Pest Risk Analysis for *Xanthomonas fragariae*

March 2013

Netherlands Food and Consumer
Product Safety Authority
Ministry of Economic affairs, Agriculture and Innovations

Instituut voor Landbouw- en Visserijonderzoek

Netherlands Food and Consumer Product Safety Authority

Utrecht, the Netherlands

Institute for Agricultural and Fisheries Research

Merelbeke, Belgium

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Dirk Jan van der Gaag[1], Maria Bergsma-Vlami[2], Johan Van Vaerenbergh[3], Joachim Vandroemme[3] & Martine Maes[3]

[1] Office for Risk Assessment and Research, Netherlands Food and Consumer Product Safety Authority, the Netherlands
[2] National Reference Centre, Netherlands Food and Consumer Product Safety Authority, the Netherlands
[3] Institute for Agricultural and Fisheries Research, Belgium

Acknowledgements.
The authors would like to thank:
- J. van der Wolf (Plant Research International, Wageningen) and H. Koenraadt (Naktuinbouw, Roelofsarendsveen) for useful comments and suggestions on a draft version of this PRA
- P. Reynaud (Anses, France) for his help to obtain information about the situation of *Xanthomonas fragariae* in France.
- the partners in the IWT research project in Flanders (BE) on Diagnosis and Prevention of *Xanthomonas fragariae* in strawberry cultivation' (IWT 070597), co-financed by a consortium of auction marketing organisations: ILVO (project coordinator), the Research Station of Fruit Cultivation at Sint-Truiden and Tongeren (PCF), the Research Centre Hoogstraten (PCH) and the Laboratory of Microbiology , Ghent University.

Version: 1.0
Date: March 2013

Citation: Van der Gaag, D.J., Bergsma-Vlami, M., Van Vaerenbergh, J., Vandroemme, J., Maes, M., 2013. Pest risk analysis for *Xanthomonas fragariae*. Netherlands Food and Consumer Product Safety Authority, Utrecht, the Netherlands - Institute for Agricultural and Fisheries Research, Merelbeke, Belgium.

Summary

Biology

The bacterium *Xanthomonas fragariae* (Xf) causes disease (angular leaf spot) on strawberry (*Fragaria* spp.). It primarily affects the leaves including the fruit calyx on which it forms water-soaked angular spots. Under conducive conditions, Xf may move down from the leaves to the plant crown. Xf is generally not destructive but it has been reported that plants may die if they become systemically affected. However, the precise role of Xf in this phenomenon remains unclear because other known strawberry pathogens, i.e. *Phytophthora* and *Verticillium*, are mostly co-isolated from those crowns and rhizomes. Although Xf can cause quantitative strawberry yield loss, the main impact usually occurs when the fruit calyx is affected which reduces the marketability of the fruit. Favorable natural conditions for this disease are wet days with temperature around 20°C or conditions which cause extended leaf wetness e.g. heavy dews or sprinkler irrigation. When these conditions occur, the number of leaf spots can be significant and the bacteria can then exude from these spots as small droplets of sticky ooze. The disease becomes difficult to diagnose under hot, dry conditions. Generally, (latently) infected planting material is the primary infection source of Xf in a strawberry fruit cultivation, but introduction with contaminated machinery, tools, field workers etc. is also possible. The bacterium may also survive in contaminated plant debris in the field from which it may be transmitted to new plantings. Natural spread of the disease from affected plants is (usually) limited to a few metres, and results from dissemination of the bacteria by splashing of rain or irrigation water. Dispersal of Xf may also occur through manipulations of the plants before planting or in the field. The bacterial exudates can also dry on the leaf surface and form flaky silvery pellets containing viable Xf cells. These cells can survive for extended periods under dry conditions and during frost and they get dispersed naturally or by human assistance. See the EPPO- and CABI-datasheet for more details on the biology of Xf (EPPO, 1997; CABI, 2011).

Distribution of *Xanthomonas fragariae*

Xf is known to be present in at least 10 EU-countries. It is possibly more widespread than records indicate. Presence in Hungary and Slovakia, for example, is plausible because the pathogen has been intercepted on plants originating from these countries.

Worldwide distribution of *Xanthomonas fragariae*:

Continent	Country[1]
Africa	Ethiopia
Asia	Taiwan
Europe	Austria, Belgium, Bulgaria, Finland[2], France, Germany, Italy, the Netherlands, Portugal, Slovenia, Spain, Switzerland
North America	Canada, United States of America
South America	Argentina, Brazil, Paraguay, Uruguay, Venezuela

[1] *X. fragariae* may be present in more countries than presently known (see text).
[2] Present, under eradication

Reason for performing the PRA

At present, EU council directive 2000/29/EC lists Xf as an Annex IIAII statutory organism on *Fragaria* plants intended for planting, other than seeds. *Fragaria* plants must either originate from a pest free country or area (within the EU) or a production place where no symptoms of Xf have been observed since the beginning of the last complete cycle of vegetation. However, the requirement of absence of symptoms does not ensure pest freedom, mainly because of the possibility of symptomless infection of planting material with Xf.

Because Xf is already present in many EU-countries and is generally not considered a pest of major economic importance, removal of Xf from the EU quarantine list has been suggested. The current PRA evaluates the risk of Xf for the EU and can be used by risk managers to reconsider the current statutory position of the pathogen.

Probability of entry and spread
International movement of latently infected strawberry planting material is the main pathway for entry and long-distance spread of Xf. Visual inspection of planting material is unreliable because of the possibility of latent infections These latent infections mostly harbour low Xf densities which may remain undetected even when analysed with sensitive molecular detection methods. The probability of entry from third countries is very high, with a medium uncertainty. *Fragaria* plants are imported from at least one country (USA) where Xf is present. Only one interception has been reported during the last 10 years, but imports are usually not tested for symptomless infections. In this PRA, the risk of entry is assessed in the absence of the current phytosanitary measures. The current quarantine status of Xf likely reduces the probability of entry . The probability of spread within the EU through trade is very high, with a low uncertainty. More than 40 interceptions on plants from within the EU have been recorded during the last 10 years, including some on plants from countries that were not yet an EU-member at the time of interception. In addition to movement of infected planting material, Xf may be spread between different strawberry production places by human assistance on contaminated machinery, tools, clothes etc. Natural spread of the disease is (usually) limited to a few metres from an affected plant, and caused by rain or irrigation splash, but factors e.g. insect vectors and wind, may enhance the distance of dispersal. No data are available on these factors and their effects. Dispersal of Xf may also occur through manipulations of the plants in the field or before planting

Area of potential establishment and endangered area
Establishment of Xf is strongly favoured by high humidity, leaf wetness and temperatures around 20˚C. Therefore, areas or cultivation systems where these conditions prevail are most endangered. However, in areas where conditions are not conducive to development and/or spread of the disease (e.g. dry conditions and no use of overhead irrigation and/or temperatures above 30˚C), Xf can stay in the plants with which it was introduced and even remain dormant and undetected for an entire production cycle. Therefore transient populations can occur in areas which are unfavourable (too dry and/or too warm) for spread of the disease.

Impact
Economic impact: minor to major, with medium uncertainty. Angular leaf spot caused by Xf has a minor to major impact in its current area of distribution. A major impact can be expected in areas where strawberries are grown in open field during humid weather or when sprinkler irrigation is applied and temperatures are around 20˚C. The impact is generally minor for strawberries produced in drier climates or cultivation systems in protected environments without overhead irrigation. The uncertainty is medium because little data on quantitative yield loss due to Xf is available, despite the decades-long presence of the pathogen in Europe. Under conditions favourable for disease, control of the pathogen is difficult. Pathogen-free planting material, hygienic measures and restricted use of overhead irrigation are the main preventive and control options for strawberry fruit growers.

Export markets: Lifting of the quarantine status in the EU may negatively affect export of strawberry planting material to countries where Xf is a regulated pest. However, EU-countries where Xf is present already export planting material to countries where Xf is regulated and without EU-regulation specific requirements could be installed for those companies that want to export strawberry plants.

Environmental impact: minimal, with low uncertainty. Xf is not known to have a (significant) environmental impact. The pathogen is already present in large parts of the PRA area without any known impact on native plants. Xf is especially known to attack the cultivated strawberry *Fragaria* x *ananassa* but there are a few reports on infection of other *Fragaria* spp.

Social impact: minor, with medium uncertainty. Occasionally, local outbreaks can lead to a high economic impact and subsequently a high social impact for individual strawberry fruit growers. The social impact at this stage of the PRA is assessed in absence of official phytosanitary measures. However, in the event of an outbreak, the current phytosanitary measures can have a large economic impact on nurseries producing plants for planting which in the worst case can lead to bankruptcy and thereby a high social impact at the farm level.

Risk reduction options

Risk reduction of Xf dissemination through control of planting material.

The current EU-requirement absence of Xf symptoms in *Fragaria* plants is not sufficient to ensure Xf freedom mainly because of the possibility of latent infections. Testing methods are available to detect latent infections but the probability of detection largely depends on the sampling intensity and detection method used. Five possible (de)regulatory options for production and trade of planting material, ranging from most to least stringent, are discussed in the PRA:

I. Pest free area.
II. Pest free production place based on visual inspections and testing for latent infections.
III. Pest freedom of the crop (field) based on visual inspections and testing for latent infections.
IV. Pest freedom of the crop (field) based on visual inspections.
V. No EU regulation (Xf can be implemented in national certification schemes).

The more stringent regulatory options I, II and III will decrease the risk of entry and spread of the pest as compared with the present regulatory measures in the EU but will increase the economic and social impact of the measures to strawberry plant nurseries. It is uncertain if such stringent measures will be cost-effective. Establishment of a pest free area (I) is not possible without widespread monitoring, while detection of symptomless Xf infections in options II and III will increase the costs of planting material and a negative result is no guarantee of pest freedom. Testing for latent infections in addition to visual inspections can reduce the risk level but cannot ensure pest freedom because of detection limits of the PCR-assays available and limits in the number of samples that can reasonably be taken and analyzed. For strawberry production areas conducive to disease development and spread (e.g. humid weather and temperatures around 20°C) the use of pathogen-free planting material will be more important than for areas which are less favourable for disease development and spread (e.g. protected cultures and/or dry and/or very warm conditions).

Uncertainties

The main uncertainties in the present PRA are:

- The current distribution of Xf in the EU. Xf may be more widely distributed than present records indicate. Xf may have been spread throughout most of the strawberry producing areas in the PRA-area through trade of latently infected planting material, but only reported in areas where conditions are favourable for disease development and where growers have suffered damage.
- The current impact of Xf in the EU: only a few publications are available on yield effects.
- The current risk associated with import and trade of planting material because of possible latent infections

- The cultivation methods in the different strawberry producing areas within in the EU, related potential impact of Xf and indication of most endangered areas
- The possibility of seed transmission: no studies known on seed transmission
- The host plant status of *Potentilla* spp. and *Fragaria* spp. other than *Fragaria* x *ananassa*
- The effect of Xf on susceptibility of strawberry plants to other diseases

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Methodology

The set-up of the present PRA follows the former scheme (version 2010) of the European and Mediterranean Plant Protection Organisation (EPPO) to a large extent but deviates from it in certain parts, e.g. most questions in the "pest categorization – part" has been skipped since the pest is already present in the PRA area and has a quarantine status.

Ratings are given according to a 5-point qualitative scale (very low, low, medium, high, very high or minimal, minor, moderate, major, massive) and uncertainty according to a 3-point qualitative scale (low, medium and high) adapted from the PRA-scheme prepared by EPPO, (http://www.eppo.org/QUARANTINE/Pest_Risk_Analysis/PRA_intro.htm, accessed March 2011). Adapted from IPCC (Intergovernmental Panel on Climate Change) definitions, low, medium and high uncertainty are defined as expressing 90, 50 and 35% confidence, respectively, that the score selected is the correct one (Mumford *et al*., 2010).

1. Introduction (Pest Risk Initiation)

1.1 What is the reason for performing the PRA?

Xanthomonas fragariae Kennedy and King (Xf) is listed as an EU IIAII quarantine pathogen on *Fragaria* plants intended for planting, other than seeds (directive 2000/29/EC). *Fragaria* plants must either originate from a pest free country or area (within the EU) or a production place where no symptoms of Xf have been observed since the beginning of the last complete cycle of vegetation. However, the requirement of absence of symptoms cannot ensure absence of the pest, mainly because of the possibility of symptomless contamination of planting material.

Xf is already present in many EU-countries, including major areas for strawberry production. However, so far, it has not become a major economic threat. It is Q-regulated (quarantine status) in the EU on *Fragaria* plants intended for planting. Certified planting material, which is visually inspected for various pests and diseases and for which the candidate nuclear stock has been tested and found free for various viruses, fungi and Xf, is commercially available. For these reasons, Q-deregulation of Xf has been suggested. The current PRA evaluates the risk of Xf for the EU and can be used by risk managers to reconsider the current quarantine status of the pathogen.

Note:
Apart from the EU, Xf is currently listed as a plant health Q-pathogen in at least Turkey, East Africa, Southern Africa, Chile and New Zealand (EPPO, 2011a).

1.2 Scientific name, taxonomy and type of pest

Xanthomonas fragariae Kennedy & King 1962 is an aerobic, gram negative rod bacterium. It is a member of the gamma subdivision of the Proteobacteria (Stackebrandt *et al.*, 1988), Order: *Xanthomonadales*, Family: *Xanthomonadaceae*, Genus: *Xanthomonas*, phenon 3 (Van der Mooter & Swings 1990). According to the Bergey's Manual of Systematic Bacteriology (Bradbury 1984), *Xanthomonas fragariae* is a phenotypically homogeneous species and clearly distinct from the other xanthomonads (Vauterin *et al*., 1995). These results have recently been confirmed in a phylogenetic analysis of *Xanthomonas* species (Parkinson *et al*., 2007).

