Scientia Terrae
RESEARCH INSTITUTE

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

Storage stability study

[redacted] PepMV, CH2 strain, isolate 1906

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

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GOAL OF THE STUDY:

The goal of the presented study is to determine the storage stability of the PPP '[redacted]PEPMV, CH2 STRAIN, ISOLATE 1906', a biological product containing a mild 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 the efficiency of the PPP depends largely on the infectivity and thus on the concentration of virus particles and the viability/infectivity thereof, both parameters were analysed upon storage at different storage conditions.

BATCH SPECIFICATIONS:

Production date: [redacted]

Batch number [redacted]

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STORAGE CONDITIONS:

Stored in final product packaging material (sealed brown PET bottles)

PART A: Cold storage

Treatment 1: Fridge 4°C (2-8°C)

Treatment 2: Freezer -18°C

PART B: Storage at room temperature

Treatment 3: Room temperature 20°C

TIME POINTS

PART A: [redacted]

PART B: [redacted]

**DAP: days after production*

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2. PART A: Cold storage

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 of the product and the viability of the virus particles**
  - **Using the concentrated product**
  - **Using 10^{-1} and 10^{-2} dilutions from the product (on time points 1 and 35 DAP):**

Bioassay: [redacted]

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

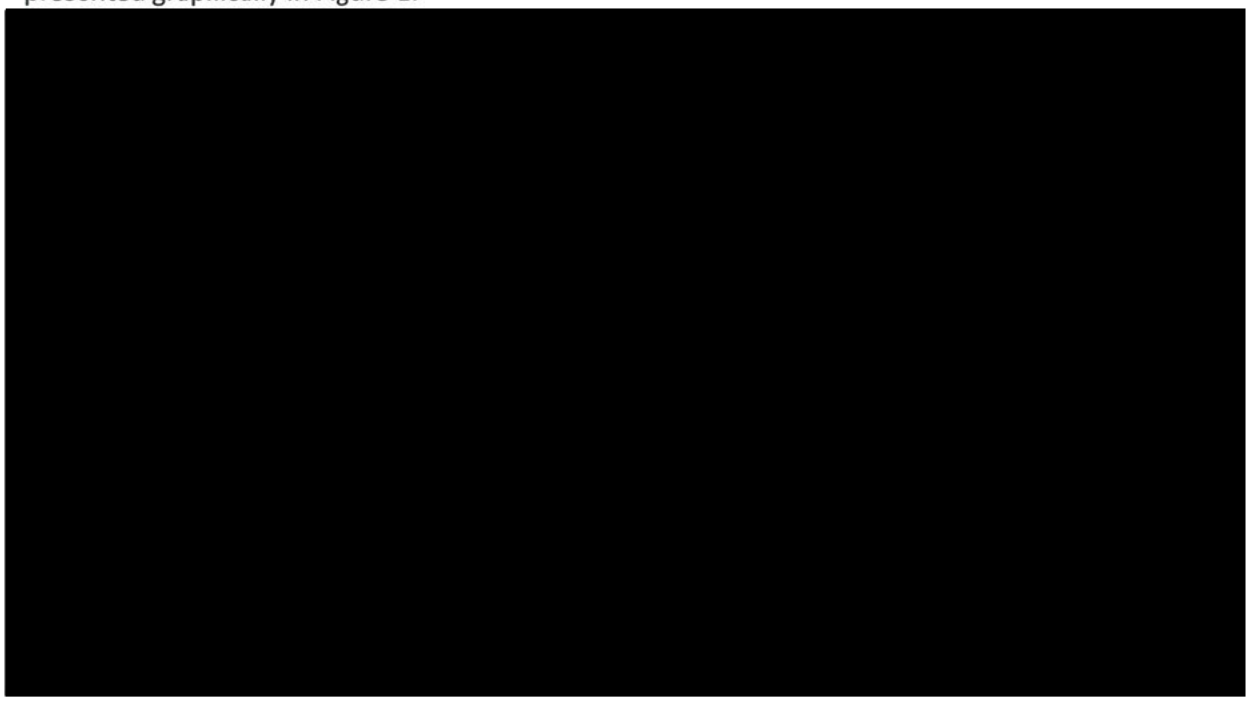
Gutiérrez-Aguirre I. et al., 2009. Real-time quantitative PCR based sensitive detection and genotype discrimination of Pepino mosaic virus. Journal of Virological Methods 162 : 46-55.

