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Erwinia pyrifoliae, a new pathogen on strawberry in the Netherlands[1]

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Abstract.
BACKGROUND: During the late spring of 2013 strawberry plants grown under protection (*Fragaria* x *ananassa* cv. Elsanta) were found at several locations in the Netherlands showing an intense brown to black discoloration of their immature fruits, their fruit calyx and the attached stems.
OBJECTIVE: Identification of a new bacterial disease on strawberry.
METHOD: Identification and characterization was based on the requirements of EPPO (European and Mediterranean Plant Protection Organization), followed by a pathogenicity test on strawberry plants for verification of the virulence.
RESULTS: The isolates exhibited biochemical profiles closely related to *Erwinia pyrifoliae* reference strain LMG 25888. The isolates were further identified as *E. pyrifoliae* based on the real time PCR assay. Pathogenicity of several isolates was tested and confirmed on potted strawberry plants (cvs. Elsanta and Selva).
CONCLUSION: *Erwinia pyrifoliae* is a pathogen on strawberry. Thus far occurrence of this pathogen on strawberry has not been reported nor its presence outside Asia.

Keywords: *Erwinia*, strawberry, bacterial disease, fire blight

1. Introduction

Worldwide strawberry (*Fragaria* x *ananassa*) is one of the most important commercially grown fruit crops [1]. Strawberry production within the European Union has decreased by approximately 6 percent during the last fifteen years despite the fact that the cultivation area of strawberry remained the same [1]. The decrease is mainly attributed to severe outbreaks of strawberry pests and diseases.

There are very few bacterial diseases known on strawberry. In 1962, Kennedy and King provided the first report of a bacterial disease on strawberry; *Xanthomonas fragariae*, the causal agent of bacterial angular leaf spot of strawberry in Minnesota, USA [2]. *Xanthomonas fragariae* was later found in New Zealand, Australia, a few Asiatic and African countries and in most European countries. *Xanthomonas fragariae* is considered the most important bacterial disease on strawberry, mainly due to its A2 quarantine status which effect the trade of nursery stock. The disease is widespread within nurseries and has been responsible for significant production losses [3].

In the 1990's, a new bacterial disease on strawberry called 'bacterial leaf blight' was observed in Italy, caused by a new pathogen *Xanthomonas arboricola* pv. *fragariae* [4, 5]. However, inconsistency in virulence of *Xanthomonas arboricola* pv. *fragariae* upon artificial inoculation on strawberry has been documented [6, 7]. Crop losses on strawberry as a result of *Xanthomonas arboricola* pv. *fragariae* infections were not significant [8]. The current genotypic classification of strawberry bacterial strains under the distinct pathovar *fragariae* within *X. arboricola* was recently demonstrated to be irrelevant [9].

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Another bacterial disease of strawberry is caused by *Pseudomonas solanacearum* (bacterial wilt), currently under the name *Ralstonia solanacearum,* occurs in nursery seedlings, but rarely observed in field grown strawberry plants intended for fruit production [10].

More recently, a finding of *Erwinia amylovora* on infected plants of *Fragaria ananassa* and *Fragaria moshata* was reported [11]. This bacterium is also the causative agent of fire blight, a disease of rosaceous plants that occurs in many countries around the world [12].

The present article describes a new bacterial disease on strawberry plants (*Fragaria* x *ananassa* cv. Elsanta) found at several locations in the Netherlands during the late spring in 2013. Strawberry plants expressed an intense brown to black discoloration of their immature fruits, their fruit calyx and the attached stems (Fig. 1A, 1B). There were no symptoms observed on the leaves. The discoloration was also observed inside the young fruits, including an intense shining of the fruit tissue. In several cases, the release of bacterial slime on the surface of the young fruits and their attached stems has been observed. Fruits did not develop at all or were in many cases heavily malformed. Crop losses up to 40% were found (Fig. 2A, 2B).

