Netherlands Food and Consumer
Product Safety Authority
*Ministry of Economic Affairs*

**National Plant Protection Organization**
POBox 9102
6700 HC Wageningen
The Netherlands

# August 2015 PEST Report - THE NETHERLANDS

## 1.1 Finding of Strawberry crinkle virus (SCV) in *Fragaria* plants for planting, variety Fleurette, without symptoms

### 1.2 Executive summary

This report concerns the official finding of SCV in the Netherlands in propagation material of strawberry, in a relatively new variety "Fleurette". The infection was not associated with virus symptoms. The infection was discovered coincidentally, when at a propagation company strawberry plants of various varieties with a physiological disorder (caused by wrong nutrient components) were tested for viruses. The identity of the virus was confirmed by a conventional RT-PCR and a real-time RT-PCR (both validated) and by sequence analysis of the PCR product from an additional conventional RT-PCR on 18 August 2015. Testing results indicate infections in planting material of all grades of the certification system in the Netherlands (SEE, SE, EE and E) at various locations. Therefore, it is concluded that Fleurette has been infected by SCV for several years, probably already from the introduction of the variety in 2010. SCV is known to occur in the Netherlands but has not been recorded earlier in nuclear stock material and certification material at this magnitude.
Real-time RT-PCR testing of all nuclear stock (SEE) plants of all other varieties produced in the Netherlands, did not reveal any other infections with SCV nor with the other aphid-transmitted viruses: strawberry mottle virus, strawberry mild yellow edge virus and strawberry vein banding virus. Because no symptoms are observed in the variety Fleurette, it is presumed that this variety could be similarly affected in other countries in Europe, since EU requirements (Council Directive 2000/29/EC, as amended) do not include testing.
This pest report is issued because of the magnitude of the contamination. The pest status for the Netherlands does not change. The organism is listed as a harmful organism in annex IIAII of EU directive 2000/29/EC.
Identity of the pest Strawberry crinkle virus, genus *Cytorhabdovirus*
Categorization of the pest EU Annexes IIAII
Location**:** Various locations in the Netherlands
Reason of the notification: First report.
How the pest was found (6) information submitted by professional operator
Information on the infested area, severity and source of the outbreak
Millions of plants are infected.
Official phytosanitary measures
All affected propagation material is placed under official control, separated both physically and in terms of hygiene procedures from other propagation material of *Fragaria.* Affected plants can be used for fruit production, following additional visual checks whereby no symptoms are observed. All recipient companies will be informed of the probable contamination of the plant material with SCV.

**4. Reason of the notification and pest status**

4.1 Select: (2) appearance of the harmful organism in nuclear stock material of a specific variety of *Fragaria*, in which its presence was previously unknown.
Select: First report

**4.4 Current Pest status**
Select: (1) Present: in all parts of the area concerned.

**4.3 Previous Pest status**
Select: (1) Present: in all parts of the Member State concerned.

**1.3 Legal provisions – select**
(2) full notification

**3. Location of presence of harmful organism**
3.1 Many locations throughout the Netherlands. The organism is probably present at all locations where the variety Fleurette is cultivated, both in the Netherlands and in other EU countries where material is marketed in the last years.
3.2 Map of the location. Not relevant.

**5. Information relating to the finding.**
5.1 How the harmful organism was found.
Select: (6) information submitted by professional operator

5.2 Date of finding: 18 August 2015 (date of identity determination by sequencing).

5.3 submission of information concerning the sampling procedure for laboratory analysis, including date, method, and sample size.
Field lots of tray plants, young plants, mother plants of Fleurette have been sampled on 8 locations in the Netherlands (10 full grown leaves per sample) in the last week July/first week of August 2015 and these leaves were RT-PCR tested individually and shown to be positive (except for 2 plants ). The two individual Pre basic mother plants (SEE) of Fleurette were sampled (again full grown leaves) in the last week of July. Both plants tested positive and were removed from the screenhouse.
On 18 August, 2015, the identity of the virus was confirmed by sequencing.

5.4 the name and the address of the laboratory:
Naktuinbouw Laboratories
Contact person: Marcel Toonen (m.toonen@naktuinbouw.nl)
Sotaweg 22
2371 GD Roelofarendsveen
The Netherlands

NPPO – The Netherlands
National Reference Centre
Contact person: Ko Verhoeven (j.t.j.verhoeven@nvwa.nl)
P.O.Box 9102
6700 HC Wageningen
The Netherlands

5.5 Diagnostic method.
(1) According to peer reviewed protocol;
The RT-PCR protocol of Naktuinbouw is based on the scientific publication by Thompson et al. (2003) Multiplex RT-PCR detection of four aphid-borne strawberry viruses in *Fragaria* spp. in combination with a plant mRNA specific internal control. Journal of Virological Methods 111: 85-93.
The real time PCR protocol is developed in collaboration with FERA (UK) and will be published as: W. A. Monger, E.T.M. Meekes, V. Harju, M. Hooftman, R. Lawson, R. Mumford (in prep.). Development and inter-laboratory evaluation of real-time PCR assays for strawberry viruses of concern to European growers.
The RT-PCR product used for sequencing is based on the forward primer described by Thompson et al. (2003) and two reverse primers developed by Naktuinbouw.

