Pest risk assessment for the European Community: plant health: a comparative approach with case studies

Prima phacie

Pest Risk Assessment: Test Method 4

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Preface

Pest risk assessment provides the scientific basis for the overall management of pest risk. It involves identifying hazards and characterizing the risks associated with those hazards by estimating their probability of introduction and establishment as well as the severity of the consequences to crops and the wider environment.

Risk assessments are science-based evaluations. They are neither scientific research nor are they scientific manuscripts. The risk assessment forms a link between scientific data and decision makers and expresses risk in terms appropriate for decision makers.

Note

Risk assessors will find it useful to have a copy of ISPM 11, Pest risk analysis for quarantine pests, including analysis of environmental risks and living modified organisms (FAO, 2004)[1] and the EFSA guidance document on a harmonized framework for pest risk assessment (EFSA, 2010)[2] to hand as they read this document and conduct a pest risk assessment.

[1] ISPM No. 11 available at https://www.ippc.int/id/13399
[2] EFSA Journal 2010, **8**(2),1495-1561, Available at http://www.efsa.europa.eu/en/scdocs/doc/1495.pdf

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Executive Summary

Keywords: *Xanthomonas citri*, citrus canker, trade of fresh fruits, trade of ornamental rutaceous plants and plant parts, Illegal entry of plant propagative material, Climex map

Provide a technical summary reflecting the content of the assessment (the questions addressed, the information evaluated, and the key issues that resulted in the conclusion)

The purpose of this pest risk assessment was to evaluate the plant health risk associated with *Xanthomonas citri* (strains causing citrus canker disease) within the framework of EFSA project CFP/EFSA/PLH/2009/01. In this PRA, all *Xanthomonas* strains causing citrus canker disease (namely pathotypes A, A* and A^w of *X. citri* pv. *citri* and pathotypes B and C of *X. citri* pv. *aurantifolii*) are considered as forming a single quarantine pest.

Pest biology

- Identity of the pest:
  Xanthomonas citri pv. *citri* (*ex* Hasse 1915) Gabriel *et al.* 1989 (Asiatic canker)

  Xanthomonas citri pv. *aurantifolii* Ah-You *et al.,* 2009 (South American canker)

  The pest will be refered to as *Xcc* (*Xanthomonas* citrus canker) in the PRA. Most of the literature cited refers to *X. citri* pv. *citri.* When necessary, mentions are made in the text when published data is specifically relevant for *X. citri* pv. *aurantifolii* or to the disease it causes.

- Life history
  The bacterium primarily survives in diseased rutaceous tissues such as lesions on leaves, twigs, branches and fruit (Das, 2003). Culturable population sizes of approximately 10^5 cells of *Xcc* per lesion were recovered from 18 month-old leaf lesions (Pruvost *et al*., 2002). Moreover, the pathogen can survive in diseased twigs (particularly on lesions formed on angular shoots) up to several years. The pathogen survives from season to season mainly in the cankers on twigs and branches (Chakravarti *et al,* 1966; Goto, 1992; Schubert *et al*., 2001). A marked decrease in population sizes in lesions was reported in association with temperature decreases in areas where a marked winter season occur (Stall *et al*., 1980). In contrast, such a decrease in *Xcc* population sizes is much more subtle in tropical areas and this decrease is more related to the age of lesions (Pruvost *et al*., 2002). Population dynamics of *Xcc* have been primariliy based on enumeration of culturable cells. In nature, bacteria that ooze onto plant surfaces die (or become non-culturable) upon exposure to drying (a phenomenon that is accelerated by exposure to direct sunlight), unless they can enter citrus tissue through natural openings or wounds (i.e. initiate infection) (Graham *et al*., 2004). Exposed bacteria remain culturable only a few days in soil and a few months in plant refuse that is incorporated into soil (Gottwald *et al*., 2002). On the other hand, an unconfirmed report (Das, 2003) suggests that bacteria can survive for years in infected tissues that have been kept dry and free of soil. Its ability to survive outside of citrus tissues is most likely limited (Graham *et al*., 2004). However, a very recent study reports the detection of *Xcc* cells marked by unstable green-fluorescent protein that were organized as biofilms on citrus leaf and fruit surfaces (Cubero *et al*., 2011). This confirms a previous report by Rigano *et al*. (2007) suggesting survival of *Xcc* on plant surfaces as bacterial aggregates; however, the viability of the aggregate cells was not determined by these authors. Moreover, a reversible viable but not culturable (VBNC) state has been suggested for *Xcc* in response to copper ions (Del Campo *et al*., 2009; Lopez, M.M., personal communication). These

results highlight the need of research on asymptomatic survival to evaluate long-term survival and epidemiological significance of (i) biofilm-associated populations as well as (ii) VBNC populations and question the assumption made until now by the scientific community that *Xcc* has a very low ability to survive asymptomatically. *Xcc* was reported to survive asymptomatically at low population levels on citrus hosts, in association with non-citrus weed and grass plants (Goto, 1970, 1972; Goto *et al*., 1978; Leite & Mohan, 1984). This includes citrus fruit surfaces from which *Xcc* could be detected at low population sizes (Gottwald *et al*., 2009). Population sizes of *Xcc* as low as 10^2 cells per gram in a biological sample have the ability to develop infection of citrus tissue (Goto *et al*., 1978). There are no studies that have extensively evaluated the asymptomatic survival of *Xcc* on plants other than citrus and there is a need of more experimental data on this issue. It remains unclear whether populations associated with asymptomatic citrus tissues are epiphytic or involved in latent infections (Stall *et al*., 1993; Timmer *et al*., 1996). Latent infections have been reported on shoots infected late in the autumn just before entering dormancy (Goto, 1992). The bacterium may survive for several weeks on non-host plant material under natural conditions (Gottwald *et al*., 2002). Saprophytic survival of *Xcc* in soil in absence of plant tissue or debris has not been conclusively established (Goto, 1970). It was suggested by Graham (1989) that the populations of the bacterium have very limited survival capability in subtropical soils. Graham *et al.* (1987) reported that the bacterium was able to persist in dry or sterilized soil in the absence of microbial activity for several months, however, with the addition of moisture, population declined rapidly. In the presence of living citrus not removed during eradication process, the bacterium could survive in the rhizoshere and on leaves and stems that resprout from the rootstock.

Attempts to detect surviving bacteria on various inert surfaces such as metal (representing vehicles, lawnmower blades, etc.), plastic (fruit crates), leather (gloves and shoes), cotton cloth (clothing), cotton gloves and processed wood (crates, ladders, etc.), bird feathers and animal fur, in both shade and sun indicate the bacterium dies within 24-72 hours (Graham *et al*., 2000) depending on the environmental conditions (mainly humidity). It was confirmed that the bacterium dies when the surface is dried, but before that, there is a considerable time period of risk for bacterial transmission (Graham *et al*., 2000).

- Host range / habitat:

Known hosts are mainly in the family of Rutaceae. *Citrus*, *Poncirus*, *Fortunella* and their hybrids are the most common host genera. The following other rutaceous genera have been reported either based on artificial inoculations or natural infections: *Aeglopsis*, *Atalantia, Casimiroa*, *Clausena*, *Citropsis*, *Eremocitrus, Evodia, Feroniella, Hesperethusa*, *Lansium*, *Melicope*, *Microcitrus*, *Murraya, Paramignya* and *Xanthoxylum* (Lee, 1918; Peltier & Frederich, 1920; 1924).