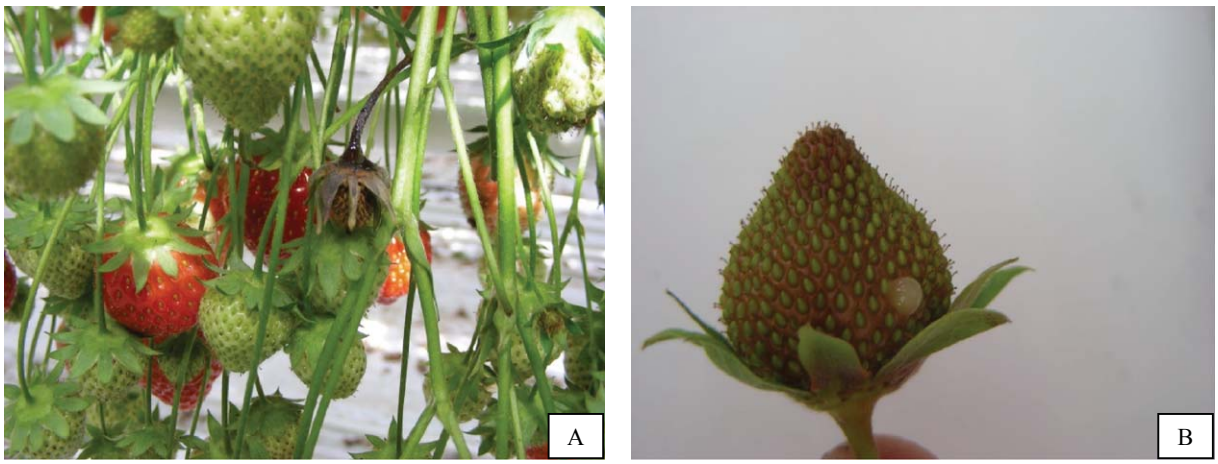

Fig. 1. Strawberry fruit (cv. Elsanta) grown in commercial holdings. Affected strawberry fruits showed intense blackening (A) and release of bacterial slime (B).

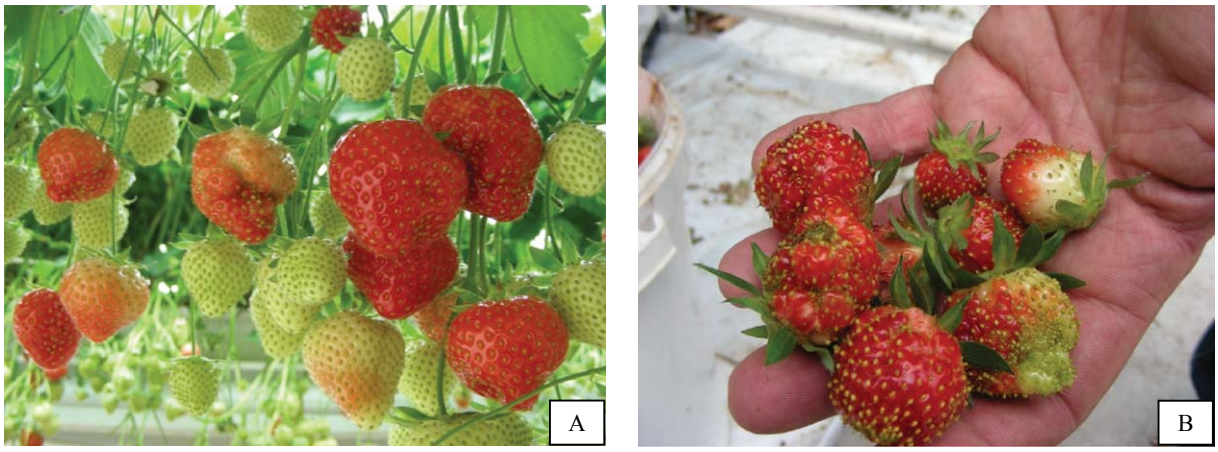

Fig. 2. (A) Strawberry fruit (cv. Elsanta) grown in commercial holdings. (B) Strawberry fruits were often heavily malformed.

2. Material and methods

2.1. Isolation and identification

Direct isolation was performed by plating tissue from the leading edge of infection of affected strawberry fruits on Yeast Peptone Glucose (YPG) agar medium. The plates were incubated for 2 to 3 days at 28°C. Pure cultures of bacterial colonies were identified based on the requirements of EPPO: application of two methodologies based on a different principle. In this study a combination of molecular and biochemical tests were used to determine species identity, followed by a pathogenicity test on strawberry plants for verification of the virulence. The specific real time PCR assay for *Erwinia pyrifoliae* [13], has been applied. A range of classical biochemical tests were applied including: gelatine hydrolysis, sorbitol, esculin and D-raffinose.