5.6 Date of official confirmation of the harmful organism's identity
See 5.2.

**6. Information related to the area, severity of the finding and source of the finding**
6.1. Size and delimitation of the infested area.
(2) number of infested plants for planting: 10 million plants that are currently (2015) produced and marketed are probably contaminated. As far as can be expected on the basis of the test results also Fleurette plants marketed in the EU in the period 2012-2014 have been contaminated (many shipments/over 4 million plants).

6.2.Characteristics of the infested area and its vicinity.
(1) Open air – production area
(1.1) field (arable, pasture);
(3) Physically closed conditions
(3.1) greenhouse;

6.3. Host plants in the infested area and its vicinity.
*Fragaria x Ananassa*, variety Fleurette.

6.4. Infested plant(s), plant product(s) and other object(s). Indication of the scientific name of the infested host plant(s).
See 6.3.

6.5. Vectors present in the area.
The principal natural aphid vector *Chaetosiphon fragaefolii* is known to occur in the Netherlands but has not been observed in propagation material of Fleurette at the locations. Transmission of the virus within the variety has taken place by clonal propagation. No spread to other varieties has occurred as far as can be determined on the basis of testing of samples of other varieties in the vicinity of propagation material of Fleurette.

6.6. Severity of the outbreak. Description of the current extent of infestation, symptoms and the damage caused, and, where appropriate, inclusion of forecasts as soon as this information is available.

At least 10 million plants are probably contaminated. It is presumed that the entire variety Fleurette is affected. No visual symptoms are present in the crop so far, and symptoms have not been observed since the introduction of the variety 5 years ago. EFSA recently assessed the impact of SCV as minimal to minor. The infection was discovered coincidentally, when at a propagation company strawberry plants of various varieties with a physiological disorder (caused by wrong nutrient components) were tested for viruses.

6.7. Source of the outbreak. As applicable, indication of the confirmed pathway of the harmful organism into the area, or of the suspected pathway pending confirmation. Attachment of information concerning the confirmed or potential origin of the harmful organism is possible.

The source of the outbreak is unknown but is presumed to originate from a clonally related source. In 2010 some candidate plants of the variety Fleurette tested positive for SCV but have been discarded. Other candidate plants at that time tested negative (individually repetitive testing) which have been used for setting up nuclear stock of this variety. It is now presumed that one or two of those candidate plants which repeatedly tested negative, in fact contained the virus albeit in very low quantities. Testing pre basic motherplants in the last 5 years (annually on UC 5) did not show any positive reaction. Further investigations on these test results are on-going.

**7. Official phytosanitary measures**

7.1. Adoption of official phytosanitary measures.
(2) Official phytosanitary measures, other than measures in the form of chemical, biological or physical treatment, have been taken;
All affected propagation material is maintained under official control, separated both physically and in terms of hygiene procedures from other propagation material of *Fragaria.* Affected plants can be used for fruit production, following additional visual checks whereby no symptoms are observed. All recipient companies will be informed of the probable contamination of the plant material with SCV.

7.2. Date of adoption of the official phytosanitary measures. In case of temporary measures, indication of their expected duration.

Not relevant

7.3. Identification of the area covered by the official phytosanitary measures. Indication of the method used to identify the area covered by the official phytosanitary measures. In case surveys were carried out, the results of those surveys.
The measures have been applied to all *Fragaria* plants for planting intended for sale or propagation of the variety Fleurette.

7.4. Objective of the official phytosanitary measures. Indication of one of the following options: (1) eradication; (2) containment, in case eradication is impossible.

Objective of the measures is to prevent propagation of contaminated plants and to prevent contamination of other varieties of *Fragaria*.

7.5. Measures affecting the movement of goods.
(2) measures do not affect import into or movement within the Union of goods.

7.6. Specific surveys. In case surveys are carried out as part of official phytosanitary measures, indication of their methodology, duration and scope.
No specific survey is carried out.

**8. Pest risk analysis/assessment. [BURO]**
Indication of the following options: (1) Pest risk analysis is not required (harmful organism is listed in Annex II of Directive 2000/29/EC. (4) Pest risk analysis exists (EFSA journal 2015; 12(4):3630 [46 pp.]).

**9. Links to relevant websites, other sources of information.**

**References:**
NPPO The Netherlands
http://www.efsa.europa.eu/en/efsajournal/pub/3630