- Means of dispersal / spread:

The bacterium is readily spread by wind and rain usually over short distances, *i.e.* within trees or to neighboring trees (Stall *et al*., 1980; Pruvost *et al*., 1999; Pruvost *et al*., 2002; Bock *et al*., 2010). Although Stall *et al.* (1980) reported that wind blown inoculum was detected up to 32 meters from infected trees in Argentina, it was later reported by Gottwald *et al*. (2001) that there is evidence for much longer dispersals (up to 11 km) in Florida, associated with meteorological events, such as severe tropical storms, hurricanes, and tornadoes. A distance of spread of up to 56 kms was found in the county of Lee/Charlotte (Florida) as a result of a hurricance in 2004 (Irey *et al*. 2006). The situation in Florida and Brazil was exacerbated by the introduction of the Asian citrus leafminer, *Phyllocnistis citrella* (Gottwald *et al*., 1997; Christiano *et al*., 2007; Gottwald *et al*., 2007). The presence of miner galleries allows bacterial concentrations of approximately 10^1 cells of *Xcc* per ml to initiate infections whereas approximately 10^4 cells/ml are required to infect unwounded leaves through natural openings (Christiano *et al*., 2007). This insect is widely present in citrus producing regions of the PRA area. Long-distance spread more often occurs with the movement of diseased or contaminated propagating material (*e.g.* budwood, rootstock seedlings, budded trees including ornamental plants) (Das, 2003). Commercial shipments of diseased fruit are also a means of long-distance spread (Golmohammadi *et al*., 2007). Workers can carry bacteria within and among plantings on hands, clothes, vehicles and equipment/tools (budding-, pruning-, hedging-, and spray- equipment). Finally, postharvest handling of fruit may be a potential source of spread, as wooden harvesting boxes that contained diseased fruit and leaves have been implicated in long-distance spread (Das, 2003). There is no record of seed transmission (Das, 2003).

Geographic Distribution

The bacterium responsible for South American canker is restricted to this continent, while the causal agent of Asiatic canker has a very large distribution. It is widespread throughout Asia, most islands in the Indian Ocean and the Arabic peninsula, although likely with a lesser prevalence. It is currently re-emerging in several African countries (only confirmed in Ethiopia, Mali, Senegal and Somalia to date) after being eradicated early in the 20th century from South Africa. Its presence in North America is currently restricted to Florida but more widespread in South America (Argentina, Bolivia, Brazil, Paraguay, Uruguay). It is absent from Central America, the Caribbean region, Europe and the Mediterranean basin. It is locally present in Oceania but absent from Australia and New Zealand.

Entry

Evaluate the probability of entry and indicate the elements that make entry most likely or those that make it least likely. Identify the pathways in order of risk and compare their importance in practice.

Xcc is not reported to be present in the PRA area (EU27) where it is considered as a quarantine organism for the PRA area under the council directive 2000/29/EC. The probability of introduction is considered high, given the reports from some other areas worldwide (e.g. USA, Brazil, Australia) stating on several introductions of *Xcc* in association with illegal entries of citrus propagative material and the numerous interceptions reported on fruit consignments in the EU. From the three pathways that have been documented herein, a single pathway (trade of fresh fruits) represents massive volumes at high frequencies, but the probability of transfer to a suitable host of inoculum brought up through this pathway is likely low, although poorly documented and therefore with a high uncertainty. The two other examined pathways (trade of ornamental rutaceous plants and plant parts and illegal entry of plant propagative material) likely consist of low volumes. Trade of ornamental rutaceous plants and plant parts is poorly documented. It seems to primarily consist of *Murraya* plants (bonsai) and a few other genera, which all presently escape the council directive 2000/29/EC.

Establishment

Evaluate the probability of establishment and indicate the elements that make establishment most likely or those that make it least likely. Specify which part of the risk assessment area presents the greatest risk of establishment.

Xcc has a relatively moderate number of host plant species (cultivated or wild), localised in specific areas in the Southern of Europe or under protected cultivation in Northern countries on a relatively low acreage. The climatic conditions of Southern Europe (based on a climex analysis), the global warning, the epidemiology of the bacterium and the absence of natural antagonists or enemies, the cultural practices and the poorly efficient IPM measures are consistent with a possible establishment of *Xcc* in the PRA area. The frequent presence of citrus trees in streets, private and public gardens could also favour establishment of *Xcc.* Although likely consisting of low volumes, the pathway that is at very high risk for establishment corresponds to the illegal entry in the EU27 of plant propagative material, consistent with several reports from other areas worldwide. The biological significance of the ‘trade of fresh fruits’ and ‘ornamental rutaceous plants’ pathway as a source of inoculum for establishment of *Xcc* needs further investigation.

Although primarily used for pests and weeds, climex-based analyses have also been used for agricultural plant pathogens (Baker *et al.*, 2000; Paul *et al.*, 2005; Pivonia & Yang, 2004; Shaw & Osbourne, 2011; Yonow *et al.*, 2004). However, the estimation of where a pathogen might live, but does not, relies on numerous parameters that may not all be taken into account in climatic mapping-based models. For example, the lack of rain during the summer months would limit the presence of the canopy wetness necessary for infection, but it was shown from Spanish data that rainfall and rain days are not good indicators of the citrus canopy wetness because of the frequent presence of dew during summer nights with temperatures over 15 and even 20°C (Vicent & Garcia-Jimenez, 2008). Thus, the microclimate in citrus orchards is of critical importance. Based on a climex map for *Xcc*, Spain (Valencia province, Baleares), Greece, Italy (Basilicata, Calabria, Campania, Lazio, Puglia, Sicilia and Toscana regions) and France (PACA region) have some of their citrus growing areas with an ecoclimatic index (EI) over 30 that could likely allow establishment of *Xcc*. Other areas with an ecoclimatic index between 10 and 30 still considered at risk for establishment include other citrus-growing areas in Portugal, Greece, Cyprus, France (Corsica), Italy (Liguria and Sardegna regions), Spain (provinces of Castellón, Alicante, Murcia, Tarragona, Sevilla, Córdoba, Huelva) and Malta.

Impact
List the most important potential impacts, and estimate how likely they are to arise in the risk assessment area. Specify which part of the risk assessment area is most at risk.

Susceptible *Citrus* cultivars are widely grown in the PRA area. Should *Xcc* become established in some parts of the PRA area, direct damage (yield loss, tree defoliation, alteration of fruit external quality, abandon of groves, higher soil erosion in areas where citrus are grown in terraces, higher soil erosion in areas where citrus are grown in terraces) would be likely high. Furthermore, the establishment of *Xcc* would threaten internationally major resources of citrus germplasm that are present in several citrus-producing areas in the EU27 (e.g. Spain, Corsica) and supply pest-free propagative material worldwide. In the absence of efficient IPM measures, costs of inspection, quarantine, eradication of trees in infected areas and certification of plants would be very high. Indirect damage would also include a possible loss of export markets, social consequences and moderate environmental consequences (e.g. soil pollution by copper, loss of biodiversity, degradation of the ability of citrus-planted surfaces to decrease atmospheric CO_2).

Overall conclusion of the pest risk assessment
The risk assessor should give an overall conclusion on the pest risk assessment and an opinion as to whether the pest or pathway assessed is an appropriate candidate for stage 3 of the PRA: the selection of risk management options, and an estimation of the associated pest risk.

Xcc is not reported to be present in the PRA area (EU27) where it is considered as a quarantine organism for the PRA area under the council directive 2000/29/EC. The directive 2000/29/EC lists in the Annex II part A, section 1 b, “*Xanthomonas campestris* (all strains pathogenic to *Citrus*)”, which includes *Xcc*, banning its introduction into EU if present on plants of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, other than seeds. Annex III part A prohibits the introduction into all EU member states of plants of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, other than fruit and seeds. Special requirements, regarding “*Xanthomonas campestris* (all strains pathogenic to *Citrus*)”, are specified in Annex IV part A , section I point 16.2, for the introduction and movement into and within all EU member states of fruit of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, originating in third countries. Citrus canker has been a destructive and costly disease in many areas. The probability of introduction is high, given the reports from several areas (e.g. USA, Brazil, Australia) stating on several introductions of *Xcc* in association with illegal entries of citrus propagative material and the numerous interceptions reported on fruit consignments in the EU. From the three pathways that have been documented herein, a single pathway (trade of fresh fruits) represents massive volumes at high frequencies, but the probability of transfer to a suitable host of inoculum brought up through this pathway is likely low, although poorly documented and therefore with a high uncertainty. Although likely consisting of low volumes, the pathway that is at very high risk for establishment corresponds to the illegal entry in the EU27 of plant propagative material, consistent with several reports from other areas worldwide.