2.2. Pathogenicity

The pathogenicity of several isolates was tested on potted strawberry plants (cvs. Elsanta and Selva). Concurrently, strawberry plants were innoculated with the *Erwinia pyrifoliae* reference strain LMG 25888. Inoculation was carried out by injecting a thick bacterial suspension (10^8 cfu/mL) in the epicalyx of three immature strawberry fruits per plant (cv. Selva). Also, strawberry flowers and immature fruitlets were inoculated by spraying bacterial suspensions (10^8–10^9 cfu/mL) on the flowers/fruitlets and subsequently incubating the inoculated plants in a plastic bag for 24 hours (cv. Elsanta; Fig. 3).

Colonies of *Erwinia* spp. were reisolated from inoculated strawberry flowers and immature fruitlets from both cultivars (Selva and Elsanta) showing typical symptoms and their identity was confirmed by real time PCR [13].

3. Results

3.1. Isolation and identification

Isolations from symptomatic immature strawberry fruits yielded *Erwinia*-like colonies on YPG agar. Initially characterization of the isolates revealed Gram negative bacterial cells, giving a negative reaction on Levan, and on media containing pectine. Subsequent biochemical characterisation revealed that the bacteria were positive for sorbitol but negative for gelatine, esculin and D-raffinose. Consistent with the biochemical profiles of the *Erwinia pyrifoliae* reference strain LMG 25888. The isolates were further identified as *E. pyrifoliae* based on the real time PCR assay [13].

Fig. 3. Plants were sealed in a plastic bag for 24 h directly after inoculation.

3.2. Pathogenicity

Infection was rapid following inoculation of with a bacterial suspension resulting in oily necrosis with abundant formation of exudate on the epicalyx and on the sepals. The symptoms that developed on fruitlets inoculated with the test isolates and the *E. pyrifoliae* reference strain LMG 25888 were similar to the symptoms observed on the origional samples three to four weeks following inoculation (Fig. 4A, 4B). Spray inoculation of strawberry flowers resulted in necrosis without ooze formation (Fig. 5A, 5B). The bacterium was reisolated from the leading edge of infection from symptomatic fruitlets and the identity of the reisolations was confirmed by PCR, indicating that *Erwinia pyrifoliae* had caused the disease.

4. Discussion

4.1. Erwinia pyrifoliae

Erwinia pyrifoliae is closely related to the main fire blight pathogen *E. amylovora*. The pathogen *E. pyrifoliae* was decribed for the first time in 1999 in Korea [14, 15]. *E. pyrifoliae* is primarily a pathogen of Asian or Nashi pear

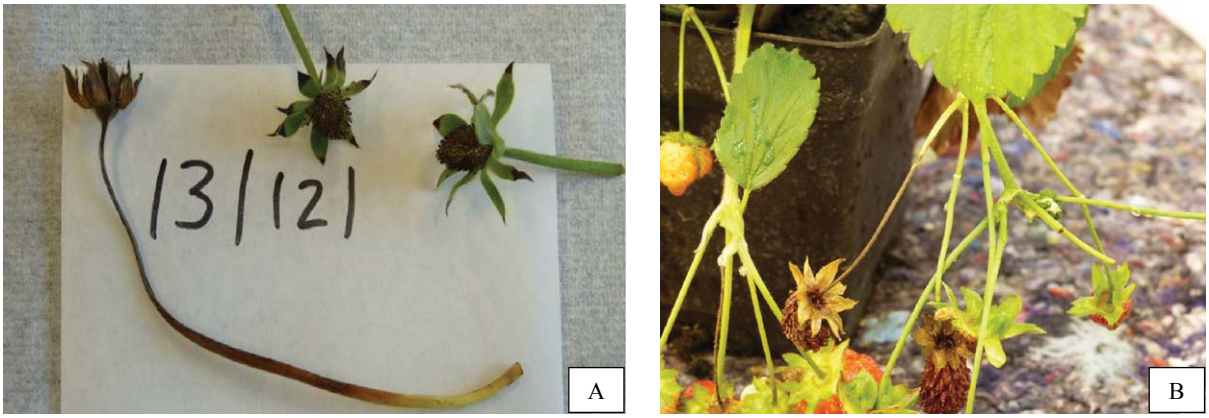

Fig. 4. (A) Young fruitlet inoculation (cv. Selva) with *Erwinia pyrifoliae* reference strain LMG 25888 at 7 dpi. (B) Young fruitlet inoculation (cv. Selva) with *Erwinia pyrifoliae* test isolate at 21 dpi.

