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

Comparison of the infectivity of VC1 batches, with high and with low nicotine content

10.2.e

Author [redacted] (senior [redacted]

Date 10 April 2017

Document Bio-assay of VC1 batches with low and high nicotine content, 7 April 2017

Period 2016

[redacted]

Introduction

During the registration procedure it was necessary to decrease the nicotine content in the VC1-batches. By an additional purification step the nicotine concentration could be decreased to values below 0.1 mg/L. To test whether the VC1-batches with low nicotine content are still effectively infectious, VC1 batches with low and high nicotine content were compared for their infectivity.

Aim

Comparison of the PepMV-infectivity of five VC1 batches with low nicotine content (< 0.1 mg/L) and five VC1 batches with high nicotine content (> 20 mg/L). All ten batches contained phosphate as buffer.

Trial site and period

The infectivity tests were conducted in greenhouses of Groen Agro Control in Delfgauw, the Netherlands in 2016.

Treatments:

Batches of VC1 were isolated from tobacco plants without further purification step (high nicotine content) and also purified (low nicotine content). The buffer of the solution was phosphate. The batches contained 0.001-0.005% PepMV isolate VC1. Shortly before treatment, batches were diluted 50-times in phosphate buffer and carborundum was added to the solution as an abrasive. Application was made by rubbing (bio-assay PepMV, method Groen Agro Control, SPV A519).

Tomato seedlings of the variety Brioso of approx. 18 days old were used in the trial. Plants were kept on rock wool plugs with regular tomato nutrient solution. The temperature in the greenhouse was kept at 20°C.

Used batches:

Five batches of VC1 with low nicotine content (<0.1 mg/L):

- Batch VC1-A-150616
- Batch VC1-B-150616
- Batch VC1-C-150616
- Batch VC1-D-150616
- Batch VC1-E-150616

Five batches of VC1 with low nicotine content (> 20 mg/L):

- Batch VC1-190516
- Batch VC1-200716
- Batch VC1-050716
- Batch VC1-120916
- Batch VC1-300916

Assessment

14 Days (± 2 days) after inoculation, seedlings were tested separately by ELISA to determine the percentage of infected plantlets. This percentage is a measure for the infectivity of PepMV in the sample (ELISA, method Groen Agro Control, SPV A517). According to "Operating procedure for the preparation of VX1 and VC1 batches" (Valto SOP1), the percentage of infected tomato seedlings in the bio-assay of 50-times diluted sample of a batch should be at least 70%.

[redacted]

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

Results

The VC1-batches with low nicotine show infectivities of 100%. Those with a high nicotine content also show infectivities of 100%.

Table 1. Infectivity of five batches VC1 with low nicotine content

Batch	Week of inoculation	Infectivity (%)
VC1-A-150616	Wk 30, 2016	100
VC1-B-150616	Wk 30, 2016	100
VC1-C-150616	Wk 30, 2016	100
VC1-D-150616	Wk 30, 2016	100
VC1-E-150616	Wk 30, 2016	100

Table 1. Infectivity of five batches VC1 with high nicotine content

Batch	Week of inoculation	Infectivity (%)
VC1-190516	Wk 20, 2016	100
VC1-200716	Wk 29, 2016	100
VC1-050716	Wk 30, 2016	100
VC1-120916	Wk 37, 2016	100
VC1-300916	Wk 40, 2016	100

Statistics

Table 3. Average infectivity and standard deviation (SD) of batches VC1 with low and high nicotine content.

Batch-type	Average Infectivity (%)	SD (%)
Low-nicotine	100	0
High-nicotine	100	0

Conclusion

The five batches VC1 with low nicotine content as well as the five batches VC1 with high nicotine content, meet the quality control requirement of an infectivity of at least 70%.