Although primarily used for pests and weeds, climex-based analyses have also been used for agricultural plant pathogens (Baker *et al.*, 2000; Paul *et al.*, 2005; Pivonia & Yang, 2004; Shaw & Osbourne, 2011; Yonow *et al.*, 2004). However, the estimation of where a pathogen might live, but does not, relies on numerous parameters that may not all be taken into account in climatic mapping-based models. For example, the lack of rain during the summer months would limit the presence of the canopy wetness necessary for infection, but it was shown from Spanish data that rainfall and rain days are not good indicators of the citrus canopy wetness because of the frequent presence of dew during summer nights with temperatures over 15 and even 20°C (Vicent & Garcia-Jimenez, 2008). Based on a climex map for *Xcc*, Spain (Valencia province, Baleares), Greece, Italy (Basilicata, Calabria, Campania, Lazio, Puglia, Sicilia and Toscana regions) and France (PACA region) have some of their citrus growing areas with an ecoclimatic index (EI) over 30 that could likely allow establishment of *Xcc*. Other areas with an ecoclimatic index between 10 and 30 still considered at risk for establishment include other citrus-growing areas in Portugal, Greece, Cyprus, France (Corsica), Italy (Liguria and Sardegna regions), Spain (provinces of Castellón, Alicante, Murcia, Tarragona, Sevilla, Córdoba, Huelva) and Malta.

The climatic conditions of Southern Europe, the presence of approximately 0.5 M ha of *Citrus* host plants in groves, nurseries, smallholdings, private gardens…, the global warning, the biology of *Xcc* and the absence of natural antagonists or enemies, the cultural practices and the poorly efficient IPM measures are consistent with a possible establishment of *Xcc* in the endangered areas of the PRA area listed above. The probability of spread may be considered likely. Natural dispersal would primarily be by rain and wind-driven rain and would spread *Xcc* at small to medium scales. Some weather events such as summer storms, which can be quite frequent in Southern Europe, have the ability to spread *Xcc* at larger distances (i.e. approximately at up to a kilometre scale). Human activities would undoubtedly favour spread of *Xcc* whatever the considered scale. Long distance spread would primarily be through human activities (e.g. movement of contaminated or exposed plant material and through machinery, clothes, etc. polluted by *Xcc* during grove or nursery maintenance operations) and the importance of which would be largely dependant on the deployment of prompt and strict quarantine measures for isolating outbreaks. Human-driven unintentional spread could also be increased due to the massive presence of citrus trees in streets, private and public gardens. It is unknown how likely intentional movement of *Xcc* by persons in the PRA area could be achieved. Although likely not well suited as a bio-terrorism agent, *Xcc* is also listed as 'dual use technology and organism' (council regulation EC 394/2006).

Susceptible *Citrus* cultivars are widely grown in the PRA area. Should *Xcc* become established in some parts of the PRA area, direct damage (yield loss, tree defoliation, alteration of fruit external quality, abandon of groves, higher soil erosion in areas where citrus are grown in terraces, higher soil erosion in areas where citrus are grown in terraces) would be likely high. Furthermore, the establishment of *Xcc* would threaten internationally major resources of citrus germplasm that are present in several citrus-producing areas in the

EU27 (e.g. Spain, Corsica) and supply pest-free propagative material worldwide. In the absence of efficient IPM measures, costs of inspection, quarantine, eradication of trees in infected areas and certification of plants would be very high. Indirect damage would also include a possible loss of export markets, social consequences and moderate environmental consequences (e.g. soil pollution by copper, loss of biodiversity, degradation of the ability of citrus-planted surfaces to decrease atmospheric CO_2).

Currently, the absence of citrus canker in the PRA area as well as in the neighbouring Mediterranean or Atlantic areas is giving to these citrus growing areas a considerable advantage for international trade of fresh citrus fruits as well as for the production of citrus plants in nurseries. This situation must be maintained and increases the impact of the entry and establishment of the disease.

Reported uncertainty should be addressed through research efforts. A comprehensive evaluation of costs linked to the establishment of *Xcc* in endangered areas of the EU27, as done in other countries (e.g. Australia, USA – Alam & Rolfe, 2006; Jetter *et al*., 2000; Spreen *et al*., 2003) should be undertaken.

Preamble

The assessments reported in the present document consider that the phytosanitary measures listed in the council directive 2000/29/EC and its annexes are implemented. They include general measures for citrus and specific measures for xanthomonads pathogenic to citrus.

Stage 1 – Initiation

1.1 Background and Initiation

The purpose of this assessment is to evaluate the plant health risk of *Xanthomonas citri*, the causal agent of citrus canker disease, within the framework of the EFSA project CFP/EFSA/PLH/2009/01: 'Pest risk assessment for the European Community plant health - a comparative approach with case studies' (PRIMA PHACIE). This evaluation will be performed within the framework of the EFSA scheme, including the risk of entry, establishment and spread of the pathogen as well as the potential impacts for the risk assessment area.

Terms of references as provided by the EFSA call: CFP/EFSA/PLH/2009/01

It is requested to generate the risk assessments on pilot organisms, including *Xanthomonas citri*, based on existing methodologies, with the aim to compare these methodologies, identify those most suitable and further develop the scientific basis for risk assessment of organisms potentially harmful to plants and plant products for the European Community. The resulting method is expected to be used by the EFSA Panel on Plant Health and will give best support for the European decision-making.

Initiation Point

This PRA was initiated within EFSA project CFP/EFSA/PLH/2009/01: 'Pest risk assessment for the European Community plant health - a comparative approach with case studies' (PRIMA PHACIE), and incorporates the latest scientific and technical knowledge on *Xanthomonas citri*, its present distribution worldwide, and the experience gained from the implementation of the measures suggested within Directive 2000/29/EC to prevent its entry, as this pathogen is not currently present in the EU.

1.2 Identification of the PRA Area

The PRA area is the 27 Members of the EU.

1.3 Available pertinent regulatory information

Previous PRA

A number of risk assessments related to *Xanthomonas citri* and scientific opinions on such risk assessments have been performed:

BIOSECURITY AUSTRALIA (2009) Final import risk analysis report for fresh Unshu mandarin fruit from Shizuoka prefecture in Japan, pp.258.

CIRAD (2004) Analyse du Risque Phytosanitaire, AGR- b1. *Xanthomonas axonopodis* pv. *citri*- Zones ARP: Martinique, Guadeloupe, Guyane. Rédaction: O. Pruvost / CIRAD – Mars 2004.

COSAVE (Comité de Sanidad Vegetal del Cono Sur) (2005) Risk assessment report for *Xanthomonas axonopodis* pv. *citri* on citrus fruits. Agreed in the II Meeting of the EWG on the Systems Approach for Citrus Canker. Buenos Aires, Argentina.

EFSA (European Food Safety Authority) (2006) Opinion of the Scientific Panel on Plant Health on a request from the Commission on an evaluation of asymptomatic citrus fruit as a pathway for the introduction of citrus canker disease (*Xanthomonas*

axonopodis pv. *citri*) made by the US Animal and Plant Health Inspection Service. *The EFSA Journal*, 439: 1–41.

EFSA (European Food Safety Authority) (2008) Scientific opinion of the Panel on Plant Health on the pest risk assessment made by France on *Xanthomonas axonopodis* pv. *citri* considered by France as harmful in French overseas departments of French Guiana, Guadeloupe and Martinique. (Question No EFSA-Q-2006-088). *The EFSA Journal*, 682: 1-22.

Fite R (2002) Pest risk assessment of citrus canker, citrus scab, citrus black Spot, south American and Mediterranean fruit flies introduction into the USA via importation of citrus fruits (Sweet oranges, mandarins, lemons and grapefruits) from country W. Draft report submitted to the USDA/Aphis April, 2002 (executive summary).

PaDIL - Plant Biosecurity Toolbox (2009) Diagnostic methods for Asiatic Citrus Canker *Xanthomonas axonopodis pv. citri Pathotypes "A"* [*http://www.padil.gov.au/pbt*].

Schubert, T.S., Miller, J.W., Dixon, W.N., Gottwald, T.R., Graham, J.H., Hebb, L.H. and Poe, S.R. (1999) Bacterial citrus canker and the commercial movement of fresh citrus fruit. An assessment of the risks of fresh citrus fruit movement relative to the spread of bacterial citrus canker (*Xanthomonas axonopodis* pv. *citri*). A report prepared for the Citrus Canker Risk Assessment Groups for Manatee County, Collier County, and Miami/Dade and Broward Counties Florida Department of Agriculture & Consumer Services.

USDA (United States Department of Agriculture) (2006) Evaluation of asymptomatic fruit (Citrus spp.) as a pathway for the introduction of citrus canker disease (*Xanthomonas axonopodis* pv. *citri*). USDA-APHIS-PPQ-CPHST-PERAL.