1.3 PRA area

The PRA area is the EU.

1.4 Does a relevant earlier PRA exist?

No. A PRA for Norway, composed in 2000, is available on the internet (http://www.mattilsynet.no/, accessed 30th July 2010) but a PRA for the EU is not known.

2. Pest Risk Assessment

2.1 Host plants and pest distribution

2.1.1 Specify all the host plant species (for pests directly affecting plants). Indicate the ones which are present in the PRA area.

Xf is known as a pathogen of the cultivated strawberry *Fragaria* x *ananassa* Duch. There are numerous cultivars of *Fragaria* x *ananassa* which significantly vary in susceptibility (EPPO, 1997). From the EPPO-datasheet (EPPO, 1997): "*F. virginiana, F. vesca, Potentilla fruticosa* and *P. glandulosa* have been infected following experimental inoculation. Among *Fragaria* spp. only *F. moschata* is not susceptible (Kennedy & King, 1962; Kennedy, 1965; Maas, 1998)". Stöger *et al.* (2008) isolated Xf from *F. vesca* and *F. chiloensis* in the field. The only known research report of a non-*Fragaria* species acting as a host plant is from Kennedy (1965) who inoculated 11 *Potentilla* spp. and found two species, *P. fruticosa* and *P. glandulosa,* as susceptible as the strawberry control (*Fragaria virginiana*). Kennedy (1965) concluded that it is unknown if either of the two *Potentilla* species are natural hosts. Besides *Fragaria* and *Potentilla* genotypes, Kennedy (1965) tested 35 other plant species and cultivars and did not find them susceptible.

Because of the limited data available, it was not considered possible to exclude certain *Fragaria* sp. as hosts of Xf*.* Thus, all *Fragaria* spp. are considered as (potential) hosts. Infection of *Potentilla* spp. has only been reported once and only after artificial inoculation. They are, therefore, not considered further in this PRA.

In the PRA-area, *Fragaria* species are present in natural ecosystems and under commercial conditions (*Fragariae* x *ananassa*).

2.1.2 Specify the pest distribution

Xf was first described in Minnesota, United States, in 1962 (Kennedy & King, 1962a) and has subsequently been reported in other regions (e.g. Bultreys *et al*., 2000). At present, the pathogen has been reported on 5 continents (Table 1). The pathogen probably originated in the United States from where it may have been introduced into other continents through international plant-trade (CABI, 2010). Observations in Sicily (Italy) suggest the pathogen has been introduced into Europe by import of infected or contaminated planting material from California, United States (Mazzucchi *et al*., 1973). An eradicated New Zealand outbreak in 1971 was also associated with planting material originating from California (Dye & Wilkie, 1973; McGechan & Fahy, 1976). In Australia, outbreaks have thus far been detected in 1975, 1994 and 2010 and have most likely been eradicated (McGechan & Fahy, 1976; Gillings *et al*. 1998; Young *et al*., 2011). The first outbreak in New South Wales was related to import of plants from California (McGechan & Fahy, 1976). The origin of the second outbreak (South Australia) was unknown; isolates were genetically different from the outbreak in 1975 in New South Wales. Maybe the bacterium had been introduced with import of plants in 1990 but that is uncertain (Gillings *et al*., 1998). The third finding was in a germplasm collection in Queensland and its origin was also unknown. It was, however, concluded based on surveys and the isolated situation of the breeding facility that Xf has most likely been eradicated (Young *et a*l., 2011).

Details and references on the distribution in the EU are presented in Annex I.

Table 1: Worldwide distribution of *Xanthomonas fragariae*

Continent	Country	Source
Africa	Ethiopia	EPPO, 2011a
Asia	Taiwan	EPPO, 2011a
Europe[1]	Austria	AGES, 2000
	Belgium	EPPO, 2011a
	Bulgaria	EPPO, 2011a
	Finland[2]	EPPO, 2011b
	France	EPPO, 2011a
	Germany	EPPO, 2011a; JKI, 2010
	Italy	EPPO, 2011a
	Netherlands	EPPO, 2011a
	Portugal	EPPO, 2011a
	Slovenia	Brence, 2002; EPPO, 2011a
	Spain	EPPO, 2011a
	Switzerland	EPPO, 2011a
North America	Canada	EPPO, 2011a
	United States of America	EPPO, 2011a
South America	Argentina	EPPO, 2011a
	Brazil	EPPO, 2011a
	Paraguay	EPPO, 2011a
	Uruguay	EPPO, 2011a
	Venezuela	EPPO, 2011a

[1] Xf might also be present in Hungary and Slovakia because the pathogen has been intercepted on plants originating from these countries, see also Table 3 (Europhyt, accessed 9th August 2011)
[2] Present, under eradication

2.2 Probability of introduction and spread

2.2.1 Identification of pathways

Plants for planting other than seeds
Trade of plants for planting other than seeds, has been shown to be an important pathway. This pathway will be evaluated in more detail below. Plants for planting of *Potentilla* spp. are not considered in this PRA for reasons mentioned above ("Host plants and pest distribution"), but remain an uncertainty in this PRA.

Seeds
It is unknown if Xf can be transmitted through seeds. Strawberry plants are mainly propagated vegetatively but seeds are also on the market (e.g. http://www.abz-strawberry.nl/). Seed might also be a pathway in breeding programmes. There are, however, no data on seed transmission and it is highly uncertain if seeds could be a pathway. Because of lack of data, seeds are not further considered in this PRA, but the relevance of seed as a pathway remains an uncertainty in this PRA.

Fruits
The calyx can become infected (e.g. Epstein, 1966; Maas, 1998; Gubler *et al*., 1999) and because the fruits are harvested and traded with the calyx present, fruits may serve as a pathway. This pathway is currently not regulated in the EU (EU directive 2000/29/EC) but the pathogen could theoretically be transferred from an infected calyx to strawberry plants in cases where fruits are placed near strawberry plants in private gardens or commercial fields. However, the probability of transfer through the calyx of detached fruit is rated as very low because natural spread of the disease is usually restricted to a few metres from an affected plant (see below "spread"). Natural dissemination is stimulated by rain or overhead irrigation and does not occur during dry weather. (see below 2.2.5 "Natural spread"). The probability that strawberry fruits with infected calyces, originating from another area, are placed without any cover directly adjacent to a strawberry crop during rainy weather is considered very low. Because the pathogen is already present in many EU-countries, the relatively low risk posed by fruits is not further evaluated in this PRA.

Natural spread
Natural spread occurs only over short distances by splash dispersal and will be discussed in the spread section of this PRA (see below: 2.2.5).

Mechanical transmission
Spread through infested machinery, tools, clothes etc. may occur especially between fields of the same company and will be discussed in the spread section of the PRA (see below: 2.2.6).

2.2.2 Probability of entry from third countries
The main pathway for entry from third countries is import of plants for planting other than seeds (further indicated as plants or planting material in this PRA) from areas where the Xf is present. The importance of this pathway has been shown by numerous interceptions which also show that the pathogen can survive transport conditions (see Annex I and questions 2.1.2 and 2.2.8 for details and references). Xf can easily be transmitted with latently infected plants and is, therefore, very likely to remain undetected during import inspections. Xf has been detected in symptomless plant material in Germany and the Netherlands (Moltman & Zimmerman, 2005; Vermunt & van Beuningen, 2008; Van der Wolf *et al*., 2009).

Data on import of *Fragaria* plants from third countries were only available for the Netherlands (Table 2). Relatively large numbers of plants are imported from the USA, where Xf is present but generally considered of minor importance on plant performance

(Dr. Richard Nelson, Plant Sciences Inc., CA, USA; Personal communication, 29/02/2012). The probability of entry from third countries is assessed very high, with a medium uncertainty. Several Xf findings in the past were associated with plants imported from the USA (question 2.2.8), although only one report of Xf on plants imported from a third country has been reported in the last 10 years (Table 3). However, plants with (symptomless) infections may have entered undetected. Xf is a problem in US nurseries and nurseries that want to export to the EU must maintain phytosanitary standards (Turechek & Peres, 2009). It is uncertain if lifting of the quarantine status of Xf in the EU will lead to lower phytosanitary standards in the US and thus higher incidence of angular leaf spot on imported planting material. Personal communication of Dr. Richard Nelson, Plant Sciences Inc., CA, USA on 29/02/2012 & 08/03/2012: regardless of EU export, Californian strawberry nurseries are under a State Certification Program. Production under this program starts with meristemed tissue culture plants, requires one field inspection each growing season and generally results in planting material with a very low to zero incidence of angular leaf spot. At present, nurseries in California which are exporting to the EU, are inspected three times each growing season by the County Agricultural Commissioner's Office and the State of California for any quarantine diseases. Q-deregulation of Xf in the EU will not have a substantial impact on the Californian phytosanitary measures, as angular leafspot free status in the young plants is still required for export into other countries (i.e. Mexico) and as part of best management practices. However, plants that were declared free of angular leaf spot after visual inspection may sometimes be positive for Xf if tested with more sensitive detection techniques.

Table 2: Import volume of plants for planting of *Fragaria* sp. into the Netherlands during 2008-2010 (source: import database of the NPPO of the Netherlands)

Year	**Country**	**Number of plants**
2008	USA	10,266,885
2009	USA	167,426
	Israel	1,600
2010	USA	1,974,829

Probability of entry from third countries: Very high. The uncertainty is medium. *Fragaria* plants are imported from at least one country (USA) where Xf is present. In the past several findings have been related to import of plants from the USA. Only one interception has been reported in the last 10 years, but symptomless infections may have entered undetected. Also, the current quarantine status of Xf likely reduces the probability of entry while the risk of entry in this PRA is assessed in the absence of the current phytosanitary measures.

2.2.3 Probability of spread within the EU by trade of plants for planting

The pathogen is present in many EU-countries. In the EU, the main producers of strawberry propagation material are the Netherlands, France, Belgium, Italy, Spain and Poland (Plantum, the Dutch association for the plant reproduction material sector, February 2011). Xf has been reported in 5 of these 6 countries. There are many Xf findings related to nursery stock trade within the EU (Table 3, see also Annex I and question 2.2.8). Also, the frequent findings on strawberry propagation material in the Netherlands each year show a very high likelihood of association (Plant Protection Service, 2010). Thus, the probability of spread with trade of planting material is very high with a low uncertainty.

Table 3: EU-notifications of *Xanthomonas fragariae* on plants for planting of *Fragaria* (Europhyt, accessed 9th August 2011)

Year	Number	Country of origin	Country of destination
1994	6	Switzerland (6x)	Germany (6x)
1995	3	Switzerland (3x)	Germany (3x)
1996	2	Switzerland (2x)	Germany (2x)
1997	5	Hungary (5x)	Germany (5x)
2000	1	Argentina	France
2002	21	Hungary (21x)	Germany (21x)
2003	13	Hungary (10x), Bulgaria (1x), Italy (1x), Slovakia (1x)	Germany (11x), Austria (1x), the Netherlands (1x)
2004	1	Hungary	Germany
2005	4	the Netherlands (2x), Hungary (1x), USA (1x)	Belgium (2x), Germany (1x), UK (1x)
2009	1	Hungary	Germany
2010	3	Spain (3x)	Belgium (2x), France (1x)
2011	1	Spain	Belgium
Total	61		

Probability of spread by EU-internal trade of infected planting material: very high, low uncertainty: many interceptions during the last 10 years.

2.2.4 Probability of establishment

How widespread are host plants or suitable habitats in the PRA area?
Fragaria species occur widespread in the EU; strawberry fruits are being produced in all EU-countries (Annex II).

Suitability of climate in the PRA area
Xf is present in various areas in Europe, despite the diverse climatic zones: Atlantic, Continental, Nemoral and Mediterranean, as defined by Metzger *et al.* (2005). However, environmental conditions greatly affect disease development and severity. Experimental results indicate that temperatures around 20°C are about optimal for disease development. Kennedy & King (1962b) found more lesions on leaves of inoculated plants grown at 18°and 22°C than at 26° and 30 °C (Kennedy & King, 1962b). Kennedy-Fisher (1997) determined numbers of bacteria in two cultivars grown at four different temperatures (16-20-23.5-27°C) after inoculation. The highest numbers of bacteria were found at 16 or 20°C. Hildebrand *et al.* (2005) determined disease development at 6 different temperatures (5–10–15-20-25-30°C). They found the highest disease severity on plants grown at 25°C although data did not seem to be significantly different from the 20°C treatment. At 10, 15 and 30°C disease development was slower than at 20 and 25°C. No lesions developed at 5°C until plants were moved to 20°C and disease severity reached similar levels as the 20°C treatment (Hildebrand *et al*., 2005). Using a specific semi-nested PCR detection, data by Roberts *et al.* (1996) indicated a sharp decline in Xf populations in inoculated strawberry plants during hot Florida summers. However, the bacteria never completely disappeared, and when the weather cooled, the Xf population increased again. High humidity is important for infection, disease development and production of bacterial ooze (Kennedy & King, 1962b; Hildebrand *et al*., 2005). Frequent rain or overhead irrigation is beneficial for dissemination, both to new leaves of infected plants and to neighbouring plants and outbreaks have been associated with rain or overhead irrigation (Epstein, 1966; Hildebrand *et al*., 1967; Melis *et al*., 2012; Kastelein *et al*. (a), in preparation; also see 2.2.5. Natural spread).

