Scientia Terrae
RESEARCH INSTITUTE

PepMV (CH2 strain) persistence in water

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:

[redacted] **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 water 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. As limited fractions of the product might enter the irrigation system of a tomato crop through the recirculated drainage water, the persistence and viability of the virus particles in water is investigated.

BATCH SPECIFICATIONS:

Production date: [redacted]

Batch numbe [redacted]

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

APPLICATION:

The PPP was applied to water (distilled water) in a ratio of 1 mL product per 10 mL water.

STORAGE CONDITIONS:

After homogenization, the water containing the above mentioned amount of PPP was stored at room temperature (app. 20°C) and at 4°C.

TIME POINTS

Three samples for each storage temperature were taken at 1, 3, 7, 14, 31, 38, 52 and 90 days after application of the PPP to the water.

2. Persistence of PepMV in water

2.1. ANALYSES

- **Determination of virus concentration using a TaqMan RT-qPCR assay (Gutierrez-Aguilar et al., 2009)**
- **Bioassays (inoculation of test plants) using the water samples treated with the PPP to check the infectivity and viability of the viral particles deposited in the water.**

[redacted]

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

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).**

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

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.

2.2. RESULTS

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

The PepMV concentration in the water samples was verified at different time points after application of the PPP to the water, using a TaqMan RT-qPCR assay (Gutiérrez-Aguirre et al., 2009). The results are presented graphically in Figure 1.

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

When the water treated with the PPP is stored at 20°C and 4°C, the virus concentration remains more or less stable for 7 days. From 14 days onwards the virus concentration starts to decrease slightly and gradually (increase in Ct-values) if the treated water is stored at 20°C. However, the virus concentration remains stable until 38 days when the treated water is stored at 4°C.

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

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

The infectivity of the water after treatment with the PPP was verified at different time points. Tomato seedlings were inoculated and analysed for PepMV (CH2 strain) infection by TaqMan RT-qPCR. Two tomato plants [redacted] were inoculated manually as previously described. At 7 days after inoculation, from each set of two plants, a sample was taken. The results are presented graphically in Figure 2.

[redacted]

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

When the treated water stored at 20°C was used for inoculation no infection was obtained after a storage period of only 3 days. Also on later time points, inoculations from treated water stored at 20°C remained negative. However, upon storage at 4°C, the treated water remains infectious for a period of at least 7 days, although a clear decrease can be seen between 3 and 7 days. Between 7 and 90 days, no bio-assays were performed, but after 90 days the treated water stored at 4°C was not infectious anymore, especially when the treated water samples were stored at 20 °C. Storage at 4°C results in a longer persistence of viable PepMV particles (between 7 and 90 days) but a water

temperature of 4°C in the greenhouse irrigation system is not in line with common practice in greenhouse tomato crops.

2.3. CONCLUSION

The analyses of the water samples after addition of the PPP reveal that a small amount of viral particles remains detectable both upon storage at 4 and 20 °C after the first days. The concentration of virus particles decreases after 7 days when the product is stored at 20°C. When the product is stored at 4°C, the virus concentration remains stable until 38 days. However, bio-assays on test plants reveal that the detected viral particles are not infectious anymore after 3 days when the product is stored at 20°C and after a period between 7 and 90 days when the product is stored at 4°C. All these results imply that the risk of virus transmission through the water is rather limited. The hygienic measures adopted in commercial greenhouse (eg. use of UV-light) will prevent further spreading of the virus to nearby crops.

10.2.e

Study director: [redacted]

Study technician [redacted]

Report finalized on 14/11/2013

Signature of study director [redacted]