USDA (United States Department of Agriculture) (2008) An updated evaluation of citrus fruit (*Citrus* spp.) as a pathway for the introduction of citrus canker disease (*Xanthomonas axonopodis* pv. *citri*). USDA-APHIS-PPQ-CPHST-PERAL

USDA (United States Department of Agriculture) (2009) Movement of commercially packed citrus fruit from citrus canker disease quarantine Area. Supplemental Risk Management Analysis. Ver. 03/03/2009-1. USDA-APHIS-PPQ-CPHST-PERAL.

Note: The following document has been published in December 2011, and has consequently not been taken into account in this PRA which was prepared earlier.

EFSA (European Food Safety Authority) (2011) Scientific opinion on the request from the USA regarding export of Florida citrus fruit to the EU. *The EFSA Journal*, 9(12): 2461, 9 pp., doi:10.2903/j.efsa.2011.2461.

Available Pest Fact Sheets/ Pest Alerts etc.:

- **CAB International (CABI) (2007)** Crop Protection Compendium. Wallingford, UK: CAB International. http://www.cabicopedium.org/cpc/home.asp

- **EPPO/CABI (1997)** *Xanthomonas axonopodis pv. citri*. *Quarantine Pests for Europe*, 2nd edn, pp. 1101–1108. CAB International, Wallingford (GB).

- **EPPO (2005)** EPPO Standards PM 7/44(1) *Xanthomonas axonopodis* pv. *citri EPPO Bulletin* **35**, 289–294.

- **Brunings, A.M. and Gabriel, D.W. (2003)** Pathogen profile. *Xanthomonas citri*: breaking the surface. *Molecular Plant Pathology* **4**, 141-157.

- **Gottwald, T.R., Graham, J.H., and Schubert, T.S. (2002)** Plant health reviews. Citrus canker: the pathogen and its impact. Plant Health Prog.:doi:10.1094/PHP-2002-0812-01-RV.

- **Graham, J. H., Gottwald, T. R., Cubero, J., and Achor, D. S. (2004)** Pathogen profile. *Xanthomonas axonopodis* pv. *citri*: factors affecting successful eradication of citrus canker. *Molecular Plant Pathology* **5**, 1-15.

In addition, there is an ongoing effort to generate a new updated datasheet of *Xanthomonas citri* in the framework of the project Prima Phacie.

Current regulatory status

What is the pest's status in the Plant Health Directive (Council Directive 2000/29/EC[3])?

EU Annex designation: II/A1 - as all *Xanthomonas campestris* strains pathogenic to citrus:

The pathogen, stated in the Council Directive 2000/29/EC as 'all strains of *Xanthomonas campestris* pathogenic to citrus', is listed under point (b): 'Bacteria', in Section I of Part A in Annex II of the Directive. Thus, its regulatory status in the Directive is among '**Harmful organisms not known to occur in the community and relevant for the entire community'** (*Section I*) and '**whose introduction into, and spread within, all member states shall be banned if they are present on certain plants or plant products'** (*Annex II, Part A*).

What is the pest's status in the European and Mediterranean Plant Protection Organisation (EPPO)? (mark box) (www.eppo.org)

EPPO List:	A1 regulated pest list	√	A2 regulated pest list		Action list		Alert list	

1.4 Strategy of data searching (*identity of data bases, data banks and information systems, key search terms and strategies applied, and the time period covered should be provided*)

Literature searches were performed consulting several sources such as:

- **Abstracting databases:** a) AGRICOLA on OvidSP (OvidSP_UI02.03.01_H11_2.101, SourceID 49793) --1970-present, b) Agris on OvidSP --1991-present, c) CAB Abstracts on OvidSP--1973-present, and d) ISI Web of Knowledge http://isi02knowledge.com/ (including Conference Proceedings Citation Index).
- **Web pages** specific to the citrus canker disease or referring to botanic data in order to identify rutaceous genera potentially present in the PRA area (Royal Botanic Garden Edinburgh: http://rbg-web2.rbge.org.uk/FE/fe.html)
- **Internet search machines:** Google Scholar searches were conducted to a) evaluate whether rutaceous genera identified in Europe had been reported or hypothesized as host species for citrus canker. b) identify previous PRAs on the same pathogen worldwide.
- **EPPO information system:** EPPO reporting services and EPPO PQR database, version 4.6 [2007-07]

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[3] **http://europa.eu.int/eur-lex/en/consleg/pdf/2000/en_2000L0029_do_001.pdf**

- **CLIMEX** data was used
- **Mopest and Pratique** outcomes were consulted
- **EUROSTAT** (data on host distribution and imports in the EU territory) and **FAOSTAT** databases
- **Agricultural land use map 2000 for Europe** (http://afoludata.jrc.it/DS_Free/AF_Agri.cfm)

Other sources:

- Information from Member States on issues related to official survey programs, imported host plant and plant products, as well as host plant distribution at a national level was acquired via a questionnaire prepared in the framework of the Prima Phacie project and distributed by EFSA to all NPPOs.
- References and information obtained from experts and from citations within references found.

The documents that were consulted to support the risk assessment activity included peer reviewed publications (reviews or papers describing primary research), conference proceedings and technical reports.

Stage 2 - Pest Risk Assessment
(Outline approach)

This system for pest risk assessment involves evaluating six risk elements,

1. climate – host interaction
2. host range
3. dispersal
4. potential consequences
5. environmental impact
6. introduction potential

Each element is divided into three categories. Assessors review data / evidence and either select a single category or spread their judgment between categories. Guidance is provided to interpret the categories.

The last risk element "Introduction potential" is composed of six sub-elements, (i) quantity imported, (ii) survival of post harvest treatment, (iii) survival during shipping, (iv) likelihood of detection at entry, (v) likelihood of movement to suitable habitat, and (vi) likelihood of contact with host. Again allocate % likelihood to appropriate categories for each sub-element. Guidance is provided as to how sub-elements should be interpreted.

Pest risk is determined via use of BBN software based on matrices that combine consequences of introduction with introduction potential.

Having apportioned your assessment across categories for each risk element, record the scores in the associated Excel spread sheet (Method 4 Inputs.xls) and e-mail the spreadsheet to Willem Roelofs. Scores will be combined using BBN software. Results of combing the scores will be provided to risk assessors for interpretation.

Contact for queries regarding operation of this approach:

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Stage 2 - Pest Risk Assessment

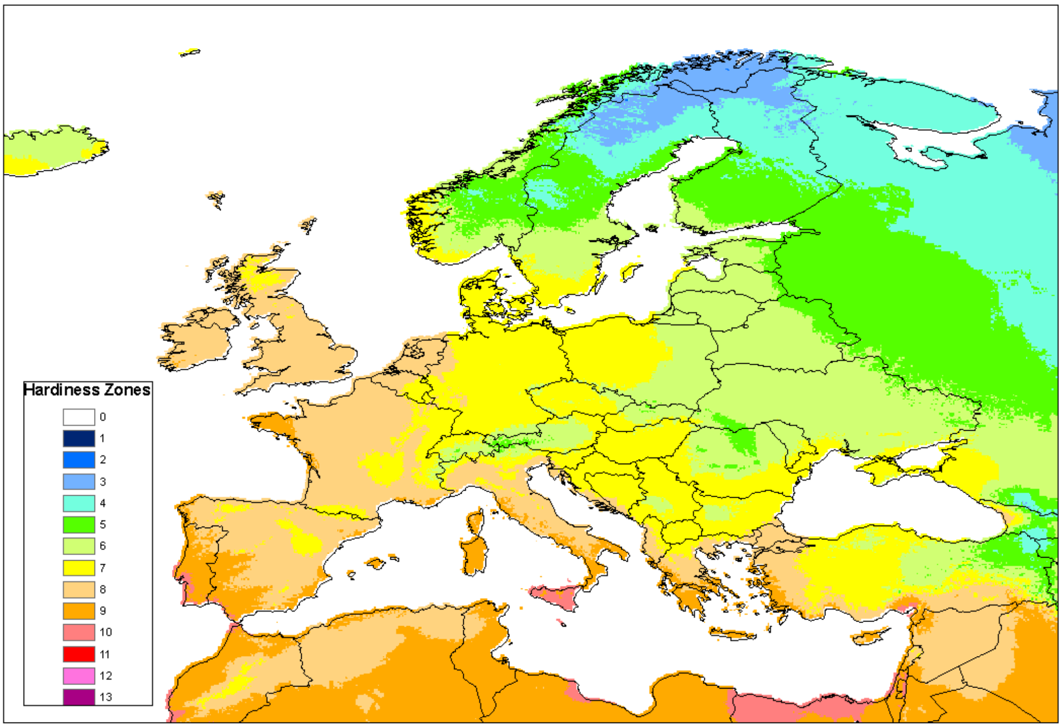
(Case study example)

Consequences of Introduction

2.1 Climate-Host Interaction

When introduced to new areas, pests can be expected to behave as they do in their native areas if host plants and climates are similar. Ecological zonation and the interactions of the pests and their biotic and abiotic environments are considered in this element. Estimates are based on availability of both host material and suitable climate conditions.