The climate may also affect survival of Xf between strawberry crops. The bacterium survives in plant debris in the soil during crop-less periods. According to Maas (2004), Xf cannot survive freely in soil. Studies are available on survival of Xf in plant debris in soil but not after decomposition. Xf is, however, unlikely to survive for long periods once the plant debris has decomposed and factors that favour decomposition of the debris in soil will probably limit survival. Kennedy & King (1962b) have shown that infected plant debris buried in soil in Minnesota remained infectious until the next spring. However, infectiousness was lost after incubation in greenhouse soil after 3.5 months. Soil temperatures in Minnesota are below 0°C and will not favour decomposition of plant debris. Winters in Minnesota are relatively cold and Xf may survive poorly in soil in climates with relatively warm winters. Experimental results indeed indicated that survival of Xf in crop debris in soil during winter and subsequent transfer to a new crop is not very likely in the Netherlands (Van der Wolf *et al*., 2008; Vermunt & van Beuningen, 2008; Kastelein *et al*., 2009). However, survival in plant debris in soil was found during a period of at least 5.5 months (Kastelein *et al*., 2009). Therefore, transfer from one crop to a next cannot be excluded.

<u>How suitable are protected conditions for establishment?</u>
Protected conditions are less suitable for establishment than non-protected conditions, unless overhead irrigation is used. Without overhead irrigation, protected conditions are unfavourable for spread and Xf may be remain on the plants on which they have been produced on and not spread any further.

<u>Effect of soil properties</u>
Xf survives crop-less periods in plant residues; it cannot survive freely in soil (Maas, 2004). Soil properties that enhance decomposition of plant tissue will decrease survival of the pathogen (see above).

<u>Effect of management practices</u>
Overhead irrigation will strongly favour establishment of Xf (EPPO, 1997). The effect of overhead irrigation has been shown for *Xanthomonas campestris* pv. *campestris* in cabbage transplants by Roberts *et al.* (2007). Incidence of the disease was greatest when trays with transplants were watered with overhead irrigation.

<u>Effect of existing crop protection measures</u>
Existing pest management practices will not be able to prevent establishment. Preventative applications of approved copper-based fungicides can be partially effective, but their application is limited because of their phytotoxicity (Howard & Albregts, 1973). They help preventing new infections and further spread of the disease but they do not completely remove infection (Tomlin, 2009). Application in the early stages of symptom development is most effective (Bardet 2008). Roberts *et al*. (1997) reported that low-rate application (0.1x of label rate) of a mixture of cupric hydroxide and mancozeb significantly reduced diseased severity in one season but not in another season. Application at the label rate significantly decreased disease severity in both seasons but control was still not complete. Moreover, the label rate proved phytotoxic and resulted in lower yields. The limited effectiveness of copper-compounds is explained by their mainly epiphytic activity. Although they help preventing initial epiphytic colonization and invasion of Xf, they are not effective against endophytic populations. In strawberry, copper-based pesticides are allowed in several EU-countries, e.g. France, Greece, Italy and Spain but not in other e.g. the Netherlands, Germany, Denmark, UK and Belgium (Pesticide databases, 2010; last access August 2010). Two other active components, benzothiadiazole (BTD) and azoxystrobin (AS) have been reported as having moderate to low effectiveness in the control of Xf. BTD reduced the average number of Xf-lesions on infected plants by 54% and AS by 21%, while total crop yield in Xf-infected strawberry plots was 9% (BTD) and 28% (AS) higher than in the untreated control (Paulus & Vilchez, 1998). It is obviously debatable if the increased yield was solely caused by the suppression of Xf. Both compounds are approved active substances in the European Union (European Commission, 2011). Antibiotics like streptomycine sulphate of kasugamycine are no longer approved as plant protection agents in the EU. In the UK, an

outbreak of Xf was eradicated by application of strict hygienic measures (Matthews-Berry & Reed, 2009), but such strict measures are not common practice. Also because of latent Xf-presence in symptomless strawberry plants it is unlikely that existing pest management practices will prevent establishment.

How likely can the pest establish starting from a low initial inoculum level? (take into account the reproductive strategy of the pest)
Xf can persist in the plant at low population levels without causing visible symtoms. With the onset of more favourable conditions, this low infection level may result in primary symptoms, building up of inoculum and secondary infections after oozing and eventually epidemic spread (Roberts *et al*., 1996).

2.2.5 Natural spread

Large amounts of Xf (>10^{12} cfu or higher) exude from leaf lesions under humid conditions (Verjans *et al*. 2012; Kastelein *et al*. (c), in prep.). The bacteria in the exudate can be disseminated to healthy leaves by splashing or as wind-driven aerosols during showers (EPPO, 1997). Observations indicate that spread of the disease is (usually) within 3 m of an affected plant (Roberts *et al*., 1987; see also below: "2.2.6. Spread by human assistance" for a description). Under extreme weather conditions (e.g. rain in combination with strong winds) natural spread might occur over larger distances but this is highly uncertain.

Under dry plant growth conditions, the Xf population inside the plant stagnates (IWT project, Belgium). Existing Xf-exudates on the strawberry leaf surfaces dry up and form a white, flaky film (Kennedy & King, 1962b). In such condition, Xf is very resistant and long-living: it was successfully isolated from a 10-year old herbarium leaf showing typical leaf symptoms (Kennedy & King, 1962b) and could even be recovered from 21 year-old air-dried leaf spots (Kong, 2010). The exudate flakes may help the bacterium to survive extended drought periods. A recent Belgian study demonstrated that viable cells of Xf were readily isolated on Wilbrink N medium from flaky films on desiccated leaves after natural overwintering (Melis *et al*., 2012). These thin biofilms on leaves may serve as a source for additional wind-driven dissemination, although data specifically on Xf are unavailable. The importance of wind-dispersal of infected plant debris has also not been studied. In the absence of rain or overhead irrigation, natural spread distances of Xf are considered very low or absent. Spread of *X. campestris* pv. *campestris* between brassica transplants in a glasshouse did not occur unless overhead irrigation was applied (Roberts *et al.* 2007). Maas (2004) mentions the potential spread of Xf by animals that come into contact with bacterial ooze, although relevant data are not available.

2.2.6 Spread by human assistance

Here, we discuss the probability and rate of spread by human assistance other than those by movement of infected planting material.

Xf may be spread by machinery and humans that come into contact with bacterial ooze or infected plant debris (Maas, 2004). The pathogen may, for example, be spread during picking of fruits. The pathogen may also be spread with machinery used in the production of propagation material. Field studies in the Netherlands have shown that Xf can be spread from infected to non-infected plants during mowing of strawberry plants over a distance of minimal 10 plants (4 m) in a row (Kastelein et al., (b), in prep.). The pathogen can be detected in relatively high quantities on the mower blades even after careful washing with water. The pathogen could also be found in high densities on the blades of a machine used for cutting runners. A recent Belgian study showed that spread of the disease from infected plants in a strawberry production field by overhead irrigation was mainly restricted to plants within a distance of up to 1 metre (disease severity 11% after 2 months), although minor spread up to 3 metres (1%) was observed after 2 months (Melis *et al*., 2012). Manual labour in the plant rows (i.e. pruning, fruit-picking) combined with overhead irrigation considerably increased the spread up to 1 (18%) and

3 metres (6%). Disease severity in newly infected plants remained low (e.g. 11%) up to 2 months after initiation of the experiment and reached moderate levels (25%) only after 3 months. Because a normal production season with strawberry cv. Elsanta in Belgium is generally shorter than two months, potential damage due to secondary spread within a production field was considered minor. Dipping of plantlets in Xf-contaminated solutions prior to planting (e.g. fungicide treatment) was indicated as another source of contamination. The Belgian study confirmed the results of Roberts *et al.* (1997), who showed that dissemination from inoculated strawberry plots by overhead irrigation only caused minimal disease (2%) in new strawberry plots, and only after a period of four months.

Xf can be transmitted to the total plant badge when the fungicide bath used for preventive fungicide treatment of planting material (current practice) is contaminated with Xf (Melis *et al*., 2012).

The persistence of Xf on various materials has been studied. Vermunt & Van Beuningen (2008) found a strong reduction in population density 17 days after infestation of wood steal, rubber, and cotton and the bacterium could not be detected after 38 days; on cotton the bacterium could not be detected after 17 days (using Wilbrink's medium and immunofluorescent microscopy). Van der Wolf *et al*. (2009) found that Xf populations decreased rapidly on wood, iron and PVC and could hardly be detected one week after infestation of these materials (Van der Wolf *et al*., 2009). It was not studied if plants could be infected through contact with the infested materials.

In conclusion, the probability of spread by human assistance (others than by trade/movement of infected plant material) is assessed very likely with a medium uncertainty. Spread within a field by machinery and humans has been shown but it is uncertain if and how frequent spread by contact occurs between production places.

2.2.7 Description of the area of potential establishment and endangered area

The recent observations from the UK where symptoms stopped abruptly where the crop was covered with a polytunnel (Matthews-Berry & Reed, 2009) and also the fact that Xf is not known as a problem in greenhouses in the Netherlands (pers. comm., H. Boesveld, Plant Protection Service, March 2011, former consultant in strawberry production) indicate that Xf does not cause significant impact in strawberries produced under protected conditions. Thus, strawberry greenhouses/polytunnels without overhead irrigation are outside the endangered area.

Outdoors, Xf may be able to establish in most regions in the EU where *Fragaria* plants are present. However, the disease potential increases with the amount/frequency of rainfall during the strawberry growing season. Thus, in areas where strawberry plants are grown during a relatively dry period and no overhead irrigation is used, Xf may not be able to establish or only be present as a transient population. In such areas, Xf can remain present during the entire growing season on the plants with which it was introduced but will not spread further. Survival and infection of a following crop through infected crop debris is also not very likely because of the low infection levels and dry conditions.

The potential impact of Xf will vary largely depending on local growing conditions and climate. In the UK for example, strawberry fruits are mainly produced in greenhouses/tunnels (information from Plantum, the Netherlands, December 2011) and strawberry fruits grown under protected conditions are outside the endangered area. More information is needed on local growing conditions to assess more precisely the more and less endangered areas in the EU.

2.2.8 How often has the pest been introduced into new areas outside its original area of distribution? (specify the instances, if possible)

Xf was first described in Minnesota in North America in 1962 (Kennedy & King, 1962a) and subsequently reported from several other regions and continents (e.g. Bultreys *et al*., 2000). Currently, the pathogen is present on all continents except Oceania and Antarctica (Table 1). It has been introduced into Oceania but has subsequently been eradicated (Wilkie, 1973; McGechan & Fahy, 1976). The pathogen may have originated from North America from which it has been introduced into other continents by trade of planting material but this is only a presumption (CABI, 2010). Observations from Sicily (Italy) suggested that the pathogen has been introduced into Europe by import of infected or contaminated planting material from California (Mazzucchi *et al*., 1973). In New Zealand and Australia outbreaks were also associated with planting material originating from the USA; these outbreaks have been eradicated (Dye & Wilkie, 1973; McGechan & Fahy, 1976; see also below "Eradication"). Observations in Florida suggested that several findings originated from planting material that had been traded from California (Howard, 1971). Within Europe, several introductions were likely due to trade of infected or contaminated planting material (see Annex I, Grimm *et al*., 1993).

Conclusions on the area of potential establishment, endangered area, probability of introduction (entry + establishment) and spread

<u>Area of potential establishment and endangered area</u>

Outdoors, Xf may be able to establish in most regions in the EU where *Fragaria* plants are present. However, the disease potential decreases in dry areas without overhead irrigation. In areas where strawberry fruits are produced during relatively dry periods, Xf may not be able to establish or will not cause significant impact unless overhead irrigation is used. Greenhouses without overhead irrigation are outside the endangered area. It is possible that Xf has already spread throughout most of the PRA area through trade of infected planting material, but that most reports to date are only from areas where conditions were favourable for symptom development and especially where growers suffered damage.

<u>Probability of introduction from third countries</u>

The probability of (new) introductions from outside the EU has been assessed "very high" with a medium uncertainty. *Fragaria* plants are imported from areas where Xf is present. In the past, several findings of Xf were associated with planting material imported from the USA, but during the last 10 years Xf has only been notified once on plants originating from a third country.

<u>Probability of spread with trade of strawberry plants within the EU</u>

The probability of spread with infected plant material has been assessed "very high" with a low uncertainty (in the absence of official phytosanitary measures). Xf is present in many EU-countries. Many findings on planting material have been reported during the last 10 years despite its quarantine status.

<u>Probability and rate of natural spread</u>

The probability of spread is high when environmental conditions are favourable (high humidity, heavy precipitation) (low uncertainty). Natural spread distances are restricted to a few meters from an infected plant (medium uncertainty), although longer spread distances cannot be excluded. Under dry conditions, the probability of spread is very low (low uncertainty).

2.3 Potential consequences

Economic impact

2.3.1 What is the economic impact of the pest in its current area of distribution?
Although angular leaf spot is generally not a destructive disease, strawberry plants with symptomatic leaves incur a certain quantitative yield reduction. This yield loss is very variable as it is highly dependent on climatic and cultivation conditions favouring disease development and spread of the pathogen (high humidity and frequent precipitation or overhead irrigation, mild temperatures) and choice of cultivar. Additionally, direct qualitative yield losses occasionally occur when infection of the fruit calyces leads to a symptom known as "black cap" and reduces the marketability of the fruit. Xf may move inside the plants to different plant parts. Several papers associated Xf with vascular decline or plant collapse (Hildebrand *et al*., 1967; Stefani *et al*., 1989; Milholland et al., 1996; Heidenreich & Turechek, no year). However, the precise role of Xf in this phenomenon remains unclear because other known strawberry pathogens, i.e. *Phytophthora* and *Verticillium*, are mostly found associated with the rhizome, roots and crown of these plants (IWT project, Belgium). Although Xf was detected in rhizome tissue at several occasions, it never resulted in plant collapse. In fact, the strawberry plants and their daughter plants remained symptomless throughout the growing season. It is not known if the endophytic occurrence of Xf makes plants more vulnerable to attack by other pathogens.