2.2. RESULTS

2.2.1. Determination of PepMV (CH2 strain) concentration in the concentrated product using the TaqMan RT-qPCR assay.

The PepMV concentration in the concentrated product was verified during storage using a TaqMan RT-qPCR assay (Gutiérrez-Aguirre et al., 2009). The Ct value of the product has to be [redacted] (approx. $5*10^5$ viral genome copies per µl product) in order to guarantee a good efficiency of the

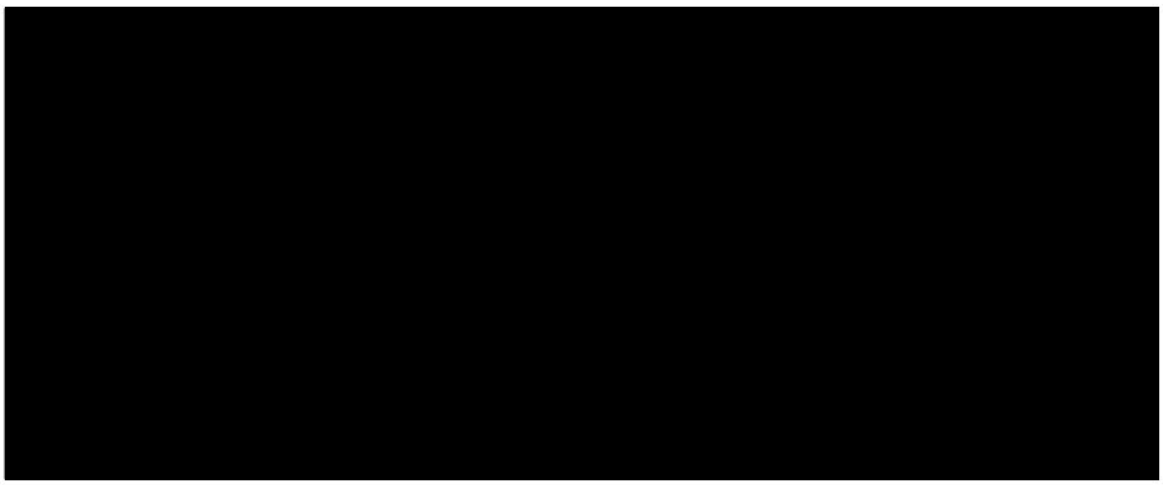
PepMV CH2 vaccine (cf. technical dossier, Tier II, Document M-MCPA, IMM 1.4.1). The results are presented graphically in Figure 1.



Until [redacted] the Ct values are below [redacted] mplying that the virus concentration is in line with the defined criteria. From [redacted] onwards the virus concentration starts to decrease slightly and gradually (increase in Ct-values) upon storage at [redacted] Upon storage at [redacted] he virus concentration appears to remain stable until [redacted]

2.2.2. Bioassays to check the infectivity of the product and the viability of the virus particles

(a) <u>Using the concentrated product</u>



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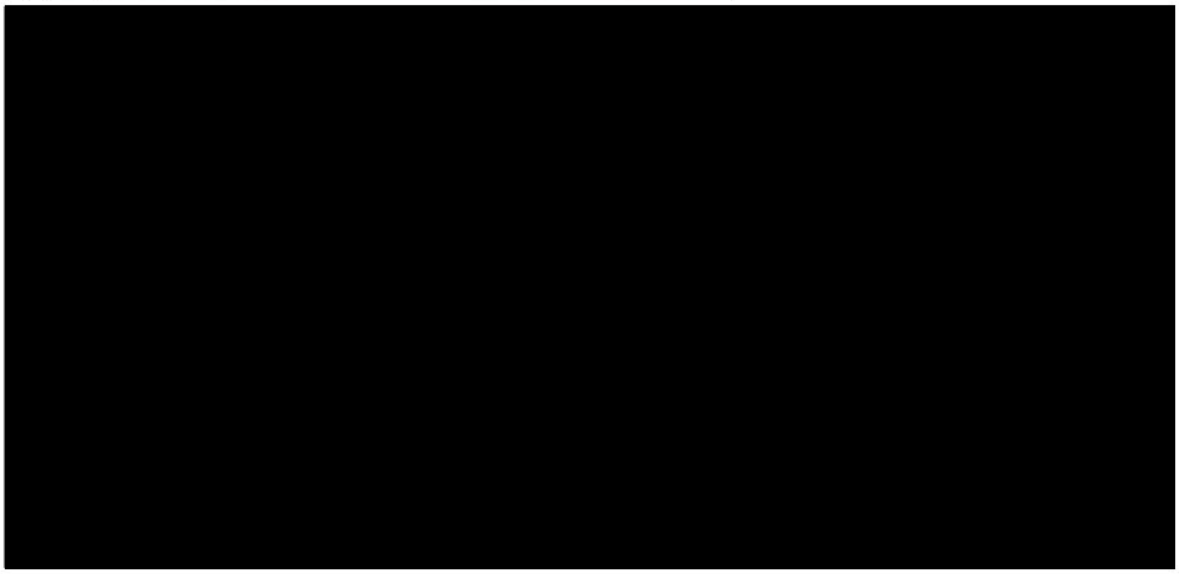
When sampled and analysed at 7 days post inoculation all test plants from all storage time points (up till [redacted] showed Ct-values below [redacted] implying a high virus titre in the test plants. These results show that, up till [redacted] the concentrated product remains highly infectious.