Due to the availability of suitable host plants and suitable climate, judge how many hardiness zones the pest has potential to establish a breeding colony in.



Source for "Hardiness Zone Map for Europe": http://www.gardenweb.com/zones/europe/
Hardiness zones for other regions are available via the following link,
http://treesandshrubs.about.com/od/treeshrubbasics/tp/worldhardinesszones.htm

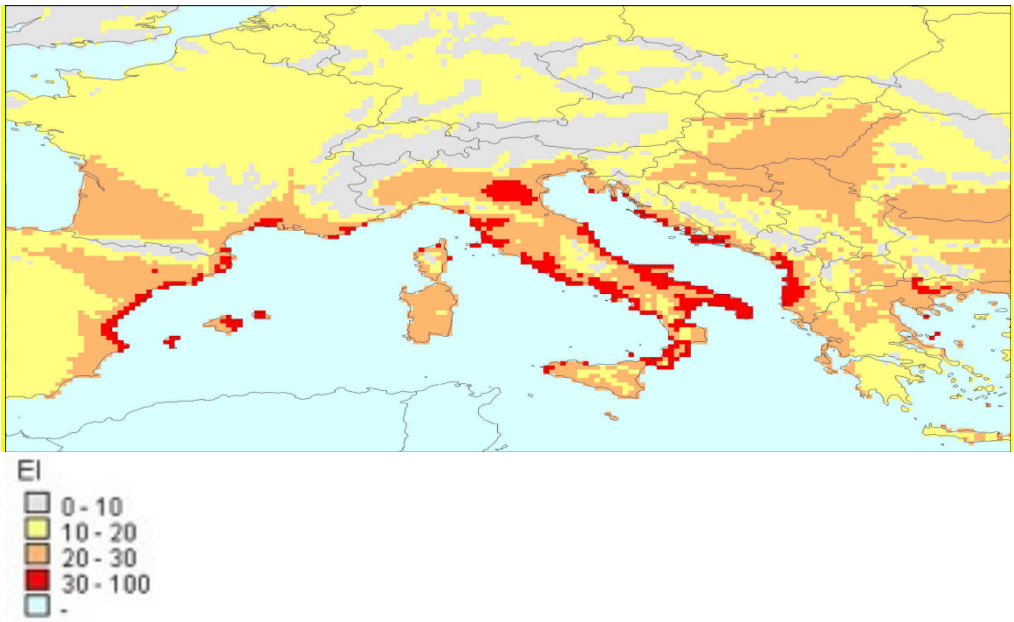
Information / evidence: *Provide reasoning then give judgment.*
Based on the current worldwide distribution of citrus canker, *Xcc* has the ability to establish in hardiness zones 8 to 12. Citrus cultivation areas in the EU are located within the hardiness zones 8, 9 and 10. *Xcc* has caused outbreaks from these zones in China (zone 8: Hubei, Jiangsu, Jiangxi, Sichuan; zone 9: Fujian, Guangdong, Hubei, Hunan, Jiangxi, Sichuan, Yunnan, Zhejiang; zone 10: Fujian, Guangdong, Hong Kong, Sichuan, Yunnan), Japan (zone 8: Honshu, Shikoku, Kyushu; zone 9: Honshu, Shikoku, Kyushu), Argentina (zone 9: Catamarca, Entre Rios, Salta, Tucuma; zone 10: Corrientes, Misiones).

Xcc has potential to establish in the PRA area for the following reasons:

- <u>Suitable climate is available in PRA:</u>

The climates most conducive to establishment of *Xcc* are tropical and subtropical environments (Gottwald *et al*., 2002). These climatic conditions are not really present in the PRA area. The estimated minimum and maximum temperatures for the occurrence of Asiatic citrus canker on sweet orange were 12 and 40°C, respectively, with an optimum range between 25 and 35°C (Dalla Pria *et al*., 2006), consistent with but slightly different from earlier data that mentioned suitable temperatures for infection between 14 and 36°C, with optima from 25 to 30°C (Civerolo, 1984). Between 20 and 35°C, all assayed leaf wetness periods in the range of 4–24 h produced 100% disease incidence (Dalla Pria *et al*., 2006). These compatible temperatures are found in the South of Europe, where Citrus are found.

Originating from Asia, *Xcc* has been disseminated to all realms. . Although primarily used for pests and weeds, climex-based analyses have also been used for agricultural plant pathogens (Baker *et al*., 2000; Paul *et al*., 2005; Pivonia & Yang, 2004; Shaw & Osbourne, 2011; Yonow *et al*., 2004). However, the estimation of where a pathogen might live, but does not, relies on numerous parameters that may not all be taken into account in climatic mapping-based models. For example, the lack of rain during the summer months would limit the presence of the canopy wetness necessary for infection, but it was shown from Spanish data that rainfall and rain days are not good indicators of the citrus canopy wetness because of the frequent presence of dew during summer nights with temperatures over 15 and even 20°C (Vicent & Garcia-Jimenez, 2008). Based on a Climex map for *Xcc* (Reynaud, 2010), some parts of Spain, France, Greece and Italy have citrus growing areas with an ecoclimatic index (EI) over 30 that could likely allow establishment of *Xcc*. Other areas have an ecoclimatic index between 10 and 30; they can be considered at risk for establishment, given the relative imprecision of climex.



Moreover, global warming in the next decades could favour establishment of *Xcc*.

- <u>No pest competition:</u>

No enemies can negatively affect establishment under natural conditions. Interactions between *Xcc* and antagonistic bacteria including *Bacillus subtilis* (Pabitra *et al*., 1996), *Pantoea agglomerans* (Goto *et al*., 1979), *Pseudomonas syringae* (Ohta, 1983) and *Pseudomonas fluorescens* (Unnamalai and Gnanamanickam, 1984) have been reported *in vitro* and *in vivo*. However, the practical usefulness of these bacteria in controlling the pathogen has not been shown. Pests that severely negatively affect citrus growth (e.g. exocortis viroid, citrus tristeza virus, *Phoma tracheiphila…* (Vernière *et al*., 2004; Moreno *et al*., 2008; Migheli *et al*., 2009) may have a limiting effect on *Xcc* infections by strongly restricting the availability of plant material at a susceptible growth stage (young flushes), although no data is available.

Xcc interacts with several bacteriophages (for examples see Goto, 1992; Kuo *et al*., 1994; Wu *et al*., 1995). Bacteriophages have the potential to biologically control plant bacterial pathogens including *Xcc*, but there is no record of commercial success in citrus groves so far (Jones *et al*., 2007).

- Suitable hosts are available in large amounts:

Crop for fruits production:

This production is concentrated in six countries but on a few surface areas of their total agricultural surface area (UAS): Greece: 1.38%; Spain: 1.26%; Italy: 1.18%; Cyprus: 3.31%; Portugal: 0.70%; France (Corsica only). This was, in 2007, 0.32% of the total UAS of EU 27 (573,000ha/181,094,000ha).

The crop is grown in monoculture (orchards and nurseries) with susceptible species most of time. *Citrus* groves in the EU are often established using rather high plantation densities (e.g. 400-500 trees/ha for mandarins and clementines).

The cultivation practices that enable a good vigour of trees also favour the development of citrus canker (Gottwald *et al*., 2002).

Moreover, overhead irrigation exacerbates the spatial and temporal development of the disease because splash disperses the pathogen. This way of dispersal is of great concern in unprotected nurseries producing young trees to be introduced to new groves (Pruvost *et al*., 1999).