Information about the impact of Xf from various sources is provided below.

Information from original research papers
CABabstracts 1910 to 2011 (week 16) were searched for papers on yield loss or impact. Two relevant papers were found: Epstein (1966) and Roberts *et al.* (1997).

Epstein (1966) reported 75–80% quantitative yield loss in an irrigated plot in Wisconsin (USA) without giving any details. No additional diseased plots were found during a survey. Increased sprinkler irrigation was suggested as the cause for a more widespread impact of the disease.

Roberts *et al.* (1997) reported quantitative yield losses of 8% in experimental plots in Florida (USA) after inoculation with Xf. Prior to planting, plants had been inoculated by dipping them in a bacterial suspension. From the inoculated plots, the pest hardly spread to non-inoculated plots at 3 m distance since maximum disease severity in non-inoculated plots was 2% (disease severity in inoculated plots was 25 and 14% in 1994 and 1995, respectively).

Information from other sources (books, trade journals, personal communications etc)
The EPPO datasheet (EPPO, 1997) states that the disease is generally not destructive but heavy losses may occur with frequent overhead irrigation.

In a PRA for *Xanthomonas arboricola* pv. *fragariae* it was stated (Scortichini, 2004): "Making a comparison with Xf, which occurs in Italy where it has not caused particular problems for farmers, *X. arboricola* pv. *fragariae* should not be a big problem. Anyway, studies and researches should be carried out to evaluate the severity of the disease in other ecoclimatic and agricultural conditions."

Yield losses up to 30 % due to infection of sepals (fruit calyx) were reported from Germany in 1995 in a trade journal (Litterst 1996).

Bardet (2008) have stated the following about Xf in France "… in open field and in autumn some foliar damages can appear caused by the fungus like *Ramularia tulasei* or *Zythia fragariae* or by the bacterium *Xanthomonas fragariae.* Generally fungicides formulated with copper applied in the first symptoms are active." Bardet (2012) asked several strawberry/plant disease experts for information about the situation of *X. fragariae* in different regions in France. Based on the experts' answers Bardet concluded the following about the situation in France: the pest appears in open field crops when weather conditions are mild to cool with constant moisture on the crop which is the case with propagation material grown in late summer and autumn. Cultivars vary in their susceptibility. In protected crops the pest is no problem.

Desmet *et al*. (2006) have reported in a Belgian trade journal that the economic impact caused by Xf is difficult to indicate in figures. Plants with leaf spots usually allow for a normal production. However, also the calyx is often affected which makes that the fruits show unattractive and may be declassified or even deemed unsalable. Production losses up to 30% can occur due to symptoms on the calyx.

In a survey carried out in Belgium in 2005, it was reported that 37% out of 115 interviewed strawberry growers had Xf in their crops (Desmet *et al*., 2009). Based on the study of the Xf symptomatology in Belgium, it was concluded that several symptoms which occur in strawberry are regularly mistaken for Xf disease by strawberry growers (Verjans *et al*., 2012), which probably led to an overestimation of Xf disease occurrence and impact.

Information obtained from H.C.H. Pijnenburg (senior consultant strawberry and vegetable production, DLV Plant, September 2011): The impact can be very high in some regions in the Netherlands and Belgium especially during periods of very wet weather and/or periods with cold nights. During dry periods damage can also be high due to overhead irrigation. Sometimes, yield periods are stopped prematurely which can lead to 30 - 50% yield loss at some production places. However, yield losses are often limited by manual removal of fruits with symptomatic calyxes. On an average, yield losses of strawberry fruit in the open field may be about 5% in the Netherlands. About half of the fruit production is in greenhouses and in total Xf may cause about 2% yield loss per year. Regularly, high damage levels also occur in some other European countries, e.g. Germany. In southern Europe, damage levels are generally lower due to the drier climate and the use of drip irrigation (instead of overhead irrigation).

S. Timmermans (Hortinova, the Netherlands, pers. comm., January 2012) assesses losses due to *X. fragariae* on an average on 1-2% on the strawberry fruit producing companies he visited in the Netherlands in recent years; many companies do have no or little problems.

In protected cultivation in the Netherlands it is not a problem (pers. comm., H. Boesveld, Plant Protection Service, March 2011, former consultant in strawberry production).

Information from R. Steffek (AGES, Austria, pers. comm., December 2011) and Steffek & Altenburger, (2009): In Austria, June bearing cultivars are mostly grown. Strawberry fruits are mainly produced in the eastern part of the country (Lower Austria, Burgenland and Styria), though there are some production areas also in Upper Austria and Tirol. Most of the production is outdoors in natural soil, though few producers use high tunnels and even fewer greenhouses for early season production. Everbearing cultivars are not important in Austria. In the north-eastern parts, annual precipitations are between 450 and 550 mm. In that area, Xf is very rare. It may occasionally occur in late summer after warm and wet periods. And even then the only symptom is water soaked lesions on the leaves and it causes no significant damage. In that area systemic infections, that lead to a collapse of the plants and even early infections causing spots and stipules and a reduced market value of the fruits are not found. However, in areas with high precipitation and everbearing cultivars are grown, the impact may be higher. A major impact has once been observed in Tirol, where a considerable acreage was ploughed in (during a rain period in summer the disease lead to a collapse of the stolons and the total plants).

Conclusion

Xanthomonas fragariae has a minor to major impact in its current area of distribution. A major impact can be expected in areas where strawberries are grown during humid weather and mild temperatures. The impact is generally minor for strawberries produced in drier climates. The uncertainty is medium because few published data on yield losses due to Xf are available.

2.3.2 What is the potential direct economic impact in the PRA area? *(without control measures)*

Xanthomonas fragariae is already present in various countries in the PRA area and the conclusion is, therefore, the same as for the question 2.3.1.

Yield/quality losses due to Xf are highly variable depending on weather and cultivation conditions (see also Q 2.2.7). Besides irrigation system (drip or overhead) and choice of cultivar, also the number of years the same plants are used for fruit production may influence potential yield losses. In some strawberry producing areas/countries new plants are planted every season, which largely prevents inoculum build up during successive growing seasons. Replanting every year has for example become common practice in Belgium and the Netherlands. However, in some other areas/countries strawberry plants are used for fruit production for 2 and sometimes more years. In Austria for example a 2 years cropping period is common practice (R. Steffek, AGES, Austria pers. comm. 19th December 2012) In Finland, strawberry plants are used for 3-5 years (S. Hannunen, Evira, Finland, pers. comm. 19th December 2012). It is assessed that a multiyear cropping period increases the potential impact of Xf, but that weather conditions and frequency of overhead irrigation are more important. In the Netherlands for example, where crops are replanted every year, overhead irrigation is used during the entire cropping period on a frequent basis (about 2-5 times per week) on about 90% of the strawberry fruit crops grown in soil in open field (H. Pijnenburg (DLV), H. Boesveld (NVWA), pers. comm. January 2012). At the beginning, small volumes of water are applied but from a few weeks on, plants are usually irrigated once every day or once every two days (5-10 mm each time). Problems with Xf occur especially after overhead irrigation to prevent damage by frost early in the season and during summer (July – September). Highest disease risk is during wet periods, and with the highest risk in September. See also Annex III for a comparison of climatic data and cultivation conditions in the Netherlands and Finland.

2.3.3 Which control measures are available in the PRA area?

See also "Probability of establishment: *Effect of existing crop protection measures"*

Pesticides

Bardet (2008) reported on plant health problems on strawberry in France: "In open field and in autumn some foliar damages can appear caused by the fungus like *Ramularia tulasei* or *Zythia fragariae* or by the bacterium *Xanthomonas fragariae.* Generally fungicides formulated with copper applied in the first symptoms are active." Copper pesticides can prevent new infections and further spread of the disease but do not kill the bacteria (Tomlin, 2009). Roberts *et al*. (1997) tested the efficacy of a mixture of cupric hydroxide and mancozeb against angular leaf spot caused by *Xanthomonas fragariae*: application of the mixture of at a reduced rate (0.1x) significantly reduced diseased severity in one season but not in another season. The mixture applied at the label rate significantly decreased disease severity in both seasons but was phytotoxic and resulted in lower yields. The mixture could not completely control the disease. Copper-containing pesticides have been authorised in several EU-countries but not in all (EU pesticides database, 2010). In strawberry, copper containing pesticides are allowed in several EU-countries, e.g. in France, Greece, Italy and Spain but for example not in the Netherlands, Germany, Denmark, UK and Belgium (Pesticide databases, 2010). Two active components, benzothiadiazole (BTD) and azoxystrobin (AS) were reported as having moderate to low effectiveness in the control of Xf. BTD reduced the average number of Xf-lesions on infected plants by 54% and AS by 21%, while total crop yield in Xf-infected strawberry plots was 9% (BTD) and 28% (AS) higher than in the untreated control (Paulus & Vilchez, 1998). It is obviously debatable if the increased yield was solely caused by the suppression of Xf. Both compounds are approved active substances in the European Union (European Commission, 2011). Antibiotics like streptomycine sulphate and kasugamycine are no longer registered in the EU as plant protection products.

(Partially) resistant cultivars

Strawberry cultivars vary in Xf susceptibility (e.g. Desmet *et al*., 2009). Two resistant genotypes have been reported (Maas et al., 2000; Maas et al., 2002). Even after artificial infiltration of Xf into the leaves, the plants remained symptomless. The resistance was not of a hypersensitive nature, and oddly enough, the Xf population in susceptible and resistant plants at the point of inoculation was comparable even 17 days post-inoculation (Hartung et al., 2003). The apparent resistance is not complete (Hildebrand et al., 2005). Moreover, resistance breeding is hampered by negative traits in the 2 resistant genotypes, such as susceptibility to other important diseases and production of small fruits (Maas et al., 2000). Cultivars that are fully resistant are scarce or not available. However, some commercial cultivars can tolerate foliar infections because the direct impact on yield is thought to be minimal (Turechek & Peres, 2009). Dr. A. Jamieson (Atlantic Food and Horiculture Research Center, Kentville, Canada) reported improved resistant genotypes of *Fragaria* x *ananassa* from resistance donors US 4808 and US 4809 derived from *Fragaria virginiana* (the 7th International Strawberry Symposium atChangping District, Beijing, China; February 18-22, 2012).

Heat treatment

Recently, heat treatment as a supplementary control measure for nursery stock has been proposed (Turecheck & Peres, 2009). Although the treatments reduced Xf numbers and symptom development considerably, the disease could not be completely eradicated without significant plant mortality. Heat treatment also had an adverse effect on flowering and bud break, which renders this technique inappropriate for disease control in production plants.

Cultivation methods

The following cultivation methods are relevant to control/prevent the disease (see also Vermunt & Van Beuningen, 2008):

- Use of pest-free (certified) planting material. Visual inspection is insufficient because the bacterium can be latently present. Laboratory testing of plants is recommended.
- Restricted use of overhead irrigation.
- Cultivation under cover (prevents spread by precipitation).
- Decontamination of hands, shoes, tools and machinery.
- Geographically separated planting material and fruit production.

Monitoring and hygiene measures and especially Xf-testing of planting material, have been successful at controlling the disease at production sites in the Netherlands (Vermunt & Van Beuningen, 2008).

Conclusion

Under humid conditions, Xf is very difficult/impossible to control. Good cultivation practices are crucial to prevent introduction and spread of the disease.

2.3.4 What is the expected economic impact when the pest would become introduced? *(with the use of control measures)*
Xf is already present in large parts of the PRA-area. In several EU-countries, no pesticides are available to control the disease. In those countries, cultural practices (for example use of certified (pest-free) planting material, no use of overhead irrigation or protected cultivation are the only ways to control the disease. Thus, the answer (final rating) to this question is the same as to question 2.3.1 (see above).

Environmental impact

2.3.5 What is the expected environmental impact in the PRA area?
Minimal (low uncertainty): Xf is not known to have a (significant) environmental impact. although it has been introduced into many new areas. The pathogen is already present in large parts of the PRA area without any known impact on native plants. Xf is known to attack the cultivated strawberry *Fragaria* x *ananassa.* There are only a few reports on infection of other *Fragaria* spp. (see Q 2.1.1)

Social impact

2.3.6 What is the expected social impact in the PRA area?
Xf can occasionally cause severe losses in strawberry fruit production. In such cases growers may lose income which can have a major social impact at the farm level. However, despite its fairly widespread presence in the EU, Xf did not yet have a major social impact beyond the farm level. Therefore, the expected social impact for strawberry fruit growers in the absence of the current phytosanitary measures are rated as minor with a medium uncertainty.

An important social impact for strawberry plant growers is the measures implemented because of the quarantine status of Xf itself. In Belgium, for example, the plant health service prohibits strawberry cultivation (both for the production of planting material and fruits) for 1 or 2 growing seasons on any field where Xf was diagnosed from, depending on the level of strawberry plants (including debris) clean-up, (L. Swillens, FASFC, Belgium, personal communication, 09/03/2012). Because strawberry fruit cultivation is a profitable field usage, a mandatory switch to other, less profitable crops will cause financial distress to the afflicted land owner.