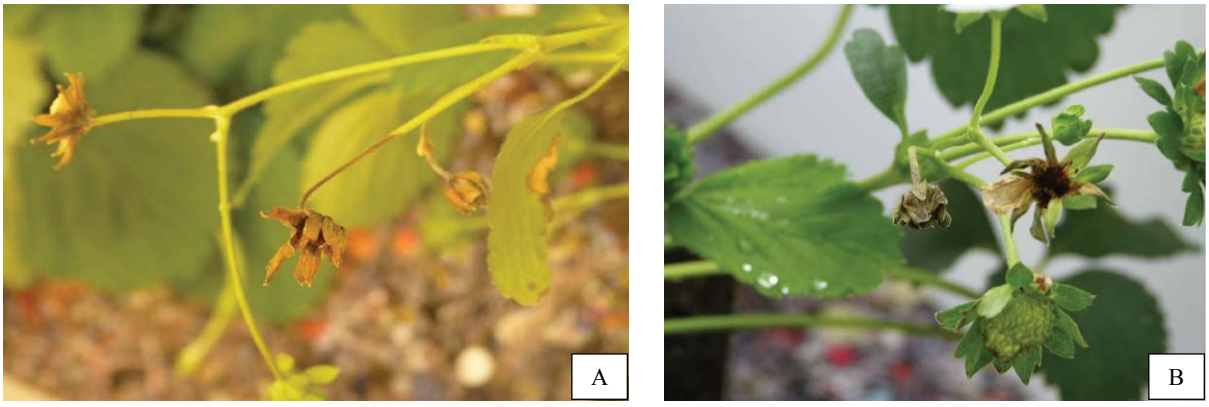

Fig. 5. (A) Flower inoculation (cv. Elsanta) with *Erwinia pyrifoliae* reference strain LMG 25888 at 6 dpi. (B) Flower inoculation (cv. Elsanta) with *Erwinia pyrifoliae* test isolate at 6 dpi.

(*Pyrus pyrifolia*) causing fire blight on Nashi pear and is considered to have a restricted geographic distribution in East Asia (Korea and Japan). However, the host range of *E. pyrifoliae* has been reported to be broader than *Pyrus pyrifolia* [15]. Although the occurrence of this pathogen on strawberry or indeed its presence has not been reported outside Asia, its real distribution could be rather uncertain because specific surveys for *E. pyrifoliae* are seldom [16].

4.2. Origin of the disease

There are no indications on the possible origin of the disease. A multilocus sequence analysis (MLSA) is currently underway to determine the relationship between isolates found on strawberry in the Netherlands and isolates from pear in Korea and Japan. The availability of pear isolates from Korea and Japan is, however, rather limited in official bacterial collections in Europe.

4.3. Impact or severity

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

4.4. Phytosanitary measures

In order to assess the distribution of this pathogen in the Netherlands, a large scale survey at strawberry fruit growers' holdings will be completed in 2015. Depending on the outcome of this survey, further phytosanitary measures will be considered. Since affected strawberry plants have already been removed, no further phytosanitary measures at the affected commercial holdings have been taken. The possible risk of *E. pyrifoliae* for pome fruit in the Netherlands will also be investigated. Several species within the genus *Erwinia* are pathogenic to pome fruit trees [16]. The fire blight disease of rosaceous plants was initially described in the late eighteenth century, and its causal agent (*Erwinia amylovora*) was identified in the late nineteenth century. In the last 15 years, some other *Erwinia* species that are pathogenic to pome fruit trees have been described, such as *E. pyrifoliae* [14] and *E. piriflorinigrans* [17]. Additional *Erwinia* species found in pome fruit trees are non-pathogenic, such as *Erwinia billingiae* [18] and *Erwinia tasmaniensis* [19]. Basic information about host range, mechanisms of survival and spread of these new *Erwinia* species is often lacking [16].

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