Hand harvest without sanitation could somewhat favour establishment.

Ornamentals and wild hosts:

Citrus species cultivated for fruit are also produced as ornamentals for gardens in South of Europe or for greenhouses in Northern areas (see case of protected environment).

Suitability of Rutaceous ornamentals and wild hosts to *Xcc* are not really documented.

A relatively low number of rutaceaous genera known to host citrus canker are present in the PRA area. But none of the available references and sources allows estimating the prevalence of these rutaceous genera, nor does it allow evaluating their spatial proximity to *Citrus* crop.

These rutaceous species are very unlikely widespread, but no precise data is available. Very few host genera have been reported in the EU 27: *Microcitrus* and *Xanthoxylum* are present in Italy but no information is available on their abundance. Other rutaceous genera are present in the PRA area but their host status is presently unknown (SLR).

Wild Rutaceous host species are very often weakly susceptible to *Xcc*, as compared with commercial citrus. A few exceptions to this rule occur (e.g. *Swinglea glutinosa*), but these species have not been identified as present in the PRA area, although some incertitude occurs there. There is to our knowledge no scientific report of *Xcc* naturally causing citrus canker on wild rutaceous genera outside the native origin of the pathogen.

No management is done on wild hosts except in case of outbreaks: eradication program includes destruction of wild hosts.

Case of protected environment:

Propagating material of citrus for fruit production and ornamentals are cultivated in nurseries in citrus producing countries (Greece, Spain, Italy, Portugal and France) and ornamentals in the Netherlands. The quantities are measured by number of plants and not by surface area and these figures are not easy to find: Greece (M Holeva personal communication): 825,813 trees en 2006 and 542,300 in 2007; France: 818,568 plants in 2005. Based on a rate of tree renewal of 7.5 % (Aubert & Vullin, 1997), estimates for Italy, Portugal and Spain would therefore be 5,771,000, 844,000 and 10,665,000 (not including ornamentals). At least for Spain, theses volumes seem consistent with information found on the internet.

The crop grown under protected conditions in the EU are most often in nurseries or greenhouses (e.g. most commercial citrus nurseries in citrus producing regions, rutaceous ornamentals in the Netherlands). This consists of areas where genetically homogeneous plants are grown at high density. *Xcc* has been sporadically recorded on nursery plants grown in a protected environment in other areas of the world (e.g. Brazil).

Citrus trees in urban public or private environments

In areas at risk, citrus trees can not only been found in groves and nurseries, but also are massively present in streets, private and public gardens.

2.1: Climate host interaction		
Rating	**Description**	**Probability Assignment** [1]
High	in four or more plant hardiness zones.	0%
Medium	in two or three plant hardiness zones.	100%
Low	in a single plant hardiness zone.	0%
	Check sum =	100%

[1] spread your judgment according to your belief / evidence

2.2 Host Range

The risk posed by a plant pest depends on both its ability to establish a viable, reproductive population and its potential for causing plant damage. For arthropods, risk is assumed to be correlated positively with host range. For pathogens, risk is more complex and is assumed to depend on host range, aggressiveness, virulence and pathogenicity; for simplicity, risk is rated as a function of host range.

Information / evidence: *Provide reasoning then give judgment*
Known hosts are primarily in the family of Rutaceae. *Citrus*, *Poncirus*, *Fortunella* and their hybrids are the most common host genera. The following other rutaceous genera have been reported either based on artificial inoculations or natural infections: *Aeglopsis*, *Atalantia, Casimiroa*, *Clausena*, *Citropsis*, *Eremocitrus, Evodia, Feroniella, Hesperethusa*, *Lansium*, *Melicope*, *Microcitrus*, *Murraya, Paramignya* and *Xanthoxylum* (Bradbury, 1986; Koizumi, 1978, Lee, 1918; Peltier et Frederich, 1920, 1924, Reddy, 1997). Some strains referred to as pathotype A*, A^{w}, B and C have a narrow host range within the genus *Citrus*, while strains with a large host range are referred to as pathotype A (Civerolo, 1984; Vernière *et al*., 1998; Sun *et al*., 2004).

2.2: Host range		
Rating	**Description**	**Probability Assignment** [1]
High	Pest attacks multiple species among multiple plant families.	0%
Medium	Pest attacks multiple species within a single plant family.	75%
Low	Pest attacks a single species or multiple species within a single genus.	25%
	Check sum =	100%

[1] spread your judgment according to your belief / evidence

2.3 Dispersal potential

A pest may disperse after introduction to a new area. The following items should be considered:

- *reproductive patterns of the pest (e.g., voltinism, biotic potential)*
- *inherent powers of movement*
- *factors facilitating dispersal (wind, water, presence of vectors, human, etc.)*

Information / evidence: Provide reasoning then give judgment

There is no known vector (besides humans) for *Xcc* (Graham *et al*., 2004). Splash dispersal of inoculum allows an efficient spread over relatively short distances in nurseries and orchards (Gottwald *et al*., 1989; Graham *et al*., 2004; Pruvost *et al*., 1999). Aerosols can also spread xanthomonads over small to medium range distances (Kuan *et al*., 1986; McInnes *et al*., 1988). *Xcc* was successfully isolated from air samples collected at eradication sites in Florida, suggesting that chipping machinery can locally spread *Xcc* (Roberto *et al*., 2001). Adults of the Asian citrus leafminer (*Phyllocnistis citrella* Stainton) are not a vector for *Xcc* (Belasque *et al*., 2005). Transportation of *Xcc* at a very localized scale can be achieved through feeding larvae (Graham *et al*., 2004). Wind-driven rains can spread *Xcc* over distances up to several miles (Gottwald *et al*., 2001; Gottwald & Irey, 2007; Irey *et al*., 2006). Some weather events such as summer storms, which can be quite frequent in Southern Europe, have the ability to spread Xcc at larger distances. However, spread over medium distances (km scale) through wind-driven rains would likely occur at much lower frequencies in the PRA area than in areas highly conducive to efficient spread, such as tropical and subtropical areas (and including Florida from which most of the literature originated). Movement of *Xcc* from commercial crops to other plants occurs by the same natural means as does movement from non-commercial plants to commercial crops. However, such movements implicitly assume host discontinuity between commercial crops and backyard or wild trees, resulting in a spread efficiency that is dependent on the host distribution topology (Cook *et al*., 2008). Diseased backyard trees have been often identified as the primary inoculum source of outbreaks in Florida commercial orchards (Gottwald *et al*., 1992; Schubert *et al*., 2001; Gottwald *et al*., 2002). This has had a major role in the failure of eradication of *Xcc* in Florida (Parnell *et al*., 2009). Cultivation practices that enable a good vigour of trees also favour the development of citrus canker. Overhead irrigation also favours symptom development and localized dispersal (Gottwald *et al*., 2002).

Xcc can transiently survive on inert surfaces and can be locally or regionally transported by clothes, shoes, orchard machinery, and harvesting equipment including boxes (Gottwald *et al*., 1992; Gottwald *et al*., 2002; Graham *et al*., 2004). Grove maintenance equipment was associated to secondary spread in a Florida outbreak (Gottwald *et al*., 1992). Uncontrolled movement of contaminated or exposed plant propagative material is at high risk and would likely result in a fast spread of *Xcc* in the PRA area. In the absence of a strict quarantine procedure, there is no evidence that *Xcc* could be contained in areas where it is present and suitable conditions occur for disease development and spread. Human-driven unintentional spread could also be increased due to the massive presence of citrus trees in streets, private and public gardens. It is unknown how likely intentional movement of *Xcc* by persons in the PRA area can be achieved. *Xcc* is listed as ‘dual use technology and organism’ (council regulation EC 394/2006) for its putative use as a bio-terrorism agent.

2.3: Dispersal potential		
Rating	**Description**	**Probability Assignment** [1]
High	Pest has high biotic potential, e.g., many generations per year, many offspring per reproduction (“r-selected”	100%

	species), AND evidence exists that the pest is capable of rapid dispersal , e.g., over 10 km/year under its own power; via natural forces, wind, water, vectors, etc., or human-assistance.	
Medium	Pest has either high reproductive potential OR the species is capable of rapid dispersal.	0%
Low	Pest has neither high reproductive potential nor rapid dispersal capability.	0%
	Check sum =	100%

[1] spread your judgment according to your belief / evidence

2.4 Potential Consequences

Introduced pests are capable of causing a variety of direct and indirect impacts. The remit of EFSA limits assessors to consider impacts on crop yield and quality (crop impacts) (2.4) and environmental impacts (see 2.5, next) e.g. impacts on ecosystem services or biodiversity itself. We recognise that other types of impacts, listed in ISPM 11, may occur.