Export markets

2.3.7 What is the expected impact on export markets for the PRA area?
Plants for planting, other than seeds, of *Fragaria* are regulated for Xf in several countries outside the EU. They are regulated in at least Chili, India, Iran, Croatia, Montenegro, New Caledonia, Serbia, Tunisia and Turkey (Client Export, NVWA, the Netherlands, last access 22nd June 2012), Norway (http://www.mattilsynet.no/english/plant_health, last access 20th June 2012), Switzerland and Mexico (Dr. Richard Nelson, Plant Sciences Inc., CA, USA; personal communication, 29/02/2012 & 08/03/2012). According to EPPO (2011a), Xf is also a regulated pest in East Africa, Southern Africa and New Zealand. At present, European nurseries already export plants of *Fragaria* to several of these countries despite the presence of Xf in Europe and the quarantine status in the destination countries. Export figures on strawberry planting material are unavailable but some qualitative information was obtained from Plantum, the Netherlands (February 2011). Within the EU, the Netherlands, France, Belgium, Italy, Spain and Poland are the main producers of strawberry planting material in the EU. The Netherlands mainly trade strawberry plants within the EU including nearly all EU-countries. However, there is also an increasing export-market in non-EU countries including Russia, South-Africa, Chile, Turkey and Japan (information from Plantum, February 2011). If the quarantine status were lifted in the EU, specific requirements could be installed for those companies that

want to export strawberry plants to third countries where Xf is a quarantine pest (e.g. plants issued from mandatory certification schemes, which is further discussed in chapter 3 of this PRA "Identification and evaluation of risk reduction options").

Export of strawberry fruit will probably not be affected because strawberry fruit is not known to be regulated in countries where Xf has a quarantine status (New Zealand has general requirements for import of fresh fruit including strawberry (MAF, 2012)). Moreover, Xf is already present in major strawberry fruit producing countries in the EU. Spain is for example the first major fresh strawberry (*Fragaria* × *ananassa*) producer in Europe and worldwide the third one behind China and the USA (Cubero *et al.*, 2009).

Conclusions on impact

Economic impact: minor to major (medium uncertainty). *Xanthomonas fragariae* has a minor to major impact in its current area of distribution. A major impact can be expected in areas where strawberry fruits are grown during humid weather. The impact is generally minor for strawberry fruits produced in drier climates. The uncertainty is medium because few data on yield loss due to Xf are available.

Under favourable conditions for the pathogen, control is difficult. Use of pathogen-free planting material, restricted use of overhead irrigation and hygiene measures are the main control options for strawberry fruit growers. The uncertainty of this assessment is medium: the pest is already present in the EU for at least several decades but little has been published on its impact.

It is possible that Xf has already been spread throughout most of the PRA area with traded propagation material, but has only been reported in areas where conditions are favourable for disease development and growers suffered damages.

Export markets: at present, strawberry plants are exported to third countries where Xf is a quarantine pest. Lifting of the quarantine status in the EU may negatively influence the export to these countries.

Environmental impact: minimal, with low uncertainty. Xf is not known to have a (significant) environmental impact. The pathogen is already present in large parts of the PRA area without any known impact on native plants. Xf is known to attack the cultivated strawberry Fragaria x ananassa. There are only a few reports on infection of other Fragaria spp.

Social impact: minor (medium uncertainty). No major social impact has been reported in the EU. Occasionally, local outbreaks can lead to a high economic impact and subsequently a high social impact for individual strawberry fruit growers. Also note that the current phytosanitary measures can have a large economic impact for young plant nurseries which in the worst case can lead to bankruptcy and thereby a high social impact at the farm level. Also see below ("Identification and evaluation of risk reduction options").

3. Identification and evaluation of risk reduction options

3.1 Indicate the pathway. The pathway is "import or trade of plants for planting of *Fragaria* sp. other than seeds"

3.2 Identification of risk reduction options

Table 3.1: overview of possible risk reduction options for the pathway "import or trade of planting material of *Fragaria* sp."

Risk Reduction Option	Reduction of risk	Justification[1]
Options at the place of production		
Detection of the pest at the place of production by inspection or testing	yes	Visual inspection not sufficient because of latent infections. Testing: probability level depends on sampling intensity, sample volume and detection limits of testing method.
Prevention of infestation of the commodity at the place of production: - use of resistant cultivars, - growing the crop in specified conditions (e.g. physical protection), - crop treatments, and/or - harvest at certain times of the year or growth stages	yes	Resistant cultivars are not (sufficiently) available. Some pesticides could reduce the risk but not ensure Xf freedom (see also question 2.3.3). Physical protection can be used to prevent splash dispersal by rain. Tissue culture in combination with protected cultivation of the plants for planting will largely reduce the risk
Establishment and maintenance of a pest-free production site, pest-free production place or pest-free production area	yes	Pest-free production site: low natural dispersal distance. Testing will be needed because of the possibility of latent infections.
Options after harvest, at pre-clearance or during transport		
Detection of the pest in consignments by inspection or testing	yes	Probability level depends on sampling and testing intensity
Removal of the pest from the consignment by treatment or other phytosanitary procedures (remove certain parts of the plant or plant product, handling and packing methods)	limited	Heat treatments have been shown to strongly reduce bacterial populations in infected planting material but not to zero. Negative effects on plants can occur depending on cultivar.
Options that can be implemented after entry of consignments		
Detection during post-entry quarantine	yes but not feasible	In principle possible but not feasible for trade of strawberry plants within the EU. Plants should be placed under quarantine conditions and at conditions that are favourable for disease development to allow detection.
Consider whether consignments that may be infested be accepted without risk for certain end uses, limited distribution in the PRA area, or limited periods of entry, and can such limitations be applied in practice	no	Pathway is propagation material
Effective measures that could be taken in the importing country (surveillance, eradication, containment) to prevent establishment and/or economic or other impacts	yes	Eradication is possible, but latent infections can make it difficult to determine the size of the area infested.

[1] a more detailed justification for those options which reduce the risk is given below.

Detection of the pest by inspection and testing

Inspection
Visual inspection only reduces the risk to a limited extent because pathogen presence can be symptomless especially under dry and/or hot conditions. Roberts *et al*. (1996) for example observed that plants which had symptoms in the spring had no symptoms the following August in Florida in several years. Moreover, planting material is often stripped of most leaves before trading, removing most symptoms (if present) in the process. Infected rhizomes do not show symptoms, despite high bacterial concentrations may be present. Symptoms in the rhizome which are currently attributed to Xf are mostly linked to infections with other pathogens (Verjans *et al*., 2012). Leaf spots may be visible on remnants of leaves still attached to the planting material. However, it has been reported that the translucent leaf spots are no longer visible after only one day at room temperature after cold storage of the planting material (EPPO, 1997).

Testing
Xf is a very slow-growing bacterium in culture and is easily overgrown by saprophytic bacteria; selective media are not yet available. Therefore, isolation plating is not suited for the detection of low Xf-numbers in symptomless plants. Several PCR detection methods each targeting different loci in the Xf genome have been developed (Roberts et al., 1996; Zimmerman et al., 2004; Weller *et al*., 2007; Vandroemme *et al.*, 2007, 2008; Vermunt & van Beuningen, 2008; Turecheck *et al.*, 2008). These methods can be used to confirm the presence of Xf in symptomatic plant material but several of them have also been used to for the detection of latent Xf-infections (Mahuku & Goodwin, 1997; Zimmerman et al., 2004; Moltman & Zimmerman, 2005; Vermunt & van Beuningen, 2008). Moltman & Zimmermann (2005), for example, applied a PCR and nested PCR, based on the multiplex PCR of Pooler *et al.* (1996), on 262 plant samples during a German survey. Symptoms were observed on 2.3% and 8.0% of the samples in the field and laboratory, respectively, whereas 17.9% and 24.8% of the samples tested positive in the PCR and nested PCR-test, respectively. Vermunt & van Beuningen (2008) used 20 leaflets in one composite sample for PCR-testing and found positive tests on 6 symptomless plant samples from a plant nursery which included samples of new cultivars from abroad. When using a PCR-test for latent testing, validation of the specificity of the test will be crucial because isolation plating will usually not be suitable for confirmation. Zimmerman et al. (2004) were able to confirm positive PCR test results by a bioassay but these bioassays are very time consuming and a positive test could not always be confirmed by the bioassay. Alternatively, PCR-primers targeting another genomic locus could be used for confirmation or one could rely on the positive results of one validated PCR assay. Validation of a PCR assay according to EPPO guidelines is described below.

Validation of the real-time (TaqMan) PCR assay developed by Weller et al (2007) for the detection of *X. fragariae* in leaves and runners of *Fragaria* spp. has recently been completed in the Netherlands (FES final report WP3, 2010). This validation has been performed according to the EPPO guideline for the validation of detection methods for plant pathogens and pests. During this validation, the limit of detection (LOD), measuring range, trueness, analytic specificity, selectivity, robustness, repeatability and reproducibility of the assay has been assessed. The LOD for detection, as determined by spiking runner- and leaf-extracts of two different *Fragaria* spp. cultivars with five *X. fragariae* isolates, varied between the 10^3 and $2x10^4$ CFU/ml. These LODs are approx 100x lower than those for the conventional PCR described by Pooler et al. (1996), making the assay more suitable for detection of lower population densities in infected plants also in cases of latent strawberry material, especially in combination with a second TaqMan PCR. Further on, the assay exhibited a high analytic specificity as it was able to detect all *X. fragariae* reference isolates used and did not give any cross reactivity with either plant DNA nor with DNA of other Xanthomonads*,* including *X. arboricola* pv. *fragariae*. The repeatability and reproducibility were both 100%. The type of the plant material (leaf or runner) does not seem to interfere with the result of the TaqMan PCR assay, however, the cultivar had a significant influence possibly due to differences in plant compounds inhibiting the amplification process. This cultivar effect did even occur after the purification step on a PVPP column (see also the paragraph below on DNA-extraction). Yet, the real-time PCR has been shown to be a valid tool for the detection of *X. fragariae* in plant material. The full validation data is available in the website of EPPO: http://dc.eppo.int/validationlist.php

The detection limit of a PCR assay is important for detection of asymptotic presence of Xf. Roberts *et al*. (1996) and Zimmerman *et al*. (2004) found higher infection percentages using nested PCR than with one round of PCR. Even a highly sensitive nested PCR assay may give false negatives as suggested by data from Roberts *et al*. (1996). They first found a decrease in the number of positive tests using the nested PCR assay while later in the season the number of positive test increased. The limit of detection in the assay of Roberts *et al*. (1996) was determined as approximately 10^5 to 10^4 CFU/ml with one round of PCR; nested PCR detected 1,000 fold less cells. The real-time (TaqMan) PCR developed by Vandroemme *et al*. (2007) has a detection limit of 3 x 10^3 CFU/g of leaf tissue. The PCR assay which has recently been validated according to EPPO guidelines and which has a detection limit of approximately 10^3 – 2 x 10^4 CFU/ml (may vary among strawberry cultivar and may even be higher for certain cultivars) will likely not detect all latent Xf infections. Nested PCR would be more sensitive but has the disadvantages of a higher risk for false positives and being more labour intensive.

Performance of a PCR based detection method will not only depend on the specificity of the primers but also on DNA extraction methods. Indeed, strawberry tissue is notorious for its PCR-inhibiting compounds and rigorous DNA extractions are often required to allow optimal sensitivity (Vandroemme *et al.*, 2008). For DNA isolation of Xf from strawberry plant material (leaf or runner), a method has recently been developed in the Netherlands (FES final report WP3, 2010). Bacteria are concentrated by application of a centrifugation step directly after extraction. DNA isolation is then performed by using a Bio-Nobile kit QuickPick SML Plant DNA (in combination with a Kingfisher Flex 96 extractor) and a purification step on a PVPP column, prior to the TaqMan PCR. Additionally, an internal control, involving *Xanthomonas campestris* pv. *campestris* can be used to ensure that no inhibition of the Taqman PCR has occurred.

The detection efficiency of latent testing will largely depend on the sample size and the plant parts sampled (e.g. Been *et al*., 2008; Van der Wolf *et al*., 2008). Leaflets from old leaves (before plants are stripped from most leaves) seem to be most appropriate as sampling unit because Zimmerman et al. (2004) found higher infection percentages in older leaves than in young leaves and crowns. Xf was not detected in the roots. The number of units to be sampled will often be a compromise between the detection efficiency required and the costs for sampling and sample analysis. For the detection of a 1.5% incidence with a 95% confidentiality, a sample of 200 units would be needed assuming a random distribution and no false negatives (which will probably not be the case in many instances). Note that in areas where conditions are unfavourable for symptom development and symptoms may even disappear while the bacterium is still present (e.g. Roberts et al., 1996), testing for latent infections in addition to visual inspections will be more effective than in areas where conditions are favourable for symptom development. When sampling and testing for latent infections is to be conducted routinely in commercial fields, the production costs of strawberry plants will increase. In the Netherlands, a rough estimation on the costs of a TaqMan PCR assay (including DNA isolation and purification as described above as well as inclusion of an additional internal control) refers to an amount of € 150 per sample. In case five composite samples, with 30 whole leaves each, need to be analysed per ha, laboratory costs would be approximately € 750 per ha (costs for sampling not included; they will probably be low compared to the laboratory costs). This amount would be approximately 0.5 - 2 % of the crop value (crop value has been assessed to vary between € 40,000 and

135,000 per ha depending on the kind of plant material). The relative costs of sampling and testing will increase for plots smaller than 1 ha because the number of units to sample will remain the same (except for very small plots) to obtain similar detection efficiencies.

A sampling (30 leaves per sample including petiole and leaf axil) and testing method for latent infections of Xf has recently been implemented by Naktuinbouw in the Netherlands. They use a multiplex PCR based on primers and probes from Vandroemme et al. (2008) and Weller et al., (2007) already mentioned above.

