



January 2014 PEST Report - THE NETHERLANDS

National Plant Protection Organization
POBox 9102
6700 HC Wageningen
The Netherlands

Findings of *Erwinia* sp. 'assigned to the *E. pyrifoliae* taxon' in two production greenhouses of strawberry fruits (*Fragaria*)

Introduction

This report concerns the first official finding of *Erwinia* sp. "assigned to the *E. pyrifoliae* taxon" in two production greenhouses of strawberry fruits in the Netherlands. Symptoms were observed in June and in October 2013. The origin of the finding is unknown. The organism *Erwinia pyrifoliae* is not listed as a harmful organism in the annexes of EU directive 2000/29/EC and is not listed on the EPPO A1 or A2 list.

Erwinia pyrifoliae is closely related to the main fire blight pathogen *E. amylovora*. *E. pyrifoliae* was described for the first time in 1999 in Korea (Kim et al., 1999).

E. pyrifoliae is primarily a pathogen of Asian or Nashi pear (*Pyrus pyrifolia*) causing fire blight on Nashi pear and is considered to have a restricted geographic distribution in East Asia (Korea and Japan).

Thus far occurrence of this pathogen on strawberry has not been reported. However, similar symptoms on immature strawberry fruits were seen in 2011 in one field cultivation in Belgium during the survey for *X. fragariae* (personal communication with Johan van Vaerenbergh, ILVO). The results of the diagnostic research in Belgium were communicated in the project report but no formal communication to plant health authorities was done. The affected crops have been removed by the growers. Specific phytosanitary measures for the coming year concern a specific surveillance to investigate possible other occurrences and possible origin of the findings. There are no indications of the occurrence of *Erwinia* sp. "assigned to the *E. pyrifoliae* taxon" in propagation material of strawberry in the Netherlands, based on testing records of propagation material for bacterial diseases.

Reason for reporting First report

Identity of the pest *Erwinia* sp. "assigned to the *Erwinia pyrifoliae* sp. nov taxon", family Enterobacteriaceae.

Categorization of the pest none

Location: Two greenhouses at different locations.

Pest status

Present, only in some areas, only in protected cultivation.

Pest significance (detailed description)

Symptomatic strawberry plants were received in June and in October 2013 from two different locations in the Netherlands, showing intense blackening of their immature fruits, their fruit calyx and the attached stems (Figure 1 to 4). No symptoms on the leaves were observed. The discolouration was also obvious inside the young fruits which presented an intense darkening/blackening of the fruit tissue at the edges and an intense shining of the fruit tissue in the middle. Release of bacterial slime was additionally observed on the surface of the young fruits and their attached stems (ooze formation). Fruits were in many cases heavily misformed. In most of the cases these symptoms were present in the whole greenhouse and the incidence was high: each of about 50% of the plants had at least one symptomatic fruit.

Date of finding

Symptomatic material was received in June and in October 2013. The identity of the pathogen was confirmed on 23 December 2013 by the National Reference Center in the Netherlands.

Detection and identification

Direct isolation was performed by plating freshly prepared extract on YPG (Yeast Peptone Glucose) agar medium. Isolations yielded *Erwinia*-like colonies. Pure cultures of the *Erwinia*-like colonies were identified based on the requirements of EPPO: two tests based on different characteristics of the pathogen (combinations of biochemical and molecular tests) were used followed by a pathogenicity test on strawberry for verification.

Identification of these isolates as *Erwinia pyrifoliae* was based on molecular tests. The partial sequences of the 16S rRNA gene placed the isolates in the genus *Erwinia* and gave a high similarity (above 99%) with *Erwinia pyrifoliae*. A clear positive reaction in the specific real time PCR assay (Wensing et al, 2011) was obtained, similarly to the positive reaction of the *Erwinia pyrifoliae* reference strain LMG 25888. Additionally, the isolates differed biochemically from *E. amylovora* and they were very closely related to biochemical profiles of *E. pyrifoliae*. After purification, characterization of two isolates revealed Gram negative bacterial cells able to utilize sorbitol but unable to utilize gelatine, esculin and D-raffinose, similarly to the *Erwinia pyrifoliae* reference strain LMG 25888.

Pathogenicity was tested on strawberry plants (cvs. 'Elsanta'). Isolates were inoculated each onto three young fruitlets/plant by injecting a thick bacterial suspension (108 cfu/ml) into the base of the young fruitlet. As positive control treatments the *Erwinia pyrifoliae* reference strain LMG 25888 was inoculated onto the same number of young fruitlets/plant. A few inoculated young fruitlets developed similar symptoms to the one observed in the original samples we received. *Erwinia pyrifoliae* reference strain LMG 25888 gave comparable symptoms. The bacterium was re-isolated from symptomatic fruitlets and identity of the re-isolates was confirmed by the specific real time PCR assay (Wensing et al., 2011) indicating that *Erwinia pyrifoliae* had caused the disease.

Impact

Severity / extent of damage:

The immature strawberry fruits showing intense blackening on fruit tissue or on their fruit calyx and the attached stems (Figures 1 to 5) are totally unmarketable. Depending on the exact time infection occurs during the production of strawberry fruits, the economic losses can greatly vary from low (when infection occurs at the last stages of the cultivation for fruit production) to very high (when infection occurs at the initial stages of the cultivation for fruit production).

Host plants or articles concerned:

Fragaria – strawberry fruit production.

Type of plant or mode of cultivation: commercial crops - indoors

Origin of the pest

There are no indications on the possible origin of the pest. A multi locus sequence analysis is being conducted to determine the relationship between the strawberry isolates found in the Netherlands and Belgium and pear isolates from Korea and Japan. The availability of pear isolates from Korea and Japan is, however, rather limited in official bacterial collections in Europe.

Phytosanitary measures

Coming year a specific surveillance at strawberry fruit growers will be completed to investigate the occurrence of *E. pyrifoliae* in the Netherlands. Depending on the outcome of this surveillance, further phytosanitary measures will be considered. Since affected crops have already been removed no further phytosanitary measures at the affected fruit companies have been taken.

Figure 1 to 4: Intense blackening of the immature fruits, the fruit calyx and the attached stems of strawberry fruits in two greenhouses in the Netherlands.



References:

- NPPO The Netherlands
- Kim W-S, Gardan L, Rhim S-L, Geider K 1999 *Erwinia pyrifoliae* sp. nov., a novel pathogen that affects Asian pear trees (*Pyrus pyrifolia* Nakai). *Int J Syst Bacteriol* 49, 899-906.
- Wensing A, Gernold M, Geider K. (2011). Detection of *Erwinia* species from the apple and pear flora by mass spectroscopy of whole cells and with novel PCR primers. *Journal of Applied Microbiology* 112: 147-158.