Information / evidence: Provide reasoning then give judgment

Xcc has a severe impact on citrus crops with regard to the quantity and quality of fruit produced. More specifically, when citrus canker occurs, the fruit yield is impaired due to the premature fruit drop, severe defoliation and dieback. Under conditions highly conducive to disease development, it is not uncommon that approximately 50% of the fruits and leaves of susceptible cultivars be infected. For example, approximately 90% of fruit infection was recorded on untreated grapefruit groves in Argentina (Stall & Seymour, 1983). Early fruit drop as high as 50% was reported for sweet orange cv. Hamlin (Stall & Seymour, 1983). In addition, severely infected young trees may be delayed in reaching their full growth (Biosecurity Australia, 2003, CABI, 2007). On the other hand, the quality of the infected fruit is compromised due to their usually blemished skin, although their internal quality is not affected (when maturing on the tree) (Gottwald *et al*., 2002). Depending on the extent of the lesions appearing on the fruit skin, the impact of such fruit quality deterioration may be highly significant, mainly at the local level.

Even if control measures are used, the impact of the disease on the crop could be significant. This is because: a) the commonly used control measures involving IPM based on cultural control, sanitary methods and chemical treatments with copper-based bactericides only reduce *Xcc* populations and are moderately efficient on susceptible cultivars (Gottwald *et al*., 2002); b) bacterial copper resistance or tolerance have been reported in Argentina and Brazil, respectively (Behlau *et al*., 2011 a and b; Canteros *et al*., 2010) and copper resistance genes have been identified on *Xcc* plasmids (Canteros *et al*., 2010). Thus, the efficiency of the copper sprays is not assured; c) eradication of diseased and exposed trees, which has been shown as the best economic option in several countries or regions where the pathogen has been eradicated (*e.g.* Australia), not become endemic or maintained at very low incidence (*e.g.* Brazil, USA) (Graham *et al*., 2004), is going to reduce the population of citrus trees and thus the fruit production. Besides, in the long term, eradication program success would be very much dependent on task force and money involved for actions and how prompt and strict the latter are; d) there is no marked resistance to *Xcc* in commercially major *Citrus* varieties used in the PRA area, that would help minimising the disease impact on the crop yield; e) there has not been report of any successful control of *Xcc* through the use of natural enemies (antagonistic bacteria, bacteriophages, other organisms).

2.4: Potential consequences		
Rating	**Description**	**Probability Assignment [1]**
High	The pest has a severe impact on the standing crop with significant host mortality; losses in storage may be total. Intervention by growers may not be possible or would be essential and expensive to counter yield and /or quality losses.	30%
Medium	The pest has a moderate impact on the standing crop but host mortality is rare; losses in storage may occur. Threat to yield and /or quality changes would justify some intervention by growers to reduce losses.	70%
Low	The pest is likely to have no or only minor impact on a standing crop and little effect on stored products. Yield and /or quality changes are within range of natural	0%

	variation. No intervention is likely to be needed.	
	Check sum =	100%

[1] spread your judgment according to your belief / evidence

2.5 Environmental Impact

The assessment of the potential of a pest to cause environmental damage proceeds by considering the following factors:

- *Introduction of the pest is expected to cause significant, direct environmental impacts, e.g., ecological disruptions, reduced biodiversity.*
- *Pest is expected to have direct impacts on endangered/threatened species listed by infesting/infecting a listed plant. If the pest attacks other species within the genus or other genera within the family, and preference/no preference tests have not been conducted with the listed plant and the pest, then the plant is assumed to be a host.*
- *Pest is expected to have indirect impacts on species that are listed in Annex II or IV of the EC Habitats Directive[4] or are key components of habitats listed in Annex I of the EC Habitats Directive.*
- *Introduction of the pest would stimulate chemical or biological control programs which will disrupt existing biological or integrated systems for control of other pests or to have negative effects on the environment e.g. biodiversity (at various levels), reduce population sizes, or increase their fragmentation.*

Information / evidence: Provide reasoning then give judgment
Xcc is usually present in commercial orchards and private gardens/amenity land (Das 2003), which are not regarded as ecologically sensitive, and do not include rare, vulnerable or keystone species.

In case *Xcc* is established in the PRA area, the most appropriate and likely control strategy would be based on an eradication program involving removal of diseased and exposed citrus trees in the quarantine areas. In addition, the pathogen has at least two uncultivated host genera (*Microcitrus* and *Zanthoxylum*) in the PRA area (see *Xcc* datasheet) and it may be possible to observe limited and reversible decline in these species, but they are not regarded as rare, vulnerable or keystone species. The eradication programs eliminating all these hosts may have a negative effect on plant biodiversity locally, but major changes in native community composition are not expected, and the physical modification of the habitats would depend on the size of the eradication area. Thus, this impact is expected to be minor and reversible.

The control programs against *Xcc* include also application of copper sprays. It is possible that the increased number of copper application needed may result in accumulation of copper in the soil (environmental pollution). Besides, the development of copper resistance is well documented for *Xcc* (Behlau *et al.*, 2011 a and b; Canteros *et al.*, 2010).

Xcc has not been implicated in affecting other organisms providing ‘Regulating services’ (*i.e.* biological control by natural enemies and antagonists, mitigation of local weather extremes, shoreline stability, river channel stability) or ‘Sustaining services’ (*i.e.* pollination, soil fertility maintenance, decomposition). *Xcc* has not being implicated in changes in nutrient cycling, nor modification of natural successions or disruption of trophic and mutualistic interactions. Moreover, *Xcc* is not known to be a vector for other pests. However, the damage caused on trees in citrus orchards or on ornamental citrus trees can be considered as an impact on

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[4] Council Directive 92/43/EEC (as amended) on the Conservation of natural habitats and of wild fauns and flora. Available at ttp://www.central2013.eu/fileadmin/user_upload/Downloads/Document_Centre/OP_Resources/HABITAT_DIRECTIVE_92-43-EEC.pdf

‘organisms providing Provisionary services’ affecting food provisions as well as genetic resources. Regarding these resources, it is known that several citrus-producing areas in the EU27 (*e.g.* Spain, Corsica) are the home of major resources of citrus germplasm that supply pest-free propagative material worldwide. Moreover, from the aspect of aesthetic impact, the damage caused on trees in citrus orchards or on ornamental citrus trees can be considered as an impact on ‘organisms providing Cultural services’.

Citrus-planted surfaces act as a carbon sink in Southern Europe (for example in Spain see http://www.intercitrus.org/NdSite/OnLineCache/FMS/87/53/e4785a346a4f471cfde0bb1870f65d82/Citricos%20huella%20de%20carbono.pdf). Eradication and/or abandon of groves would affect the positive effect of citrus against atmospheric CO_2.

2.5: Environmental impacts		
Rating	**Description**	**Probability Assignment** [1]
High	Two or more of the above would occur.	0%
Medium	One of the above would occur.	90%
Low	None of the above would occur; it is assumed that introduction of a non-indigenous pest will have some environmental impact (by definition, introduction of a non-indigenous species affects biodiversity).	10%
	Check sum =	100%

[1] spread your judgment according to your belief / evidence

Introduction Potential

3.0 List and describe the pathways for pest entry into the EU

For each pathway copy 3.1 to 3.6 and give responses by pathway

Pathway 1: Trade of fresh fruits
Fresh citrus fruits include oranges, lemons, limes, grapefruit, pummelos, mandarins, tangerines, clementines and satsumas.

Pathway 2: Trade of ornamental rutaceous plants and plant parts
Rutaceous species that are traded as ornamentals primarily consist of *Murraya* (whole plants and foliage) and to a lesser extent *Eremocitrus*, *Microcitrus* and *Severiana.* The importation of ornamental *Citrus, Poncirus* and *Fortunella* is banned by the council directive 2000/29EC.