<u>Prevention of infection of planting material at the place of production</u>

Resistant cultivars
No cultivars have been developed that are fully resistant to this disease. According to Maas (2004), work is in progress to develop resistant cultivars. See question 2.3.3 for more details.

Pesticides
There are some pesticides with a limited effect on Xf but they cannot fully prevent infection of the crop. See question 2.3.3 for more details on the efficacy of pesticides.

Tissue culture
Propagation of *Fragaria* plants through tissue culture is under development and may become more important in the future (pers. comm. H. Koenraadt, Naktuinbouw, December 2011). This system could contribute to the production of disease free planting material. However, bulk production of later generations of *in vitro* derived planting material will occur in the field and can, therefore, still be exposed to Xf-infection.

Physical protection
The risk of infection and further spread can be strongly reduced by growing the plants under protected conditions without using overhead irrigation. However, plants may harbour Xf without expressing symptoms because of the unfavourable conditions for disease development. Random sampling and testing could, therefore, be used in combination (see also above "testing").

Pest-free production site or place
This option is effective but strict hygienic measures will be needed to maintain a pest-free status in areas where Xf is present. Visual inspections and testing will be needed to confirm the absence of the pathogen. In principle, the requirements can be limited to a defined proportion of a production place (a production site which could be defined as a field plot within the production place) because of the low natural dispersal capacity of the pathogen. However, the disease might have been spread by human assistance (e.g. machinery) to other plots and measures imposed on the whole production place will, therefore, reduce the risk more than measures limited to the field where Xf has been found. Nurseries can use hygiene measures (e.g. disinfestation of machinery, shoes etc) to avoid spread between plots (e.g. Vermunt & van Beuningen, 2008). Removal of *Fragaria* plants in the direct vicinity (e.g. 10 m) of the production place or site will reduce the risk of natural infections from outside the production place or site. However, it is assessed that the risk reduction level will be mainly determined by sampling intensity (see above "Testing").

Pest-free area
This option is effective. Intensive surveys including sampling and testing will be necessary to ensure pest freedom of the area since Xf can be symptomlessly present. Preferentially, any pest-free area would employ strawberry cultivation methods that are highly conducive to angular leaf spot development, to increase the detection chance in case of Xf-infection.

Options after harvest or during transport

Treatment
Heat treatment (HT) has been shown to be effective against systemic pathogens and has been used to significantly reduce angular leaf spot infections on strawberry nursery stock (Turechek & Peres, 2009). However, there is a strong cultivar limitation to be considered when using these treatments. Still, for those cultivars where HT is an option, disease can be reduced throughout the propagation cycle and its repeated use will lower pathogen populations overall.

Options that can be implemented after entry

Eradication
Eradication is possible in some cases because of the slow natural spread of the pathogen. The pathogen has been reported as eradicated in New Zealand, Australia (New South Wales) and the UK (Gillings *et al*., 1998; Matthews-Berry & Reed, 2009). The measures taken in these countries were:

New Zealand: all strawberry plants within infected plots were destroyed; adjacent plots were sprayed with Bordeaux mixture. The area was quarantined for two years (Dye & Wilkie, 1973).

Australia (New South Wales): all strawberry plants in the infected area together with all plants within 10 metres of an infected block were removed and burnt. The area was cleared of all dead strawberry leaves and drenched with 1% formalin. The area was quarantined for two years (McGechan & Fahy, 1976)

UK: growers were given the choice of either immediate eradication which required the immediate removal of all plants from infected plants or a containment option which allows infected plants to be retained for fruit production but with hygiene measures to reduce the risk of spread of the bacterium to uninfected plants on the place of production. With the second option, hygiene measures were to be continued until the disease had not been detected during an entire season (Matthews-Berry & Reed, 2009). Growers (two in total) chose the containment option. At both production sites, the disease was no longer observed in the subsequent season. The infected crops were covered with polytunnels the season following the identification of Xf and it is possible the covered cultivation contributed to the successful containment and eventual eradication of the pathogen which has not been detected at either site in subsequent seasons.

In conclusion, there is a good chance of eradication of a limited Xf-outbreak. However, latent infections may prevent complete sanitation. The absence of symptoms is no certainty that the pathogen has been eradicated. Also, Xf in planting material may have been spread extensively in a new area before it is detected, significantly reducing eradication success. Eradication strategies may, therefore, be practically impossible in areas with extensive planting material traffic.

3.3 Certification (Council Directive 2008/90/EC)
Council Directive 2008/90/EC of 29 September 2008 replaces Directive 92/34/EEC "on the marketing of fruit plant propagating material and fruit plants intended for fruit production" from 30th September 2012 on (http://ec.europa.eu/food/plant/propagation/index_en.htm; accessed November 2010). The directive contains the general requirements for the production of propagation material and fruit plants. Propagation material includes seeds and all plant material intended for the propagation and production of fruit plants. Fruit plants are defined as "plants intended to be planted or replanted, after marketing". The directive stipulates

that plant propagation material and fruit plants of genera and species listed in the Annex, including *Fragaria* L. may only be marketed if they are either CAC (Conformitas Agraria Communitatis), pre-basic, basic or certified material. The conditions to be met are most strict for pre-basic and basic material and least strict for CAC-material. Certified material should be produced directly from basic or pre-basic material. Certification schemes can be implemented on a voluntary basis by EU member states and a certification scheme for the production of certified pathogen tested material of strawberry has been described by EPPO (2008). It includes the following successive steps:

1. selection of candidate nuclear stock
2. production of nuclear stock
3. maintenance of the nuclear stock
4. production of propagation stock
5. production of certified material

Production steps 2 – 4 should be performed under conditions ensuring freedom from infection via pollen, aerial or soil vectors, e.g. in an insect-proof glasshouse or a gauzehouse. Such conditions cannot ensure prevention of contamination with Xf but this will be minimized considering the low natural dispersal capacity of the pathogen. Production of certified material should be grown under conditions minimizing infections. The scheme indicates a zero tolerance in visual inspection for Xf at each stage of certification. However, visual inspections cannot ensure pest freedom since Xf can be symptomlessly present (see above).

In several European countries, certification schemes have been implemented. In the Netherlands, the certification scheme is under supervision of Naktuinbouw (http://www.naktuinbouw.nl/en/topic/naktuinbouw-elite). One of the requirements is that candidate nuclear stock should be tested and found free of Xf. Propagation material derived from nuclear stock is visually inspected for symptoms of Xf. In case of any (suspicious) symptoms a sample is taken and tested for Xf. In case Xf is found, an area is demarcated around the diseased plants: 150 m in the length of the strawberry bed in both directions and an area of 25 m at both sides of the strawberry bed or if a tram line is present within this 25 m, the tram line is used as demarcation border. Diseased plants and those in a buffer area around them should be removed and destroyed according to the phytosanitary instructions of the NPPO of the Netherlands. The buffer includes plants in the length of the strawberry bed, 20 m in both directions from the diseased plants and plants in 2 strawberry beds at both sides of the strawberry bed with the diseased plants (thus 2 beds at the left and 2 beds at the right side; one bed is 1.5 – 1.6 m width). If more than 2 foci are found in a plot or more than 10% of the plot is within the buffer/diseased area, all plants in the plot have to be removed and destroyed. Any remaining plants in the demarcated area may only be traded as CAC-material. The other plants on the same plot but outside the demarcated area may only be traded as E-plants, the lowest category in the certification pyramid system (pers. comm. F. Claassen, Naktuinbouw, 17th September 2012; http://www.naktuinbouw.nl/en/topic/naktuinbouw-elite; last access 24th September 2012).

3.4 Current phytosanitary legislation (Council Directive 2000/29/EC)

Xf is regulated for plants of *Fragaria* L., intended for planting, other than seeds (Annex II of 2000/29/EC). Specific requirements are formulated in Annex IV Part A section I article 19.2 and section II article 12 for plants originating from outside the community and plants originating in the community, respectively. These requirements are that no symptoms may have been observed at the place of production since the beginning of the last complete cycle of vegetation (or plants should originate from a pest-free country or area). The requirement that the whole production place should have been free of symptoms will probably be more strict than the requirements in (most) national certification schemes. Certification schemes may only demand that symptomatic plants (and a buffer around these plants) are removed. However, the current legislation also does not ensure pest freedom of the production place because of the possibilities of latent infections (see above).

3.5 Selection of risk reduction options

A 100% certainty level that plants are free of Xf is difficult to achieve in areas where the pathogen is present because of the possibility of latent infections, the possible risk of spread with animals, machinery etc. To reduce the risk of entry and spread of Xf with import and trade of planting material of *Fragaria* plants, visual inspections could be combined with testing. Below five possible (de)regulatory options are discussed ranging from most stringent to least stringent (deregulation).

<u>Option I: pest-free area</u>

In this option, production of planting material is conducted in a validated pest-free area (country, region). Extensive monitoring and testing of all strawberry production sites in the area is needed and import of new planting material in the area will require thorough laboratory testing to ensure an Xf-free status. Ideally, the pest-free area would have climatic conditions or employ cultivation methods that are highly conducive to angular leaf spot development. This will ensure quick symptom development and therefore timely detection in case of an Xf-outbreak. Although this option would be very effective, maintaining a pest-free area for Xf would be laborious and costly. Most strawberry producing areas currently considered free of Xf are geographically isolated (UK, Australia, New Zealand), but still have reported several Xf-outbreaks and subsequent eradications (Dye *et al.*, 1973; Mc Gehan & fahy, 1976; Gillings *et al.*, 1998; Matthews-Berry & Reed, 2009; Young *et al*., 2011). The current intensive trade of strawberry planting material and the possibility of latent Xf-infections could make the establishment of pest-free areas on the European continent very hard if not impossible to achieve. Indeed, the current quarantine measures are unable to prevent introduction and general spread of the pathogen in the EU.

<u>Option II: pest-free production place based on visual inspections and testing for latent infections</u>

This option requires that no symptoms may have been observed at the place of production since the beginning of the last complete cycle of vegetation and randomly chosen plants must have been tested and found free of the pest. A place of production has been defined as "any premises or collection of fields operated as a single production or farming unit" (FAO, 2007). Thus, the finding of the pest on one field will have consequences (quarantine measures) for the entire production place. Option II is more stringent than the current legislation because pest freedom is now only based on visual inspections. Note that testing in addition to visual inspections will reduce the risk level but cannot ensure pest freedom because of detection limits of the PCR-assays available and limits in the number of samples that can reasonably be taken and analyzed as discussed above (see the paragraph "Testing" in Chapter 3.2). The implementation of testing regimes for the production of strawberry plants will increase the economic impact of the measures for plant nurseries because it is expected that more infections will be found with more intense testing. Detection of the harmful organism would result in a trade-prohibition for the afflicted company. Also, the production costs will increase due to the costs for sampling and testing (see 3.2 "Testing"). It is uncertain if this option will be cost-effective compared to the present less stringent regime based on visual inspection alone. Xf is already fairly widespread and the pathogen does not cause much damage in most instances.

<u>Option III: pest freedom of the crop (field) based on visual inspections and testing for latent infections.</u>

This option requires pest freedom of the crop or field (a defined portion of a place of production). It requires that no symptoms may have been observed in the crop or field since the beginning of the last complete cycle of vegetation and randomly chosen plants must have been tested and found free of the pest. In case of the finding of the pest, quarantine measures are limited to the crop or field in which Xf has been found.

The risk reduction level of this option will be lower than that of option II (pest freedom of the production place). When Xf is found in one field it may have been transmitted to other fields by machinery, personnel etc. where it may be present at undetectable levels. On the other hand, the economic and social impact for plant nurseries will be lower than that of option II because trade will still be allowed from other fields if no indications of pest presence has been found in those fields. The costs for testing and detection limits will, however, be the same for both options.

Option IV: pest freedom of the crop (field) based on visual inspections
This option is the same as option III with the exception that the crop or field is only visually inspected. In case of a pest find, the whole field is rejected. A less strict approach (with a lower level of risk reduction) would be to remove only the symptomatic plants and a buffer around these plants. In the Naktuinbouw-certification scheme for example a buffer zone (25 m at both sides and 150 m in the length of the strawberry bed in both directions) is used for the production of certified plants (see above: question 3.3). The larger buffer zone in the length of the strawberry bed is recommended because the bacterium may have been spread by machinery over longer distances in that direction. Note that it is unknown how far the disease can be spread by machinery under practical conditions but the risk of infection is assessed to decrease with the distance from the source. In case of more than 2 foci within a plot or a relatively large proportion is affected the whole plot could be rejected. This option will reduce the risk to a lower extent than option III because infected plants may not always develop symptoms, especially not during warm conditions (temperatures above 30°C).

Option V: no EU regulation
In this option, pest freedom will be mainly determined by voluntary measures (including voluntary entering of certification schemes) by plant nurseries and their interest to grow pest-free planting material (i.e. strawberry fruit producers may require planting material that is free of the pest). Xf could be implemented in national certification schemes as far as it has not been done yet (see Q 3.3). These certification schemes require several measures to reduce the risk of infection by pathogens (see above 3.3 "Certification"). Sampling and testing for Xf could be part of certification schemes especially for the early propagation steps in the pyramid system. Compared to the current EU Xf-regulation, option V can increase the transparency about the Xf-contamination risk of a certain batch of planting material. The current visual inspections are insufficient to ensure Xf freedom as suggested by its quarantine status. Currently, growers may be reluctant to have their planting material tested for Xf because of the quarantine status, inhibiting detection at an early stage. A disadvantage of risk reduction option V could be an increased Xf-incidence if growers primarily opt for lower quality planting material (i.e. CAC-material in stead of certified material).

