil using the TaqMan RT-qPCR assay.

The PepMV concentration in the soil was verified at 8 months after application of the PPP into the soil, using a TaqMan RT-qPCR assay (Gutiérrez-Aguirre et al., 2009). The results are presented graphically in Figure 1.

[redacted]

Until 14 days, the Ct values remained betwee[redacted] After 31 days onwards the virus concentration starts to decrease slightly and gradually (increase in Ct-values). During the experiment, the virus concentration remains slightly higher when the soil is maintained at 4°C. At 52 DPA (days after application), the Ct-values are abov[redacted]r both temperatures, which indicates that the virus is no longer reliably detectable.

2.2.2. Bioassays to check the infectivity of the soil containing PepMV and the viability of the virus particles

After the application of the PPP into the soil, the infectivity of the soil containing PepMV was verified at different time points. As the soil stored at 4°C was considered the most relevant for this test (virus concentration/infectivity is expected to be higher), this treated soil was used for inoculation on tomato test plants. Tomato seedlings were inoculated with samples from the treated soil and analysed for PepMV (CH2 strain) infection by TaqMan RT-qPCR. Two tomato plants [redacted] were inoculated manually as previously described (2.1). At 7 days after inoculation, from each set of two plants, a sample was taken. The results are presented in Figure 2. All samples tested negative using the above mentioned TaqMan RT-qPCR assay. Therefore, inoculation at later time points was considered irrelevant. These results imply that the virus particles detected with the direct TaqMan RT-PCR assay (2.2.1.) were not viable anymore, or that the amount of viable particles that persisted in the soil was not sufficient to cause infection.

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2.3. CONCLUSION

The analyses of the soil samples after addition of the PPP in these laboratory experiments reveal that a small amount of viral particles is detected both upon storage at 4 and 20 °C. The concentration of virus particles decreases after 31 days at both temperatures. A slightly higher virus concentration remains detectable when the product is stored at 4°C (Figure 1). However, the bioassays with the treated soil samples show that, although the virus is detected by direct RT-qPCR assays, the remaining virus particles are no longer viable or the amount of remaining viable particles is not enough to cause infection (Figure 2). The exposure of the PPP to the soil in the experiment was a lot higher than the exposure that can be expected in normal conditions during application of the PPP in a tomato crop grown in the soil. Therefore, based on these results, it can be concluded that there is no risk for PepMV dissemination through or persistence in the soil when the PPP is used as a plant vaccine in tomato crops.

3. Part B: Persistence of PepMV in soil in commercial greenhouse (bio) experiments

10.1.c Wob juncto 63.2.a Vo 1107/2009 juncto 39.2.a Vo 178/2002

3.1. ANALYSES

- **Determination of virus concentration using a TaqMan RT-qPCR assay (Gutierrez-Aguilar et al., 2009)**

A TaqMan RT-qPCR assay results in Ct values (threshold detection cycles). Note that the Ct value is inversely correlated with the virus concentration: the higher the Ct-value, the lower the virus concentration. [redacted] he standard curve and the details of the method are provided in the technical dossier (Tier II, Document M-MCPA, IMM 1.4.1).

Reference

Gutiérrez-Aguirre I. et al., 2009. Real-time quantitative PCR based sensitive detection and genotype discrimination of Pepino mosaic virus. Journal of Virological Methods 162 : 46-55.

3.2. RESULTS

3.2.1. Determination of PepMV concentration in soil using the TaqMan RT-qPCR assay after application of the PPP as plant vaccine in commercial greenhouse conditions.

The PepMV concentration in soil samples taken at two different depths at 8 months after the application of the PPP to tomato plants in a commercial, biological tomato production site was verified using a TaqMan RT-qPCR assay (Gutiérrez-Aguirre et al., 2009). In total, 12 samples were analysed: 3 replications of each depth at each greenhouse. The results are presented in Figure 3. All the samples tested negative [redacted] Therefore, further testing using bio-assays, as described above, was deemed irrelevant.

[redacted]

10.1.c Wob juncto 63.2.a Vo 1107/2009 juncto 39.2.a Vo 178/2002

3.3. CONCLUSION

The analyses of the soil samples taken at 8 months after application of the PPP containing a mild isolate of PepMV in commercial greenhouse (bio) conditions, in order to protect tomato plants against a secondary infection with other variants of PepMV, reveal that no virus particles are detectable. Based on these results, it can be concluded that there is no risk for transmission of the virus from the PPP from one cropping cycle to the next through contaminated soil.

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Study director: [redacted]

Study technicia[redacted]

Report finalized on 17/11/2013

Signature of study direct[redacted]