(b) Using 10^{-1} and 10^{-2} dilutions of the concentrated product



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[redacted]

[redacted]

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All test plants inoculated at 1DAP showed high virus concentrations after 7 days, regardless of the storage conditions (Figure 3 and 4). No difference in infectivity could be seen between the non-diluted and the diluted products. However, upon storage at [redacted] test plants inoculated at [redacted] with [redacted] dilutions of the concentrated product showed lower virus concentrations (Figure 3). Using the [redacted] dilution as inoculums, the plants were still negative 7 days after inoculation [redacted] These results imply that, although the concentrated product remains infectious (Figure 2) and the number of virus particles in the product remains high up till [redacted] (Figure 1), the viability of the virus particles has decreased at [redacted] Upon storage [redacted] test plants inoculated at [redacted] with the [redacted] dilution of the concentrated product showed high viral titres (Figure 4), with a Ct value similar to the one obtained for the concentrated product. Also the [redacted] dilution was still infectious but the virus concentration in the test plants was lower [redacted] Figure 4), indicating that also here the viability of the virus particles has decreased. [redacted] Therefore an additional storage stability test, including bioassays with a 10^{-2} dilution of the concentrated product, was initiated but the results are not yet available.

2.3. CONCLUSION:

The bioassays with the concentrated product show that the infectivity remains high until the end of the experimental period [redacted] Figure 2). Also the concentration of virus particles in the concentrated product remaines stable up till [redacted] upon storage at [redacted] (Figure 1). [redacted]

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The bioassays with [redacted] dilutions of the concentrated product were only performed at [redacted] so there is no information on a possible decrease in viability of virus particles between 1 and [redacted] Therefore an additional storage stability test, including bioassays with [redacted] dilutions of the concentrated product, was initiated but the results are not yet available. [redacted] **As soon as the results of the additional storage stability experiment are available, this conclusion will be reevaluated.**

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3. Part B: storage at room temperature

3.1. 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 of the product using the concentrated product**

[redacted]

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

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3.2. RESULTS

3.2.1. Determination of PepMV (CH2 strain) concentration in the concentrated product using the TaqMan RT-qPCR assay.

The PepMV concentration in the concentrated product was verified during storage using a TaqMan RT-qPCR assay (Gutiérrez-Aguirre et al., 2009). The Ct value of the product has to be lower than [redacted] (approx. $5*10^5$ viral genome copies per µl product) in order to guarantee a good efficiency of the PepMV CH2 vaccine (cf. technical dossier, Tier II, Document M-MCPA, IMM 1.4.1). Over a 35 days periods, no significant changes in Ct value upon storage at room temperature could be seen. The Ct values remained lower than [redacted] over the entire test period. The results are presented graphically in Figure 5.

[redacted]

3.2.2. Bioassays to check the infectivity of the product using the concentrated product

[redacted]

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When sampled and analysed at [redacted] post inoculation all test plants from all storage time points (up till [redacted] showed low Ct-values implying a high virus titre in the test plants. These results show that the concentrated product remains infectious up till [redacted] when stored [redacted]

3.3. CONCLUSION:

The bioassays with the concentrated product show that the infectivity remains high until the end of the experimental period [redacted] igure 6) when stored [redacted] Also the concentration of virus particles in the concentrated product remained stable up [redacted] when stored at [redacted] (Figure 5). However, bioassays using dilutions of the concentrated, stored product where not performed, so no information is available on a possible decrease in viability of the virus particles when the product is [redacted] Therefore an additional storage stability test, including bioassays with [redacted] dilutions of the concentrated product, was initiated but the results are not yet available. Although no decline in virus concentration or infectivity of the product was seen in this experiment, storage of this PPP at room temperature is not recommended. As soon as the results of the additional storage stability experiment are available, this conclusion will be reevaluated.

Study director [redacted]

Study technician [redacted]

Report finalized on 22/10/2011

Signature of study director:

[redacted]

Disclaimer:

Scientia Terrae *performs research based on the on this moment leading scientific views and knowledge. 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.*