Conclusions on risk reduction options

The current EU-requirement, the absence of Xf symptoms in *Fragaria* plants for planting, is not sufficient to ensure pest freedom mainly because of the possibility of latent infections. Testing methods are available to detect latent infections but the probability of detection largely depends on the sampling intensity. Five possible (de)regulatory options have been discussed ranging from most stringent to least stringent:

I. Pest-free area
II. Pest-free production place based on visual inspections and testing
III. Pest freedom of the crop/field based on visual inspections and testing
IV. Pest freedom of the crop/field based on visual inspections
V. No EU regulation (Xf can be implemented in national certification schemes)..

The implementation of more stringent requirements for the production of strawberry plants (options I, II and III) will decrease the risk of entry and spread of the pest but will increase the economic an social impact of the measures for plant nurseries. It is uncertain if such stringent measures will be cost-effective as Xf is already fairly widespread in the PRA area and the pathogen does not cause much damage under dry conditions. In disease conducive strawberry production areas (e.g. production for the fresh market during humid conditions) the use of Xf-tested planting material will be more important than in production areas which are less favourable for disease development and spread of the disease. Therefore, more data are needed on Xf-distribution and cultivation methods in the different EU strawberry production areas to improve the risk assessment of further EU spread of Xf and determine the most cost-effective risk reduction option(s).

4. Uncertainties

The main uncertainties in the present PRA are:

- The current distribution of Xf in the EU. Xf may be more widely distributed than presently known. Xf might have been spread throughout most of the strawberry producing areas in the PRA-area through trade of latently infected planting material, but only reported in areas where conditions are favourable for disease development and growers suffered damages.
- The current impact of Xf in the EU: only a few publications are available on yield effects.
- The current risk associated with import and trade of planting material because of possible latent infections
- The cultivation methods in the different strawberry producing areas within in the EU, related potential impact of Xf and indication of most endangered areas
- The possibility of seed transmission: no studies known on seed transmission
- The host plant status of *Potentilla* spp. and *Fragaria* spp. other than *Fragaria* x *ananassa*
- The effect of Xf on susceptibility of strawberry plants to other diseases

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Annex I : Distribution of *Xanthomonas fragariae* in the EU

Information on the distribution and prevalence of Xf in several EU-countries was obtained from literature, reports, websites and the EPPO PQR-database (EPPO, 2011a):

Austria
According to the website of the Austrian Agency for Health and Food Safety (AGES), Xf is locally present in Austria (AGES, 2010)

Belgium
According to Bultreys *et al*. (2000), Xf was introduced into Belgium in 1998 through import of infected or contaminated strawberry plants from the Netherlands and France. According to ILVO (1998 – 2000), the experimental station of Hoogstraten made the first observation in 15 different strawberry fruit production companies in Flanders in 1997. In 2003-2004, the pest was present on 20 to 25 % of strawberry fruit production companies in Flanders.
Official pest status (2011-01): present, subject to official control (EPPO, 2011a)

Bulgaria
No information found in literature. However, one interception of Xf on plants originating from Bulgaria has been reported in Europhyt (see Table 3).
Official pest status (2011-01): present, limited distribution (EPPO, 2011a)

Cyprus
Absent or no information
Official pest status (2011-01): absent, not known to occur (EPPO, 2011a)

Czech republic
Absent or no information
Official pest status (2011-01): absent (EPPO, 2011a)

Denmark
Not known to occur in Denmark (NPPO of Denmark, January 2013).

Estonia
First finding in 2009 at a strawberry fruit production site (IPPC, 2009). The planting material used originated from another EU member state.
Official pest status (2011-06): "found in 2009 in one place of production, eradicated. Not found during the 2010 official surveys"; absent, pest eradicated (EPPO, 2011a)

Finland
First finding at a strawberry fruit production site in July 2011. The strawberry plants had been delivered by a Dutch company in 2010. The bacterium had possibly been introduced with the planting material because there are no previous records of *X. fragariae* in Finland. All plants of the infested lot will be destroyed and cultivation of strawberry will be prohibited for two years at the production site (EPPO, 2011b).

France
First finding of Xf at a strawberry fruit production site in 1973 in the South-West of France (Rat, 1975). Bardet (2008) reported on plant health problems on strawberry in France: "In open field and in autumn some foliar damages can appear cause by fungus like *Ramularia tulasei* or *zythia fragariae* or by bacteria *Xanthomonas fragariae.* Generally fungicides formulated with copper applied in the first symptoms are active." In some papers about findings in other countries it was reported that infected planting material had originated from France (Panagopoulus *et al*., 1978; Bultreys *et al*., 2000). Details on the situation of *Xanthomonas fragariae* on strawberry in different "départements" in France according to French experts are given by Bardet (2012):

Département (region)	Situation
Nord & Pas de Calais (Nord Pas de Calais)	Not a problem until today. Some cases but still rare
Morbihan (Bretagne)	Anecdotal presence only in open field
Maine et Loire (Pays de la Loire)	Very low prevalence in 2011 compared to 2010 when cv. Candiss was heavily attacked
Loir-et-Cher (Centre)	Rare and not a problem until today
Dordogne (Aquitaine)	No problems in protected crops. Symptoms only visible in open fields in late summer and autumn. No effects on production.
Lot et Garonne (Aquitaine)	On soil and soilless crops in autumn
Haut Loire (Auvergne)	Very low prevalence in open field.
Vaucluse (Provence-Alpes-Côte d'Azur)	Not a problem
Alpes-Maritimes (Provence-Alpes-Côte d'Azur)	Not often seen but estimated 1% of plants are affected

About the situation on young plant nurseries it was stated by an expert that *X. fragariae* is not of major concern and that the pest is present throughout Europe and the USA (Bardet 2012).

Official pest status in France (2011-01): present, only in some areas (EPPO, 2011a)

<u>Germany</u>
Xf has been reported to be present in Germany since 1994 (Billen, 1995). Moltman & Zimmerman (2005) applied a PCR and a nested PCR method on 262 plant samples taken during a survey in Germany. Symptoms were observed on 2.3% and 8.0% of the samples in the field and laboratory, respectively whereas 17.9 and 24.8% of the samples tested positive in the PCR and nested PCR-test, respectively. According to JKI (2010), the pathogen is at least symptomlessly present in the entire country; symptoms occur especially in southern Germany.
Offical pest status (2011-01) from EPPO (2011a): present, limited distribution and low prevalence.

<u>Greece</u>
Xf has been reported from Greece in the past. Panagopoulus *et al*. (1978) reported that the disease caused by Xf was observed in one field on West Peloponnesos in 1975. The disease had probably been introduced on infected material imported from France. In a subsequent survey of strawberry plantings in many localities in Greece no new findings were done. It was suggested that eradication of the pathogen was likely to succeed by destruction of the plants.
Official pest status: absent, pest not longer present (EPPO, 2011a)

<u>Hungary</u>
No information found in literature. However, many interceptions on plants originating from Hungary have been reported in Europhyt since 1997 (see Table 3).

<u>Ireland</u>
Absent or no information

<u>Italy</u>
Mazzucchi *et al*. (1973) reported Xf as a new pathogen for Italy on Sicily. The disease caused by the pathogen was observed in the Metaponto area in 1977 – 1984 (Surico & Varvaro, 1985). In 1993, Xf was isolated from field strawberries in Latium, Campania, Emilia-Romagna and in Sicily (Scortichini & Rossi, 1994). *Xanthomonas fragariae* has also been reported from the regions Piemonte, Valle d'Aosta and Emilia-Romagna (EPPO, 1998).

Latvia
Absent or no information
Official pest status (2011-01): absent (EPPO, 2011a)

Lithuania
Absent or no information

Luxembourg
Absent or no information

Malta
Absent or no information
Official pest status (2011-01): absent (EPPO, 2011a)

Netherlands
Offical pest status: present, in several areas where host crops are grown (Plant Protection Service, 2010).

Poland
Absent or no information

Portugal
Xf was reported as a new pathogen for Portugal in 1981 (Fernandes & Pinto-Ganhao, 1981).
Official pest status (2011-01): present, widespread (EPPO, 2011a)

Romania
Severin *et al.* (1985) isolated a pathogen very similar to Xf except acid production from arabinose. Several papers on Xf refer to this paper (e.g. Matthews-Berry & Reed, 2009). However, the pathogen might have been *X. arboricola* pv. *fragariae* in stead of Xf. In general: a pure culture of Xf is distinguishable from other phytopathogenic xanthomonads by at least seven characteristics (no growth at 33°C; no hydrolysis of aesculin; no acid from arabinose (whereas, *X. arbroricola* pv *fragariae* produces an acid from arabinose), galactose, trehalose, cellobiose; 0.5-1.0% maximum NaCl tolerance) (Kennedy & King, 1962b; Bradbury, 1984).
Official pest status: absent, confirmed by survey (EPPO, 2011a; NPPO of Romania, January 2013)

Slovakia
Not known to occur (EPPO, 2005). However, Xf was intercepted on plants for planting originating from Slovakia in 2003 (Europhyt, see also Table 3)
Official pest status (2011-01): not present (EPPO, 2011a)

Slovenia
Present (Brence, 2002).
Official pest status (2011-01): present only in some areas (EPPO, 2011a)

Spain
Xf was reported as a new pathogen for Spain in 1985 (Lopez *et al*., 1985). Xf is present in Andalucia (Anonymous (2007) Incidencia de plagas y enfermedades en las Comunidades Autónomas en 2006. *Phytoma-España* no. 187, 19-52 and no. 188, 16-56; source referred to by EPPO, 2007)
Official pest status (2011): it occurs wherever strawberries are grown (EPPO, 2011a)

Sweden
Not known to occur in Sweden (NPPO of Sweden, December 2012)

United Kingdom
The pathogen was identified in strawberry fruiting crops in October 2004. Plants originated from the Netherlands. The pathogen was eradicated by hygiene measures (Matthews-Berry & Reed, 2009).
Official pest status (2011-01): absent (EPPO, 2011a)

Annex II: Acreage and volume of strawberry production in the EU

Acreage (in ha) per EU-country from 2006 - 2009. Source: FAOstat (http://faostat.fao.org/site/567/DesktopDefault.aspx?PageID=567; accessed 28 April 2011).

Country	2006		2007		2008		2009	
Austria	1073		1398		1560		1253	
Belgium	1243		1115		1067			M
Bulgaria	1396		1240		1182		5807	
Cyprus	105		89		88		80	
Czech Republic	2526		2553		2467			M
Denmark	915		900	F	900	F	900	F
Estonia	814		648		564		597	
Finland	3451		3340		3225		3270	
France	3403		3266		3021		3000	F
Germany	14214		13013		13032		12800	
Greece	322		393		400	F	400	F
Hungary	484		501		599		569	
Ireland	110	F	110	F	110	F		M
Italy	5746		6033		6409		3100	
Latvia	529		341		360		319	
Lithuania	1845		1821		1640		1558	
Luxembourg	3		3		3		3	
Malta	20	F	20	F	20	F	20	F
Netherlands	1700		1700		1700			M
Poland	55600		52309		54160		53551	
Portugal	1600	F	1550	F	1550	F		M
Romania	2397		2826		2591		2507	
Slovakia	255		271		239		198	
Slovenia	104		108		124		110	
Spain	8296		8078		8134		7100	
Sweden	2203		1800		2000		2000	
United Kingdom	4500		4656		4656	F		M
TOTAL	114854		110082		111801		99142	
[ ] = Official data | F = FAO estimate | M = Data not available								

Production volume (in 1,000 tonnes) per EU-country in 2010
Source: Tike (2012) from Eurostat, statistical databases (http://epp.eurostat.ec.europa.eu).

Country	Production volume (1,000 tonnes)
Austria	16.4
Belgium	33.0[1]
Bulgaria	5.7
Cyprus	1.8
Czech Republic	2.7
Denmark	5.9
Estonia	0.5
Finland	10.3
France	46.6
Germany	156.9
Greece	42.5
Hungary	4.2
Ireland	No data
Italy	153.9
Latvia	0.6
Lithuania	2.1
Luxembourg	0.0
Malta	0.6
Netherlands	43.0
Poland	191.8
Portugal	No data
Romania	21.3
Slovakia	1.4
Slovenia	2.1[1]
Spain	275.3
Sweden	11.5
United Kingdom	103.0

1) Year 2009

Annex III: Potential impact of Xf: climate and cultivation practices

Introduction
Yield/quality losses due to Xf are highly variable depending on weather and cultivation conditions which makes it difficult to provide a more detailed impact assessment for the different strawberry fruit production areas in the EU. Frequent rainfall and overhead irrigation in combination with temperatures around 20°C are generally known as important disease favouring factors. Indeed, experimental data have shown that temperatures of 20 – 25°C are optimal for disease development after infection and also that high humidity and overhead irrigation/precipitation favours the disease and/or spread of other bacterial diseases (see Q 2.2.4 "Suitability of climate in the PRA area" for details and references).

Here, we present climatic conditions and cultivations practices in the Netherlands where Xf outbreaks in fruit crops has been reported by experts (see Q 2.3.1). The climatic data are from the province Noord-Brabant where nearly 80% of the strawberry fruit production is located in the Netherlands (CBS, 2009). The data can be used for comparison with the conditions in other strawberry fruit producing areas in the EU. Here, the data are compared with data from Finland kindly provided by S. Hannunen (Evira, Finland, January 2013) and an assessment was made of the potential impact of Xf for strawberry fruit production in Finland.

Cultivation practices and climatic conditions
Cultivation practices for strawberry fruit crops that are grown in soil in the open in the Netherlands were kindly provided by H. Pijnenburg (DLV) and H. Boesveld (NVWA) and in Finland by Tuija Tanska (Puutarhaliitto ry). In Finland about 99.5% of the production is in soil in the open. For Finland, data were gathered from 2 provinces with the largest and second largest strawberry fruit producing acreages. One of these provinces is located in the centre of Finland (Pohjois-Savo) and the other is located in south western part of the country (Varsinais-Suomi). The data are summarized below:

the Netherlands:

- Crops are replanted every year
- Harvesting period: end of May until the end of September with a peak from mid June till mid August
- Average yield volume per ha: 15,600 kg/ha (CBS, 2009; average for both soil-grown and table-grown strawberries in the open)
- Climate: Figs III.1-5
- Sprinklers (overhead irrigation) are generally used (on about 90% of the fields) during the entire cropping period on a frequent basis (about 2-5 times per week). At the beginning, small volumes of water are applied but from a few weeks on plants are usually irrigated once every day of once every two days (5-10 mm each time). Overhead irrigation is continued during the harvesting period at the same frequency.

Finland:

- Crops stay on the field for 3-5 years
- Harvesting period:
    - o In Pohjois-Savo harvesting starts in an average year in the last week of June - first week of July (in a "good" year already in the 3rd week of June and in a "bad" year in the 2nd week of July). Most commonly (i.e. for the common varieties and when plants have not been planted the same year), harvesting ends by the end of July.
    - o In Varsinais-Suomi harvesting starts in an average year in mid-June, i.e. $2^{nd}/3^{rd}$ week of June (in a "good" year already in the 1st week of June and in a "bad" year in the 4th week of June). Most commonly (i.e. for the

common varieties and when plants have not been planted the same year), harvesting ends 3rd week of July.

- Average yield volume per ha: 4,894 kg/ha in Pohjois-Savo; 5,515 kg/ha in Varsinais-Suomi; 4,312 kg/ha in whole Finland (Tike, 2012)
- Climate: Figs III.1-5
- About 30 % of the growers have drip irrigation systems. The remaining 70 % of the growers use sprinklers. Some do no irrigate at all. Overhead irrigation (sprinklers) is used in the spring to protect the plants against frost, whenever frost is forecasted (for this purpose sprinklers are also used in the fields that have a drip irrigation system). Overhead irrigation is used before the harvesting period. During the harvesting period fields are usually not sprinkled because it increases the risk of mold damages. After the harvesting period the fields are sometimes sprinkled to ensure successful induction of flowers. When sprinkling is used to water the plants, a relatively large amount of water is applied (no assessment of the amount of water) at a time. Sprinkling is used on an average once a week.

Discussion

According to Dutch crop experts, problems with Xf in strawberry fruit crops in the Netherlands especially occur after overhead irrigation to prevent damage by night frost early in the season and during summer (July – September) during wet periods with the highest risk in September. In Finland, the harvesting period generally ends before August, thus before climatic condtions seems to be most favourable for Xf (higher RH than in June and July). Climatic conditions in Finland (temperature and rainfall) in June and July when strawberries are generally harvested are very similar to those in the Netherlands except that the RH is lower (Figs III.1-5). Overhead irrigation is used in Finland but less commonly and at a lower frequency than in the Netherlands. In the Netherlands the plants are only used for one season while in Finland plants may be used up to 5 years which could lead to a build-up of Xf inoculum over years. However, weather conditions and irrigation practices during the harvesting period are generally considered the most important disease determining factors. Also, in other European countries strawberry crops stay in the field for more than one year while especially in the Netherlands and Belgium, where plants are replanted every year, Xf can have a major impact (see also Q 2.3.2.). Thus, it is assessed that the potential impact of Xf for strawberry fruit production in soil in the open is higher for the Netherlands than for Finland under the current cultivation practices. Wet periods can, however, also occur under Finish conditions and it is assessed that introduction of Xf can lead to quality and yield losses in strawberry fruit production in Finland. It should be noted that no Xf documentation on quality or yield losses in strawberry fruit production in Europe have been found except from a few papers in trade journals (see Q 2.3.1). Therefore, it remains uncertain what the impact of Xf actually is and to which extent outbreaks can be related to weather conditions and/or cultivation conditions.

Climatic data of the Netherlands and Finland (long term average 1981-2010)
(sources: KNMI at http://www.klimaatatlas.nl/ and Penntti et al., 2012)

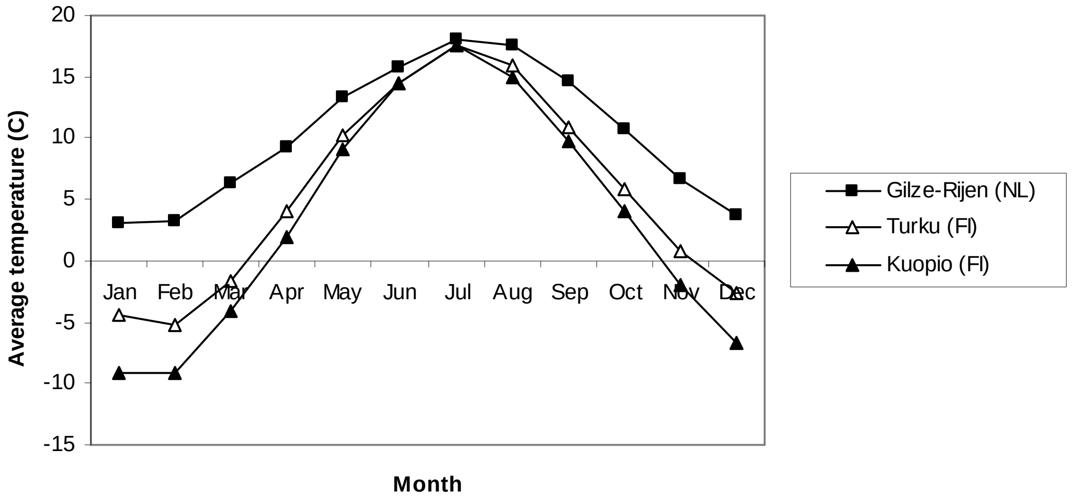

Fig III.1. Average monthly temperature (°C) at Gilze-Rijen (the Netherlands) and at two places in Finland (Turku and Kuopio).

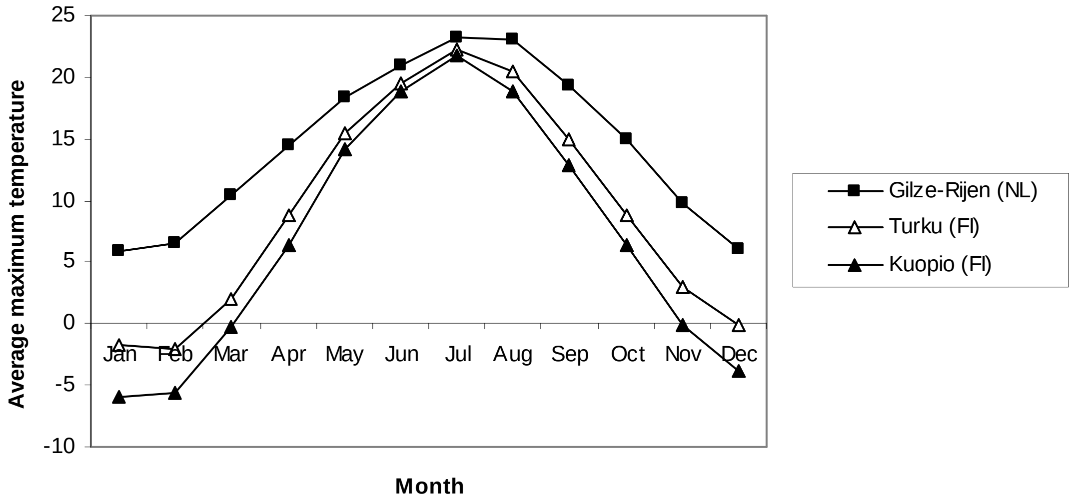

Fig III.2. Average monthly maximum temperature (°C) at Gilze-Rijen (the Netherlands) and at two places in Finland (Turku and Kuopio).

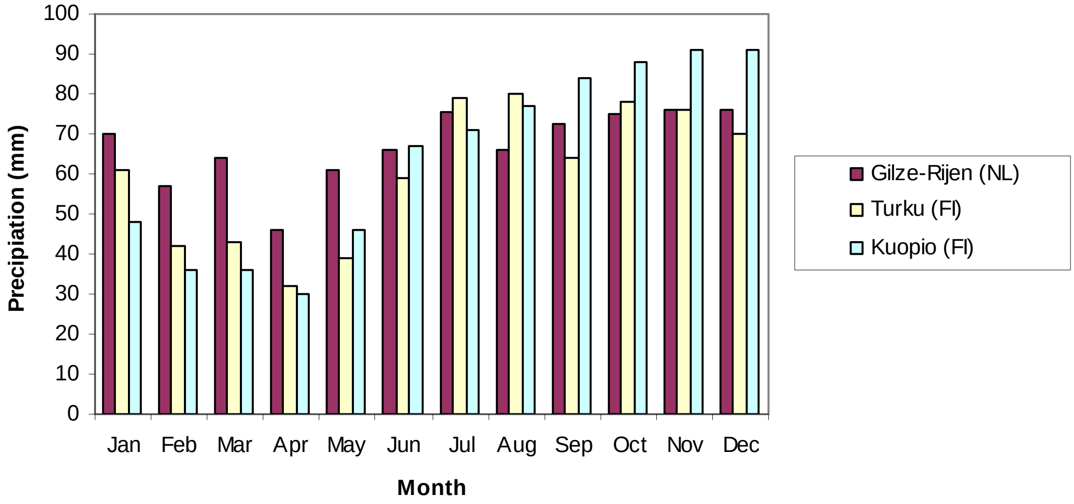

Fig III.3. Monthly precipitation (mm) at Gilze-Rijen (the Netherlands) and at two places in Finland (Turku and Kuopio).

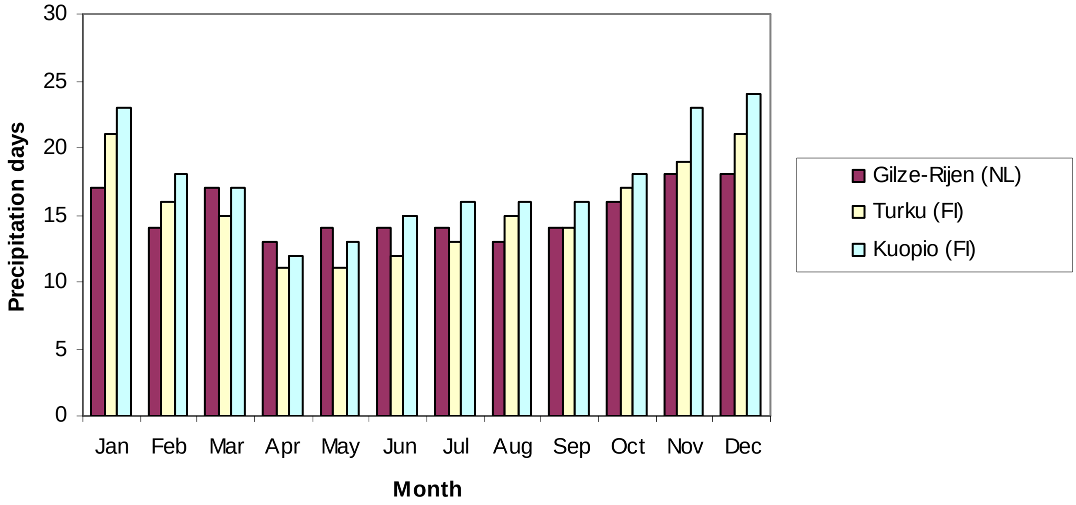

Fig III.4. Number of precipitation days (days with ≥ 0.1 mm of precipitation) at Gilze-Rijen (the Netherlands) and at two places in Finland (Turku and Kuopio).

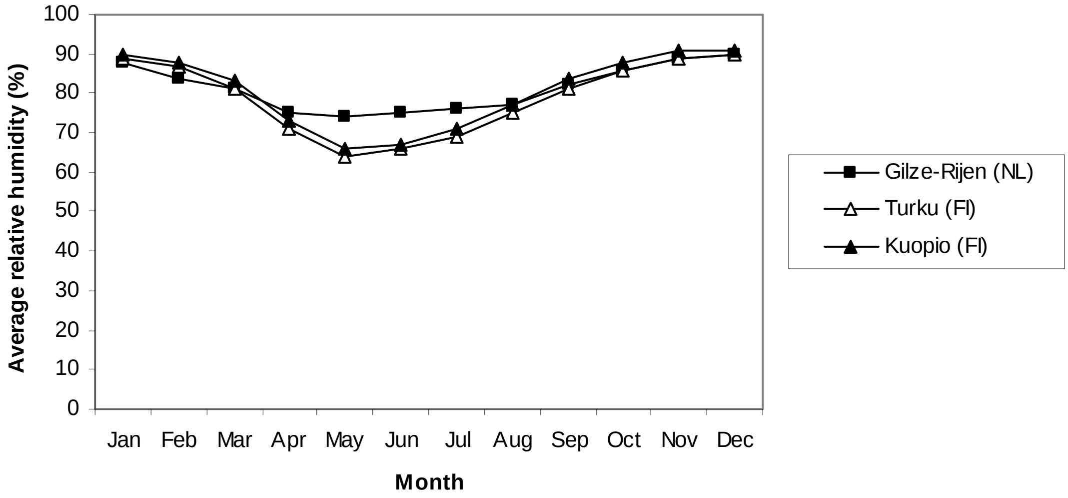

Fig III.5. Average relative humidity at Gilze-Rijen (the Netherlands) and at two places in Finland (Turku and Kuopio).