Pathway 3: Illegal entry of plant propagative material
This could be any commercial variety (see pathway 1) as well as ornamentals (primarily calamondin, lemons, limes, sour oranges, kumquats). This covers putative introduction through mail services (including trade of plant propagation material trough internet with any official certificate), international travellers (air and sea), or any other means of introduction

Pathway 4: Entry of fruits through the passenger pathway
This includes fruit of all type of rutaceous species that could be transported by travellers and waste from passenger ships and planes.

The most relevant pathways (pathways 1 to 3), will be analyzed further in this document.

Pathway 1: Trade of fresh fruits...

3.1 Quantity of commodity imported annually

Quantity of commodity imported annually: The likelihood that an exotic pest will be introduced depends on the amount of the potentially-infested commodity that is imported. For qualitative pest risk assessments, the amount of commodity imported is estimated in units of tonnes, or other metric such as standard 40 foot long shipping containers.

Information / evidence: Provide reasoning then give judgment

With more than 6 M tons circulating per year (FAOstat, 2007), fresh citrus fruit is the second in volume among fruits traded in the EU after bananas. Among these, more than 2 M tons are imported from outside the EU. Citrus fruits originate from countries where citrus canker is widespread: approximately 420 ktons from Argentina, 110 ktons from Uruguay, 78 ktons from Brazil and 70 ktons from China (Eurostat, 2008). Major volumes of citrus fruits are imported by EU countries where citrus industry takes an important place in crop production (see examples in the Table below). There are important international intra-EU movements of citrus fruits. Important quantities of fresh citrus fruits are re-exported through the EU by several member countries (i.e. Netherlands, Belgium, Germany…). For instance in 2009, Belgium imported almost 60 ktons of fresh grapefruit (one third arriving from Florida and Argentina) and re-exported almost half of these fruits to other European countries. Netherlands is by far the main European citrus re-exporting country. In 2009, Netherlands imported around 450 ktons of sweet orange and almost 170 ktons of grapefruit from various countries (including Florida, Argentina, Brazil and Uruguay) and re-exported almost 200 ktons of sweet orange and 115 ktons of grapefruit to other EU countries, including citrus producing countries (Eurostat). Minor pathways such as kaffir limes (*Citrus hystrix*) used for cooking purpose also exist on a regular basis but for small volumes.

	Sweet orange (2008)										
From	**To**	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	**Total** (ton)
Argentina	Spain				197	4,930	15,035	9,391	1,785		**31,338**
Brazil	Spain					1,775	1,536	3,778	48		**7,137**
Uruguay	Spain			178	890	2,327	1,747	8,077	3,157		**16,376**
Argentina	Italy				124	454	1,304	1,959	321		**4,162**
Brazil	Italy						264	192			**456**
Uruguay	Italy			48	291	834	1,071	1,409	963		**4,615**
Argentina	Portugal				71	1,268	2,272	3,640	821		**8,071**
Brazil	Portugal					72	1,188	1,128	240		**2,628**
Uruguay	Portugal				219	10,392	7,875	9,064	14,196	247	**41,993**
		Lemon (2008)									
Argentina	Italy	50	478	4,746	11,248	18,353	19,261	5,363	163		**59,663**
Uruguay	Italy				43	481	1,031	540	207		**2,303**
Argentina	Portugal			310	128	531	640	130			**1,739**

3.1: Quantity of annual imports (Examples provided for tonnes and containers, other units can be used)			
Rating	**Tonnes imported into PRA area (per year)**	**Number of containers (per year)**	**Probability Assignment** [1]
High	> 1,000,000	>100 containers	100%
Medium	100 -1,000,000	10 - 100 containers	0%
Low	< 100	< 10 containers	0%
		Check sum =	100%

[1] spread your judgment according to your belief / evidence

3.2 Survive postharvest treatment:

For this sub-element, postharvest treatment refers to any manipulation, handling or specific phytosanitary treatment to which the commodity is subjected. Examples of postharvest treatments include culling, washing, chemical treatment, cold storage, etc. If there is no postharvest treatment, estimate the likelihood of this sub-element as High.

Information / evidence: Provide reasoning then give judgment
Cleaning, sorting and treatment of fruits: sorting of fruits may allow the removal and destruction of many (but not all) symptomatic fruits. Further treatments performed in packinghouse lines before export, such as the prewash of fruits with water and detergent (SOPP) and treatment with chlorines would have a partial negative effect on *Xcc* surface populations (Gottwald *et al*., 2009). No chemicals are known to have a marked negative effect on *Xcc* present in fruit lesions.

Numerous successful bacterial isolations from interceptions (Golmohammadi *et al*., 2007; LNPV, unpublished data; Fera, unpublished data) are in agreement with experimental data showing the isolation of viable *Xcc* from symptomatic fruits shipped from South America and analyzed in Spain upon arrival, even when treated by homologated chemicals (Golmohammadi *et al*., 2007).

3.2: Likelihood of surviving post harvest treatments		
Rating	**Description** (likelihood of survival is ....)	**Probability Assignment** [1]
High	> 10% (greater than one in ten survive)	100%
Medium	Between 0.1% - 10% (between one in one thousand to one in ten survive)	0%
Low	< 0.1% (less than one in one thousand survive)	0%
	Check sum =	100%

[1] spread your judgment according to your belief / evidence

3.3 Survive shipment

Estimate survival during shipment; assume standard shipping conditions.

Information / evidence: Provide reasoning then give judgment
Fruit transportation is under cool conditions (Wills *et al*., 1998), which have no negative effect on the survival of the bacteria (Goto, 1962). More specifically, shipping temperatures

for oranges and mandarins are fairly standard at 1°C and 4°C respectively, whereas lemons and limes are normally shipped at 10°C. Grapefruit temperatures range from 10 to 15°C depending on the time of the year and conditions of the trees at harvest. The cooler temperature provides better decay control while the warmer protects against chilling injury (Wardowski, 1981). It is thus very likely that *Xcc* survives the transport. However, *Xcc* does not multiply at temperatures used for fruit transportation (Civerolo, 1984). Furthermore, *Xcc* exponential multiplication primarily preceeds lesion development (Graham *et al*., 1992) and *Xcc* population sizes in canker lesions are known to remain stable or slightly decrease over time (Stall *et al*., 1980; Pruvost *et al*., 2002; Bui Thi Ngoc *et al*., 2010).

Numerous successful bacterial isolations from interceptions (LNPV, unpublished data, Fera, unpublished data) are in agreement with experimental data showing the isolation of viable *Xcc* from symptomatic fruits shipped from South America and analyzed in Spain upon arrival, even when treated by homologated chemicals (Golmohammadi *et al*., 2007).

3.3: Likelihood of surviving during shipping		
Rating	**Description** (likelihood of survival is ....)	**Probability Assignment** [1]
High	> 10% (greater than one in ten survive)	100%
Medium	Between 0.1% - 10% (between one in one thousand to one in ten survive)	0%
Low	0.1% (less than one in one thousand survive)	0%
	Check sum =	100%

[1] spread your judgment according to your belief / evidence

3.4 Not be detected at the port of entry

Unless specific protocols are in place for special inspection of the commodity in question, assume standard inspection protocols for like commodities. If no inspection is planned, estimate this sub-element as high.

Information / evidence: *Provide reasoning then give judgment*
Canker symptoms could be confused with abiotic stresses (shocks, physiological disorders…) or to other diseases (scab, back spot) by inexperienced inspectors. Survival of *Xcc* in association with wooden boxes has been reported (Das, 2003).

Citrus fruits can be imported from countries where the disease is present only if they are produced in an canker free area or in a canker free place of production. Despite this prerequisite incorporated into the current EU legislation, numerous interception reports by EU members are indicative of a regular presence of diseased fruit in consignments, primarily from Asia and South America.

***Xcc* interceptions reported on fruit consignments over the last 5 years by the EPPO reporting service**

Country	Year	Origin	Number
France	2009	Argentina	1
Greece	2007	Uruguay	1

Greece	2010	Uruguay	1
Spain	2009	Argentina	2
Spain	2006	Uruguay	1
UK	2006	Bangladesh	9
UK	2007	Bangladesh	21
UK	2008	Bangladesh	17
UK	2009	Bangladesh	18
UK	2010	Bangladesh	28
UK	2006	India	1
UK	2007	India	8
UK	2008	India	8
UK	2009	India	9
UK	2007	Pakistan	1
UK	2008	Pakistan	3
UK	2009	Pakistan	2
UK	2009	Thailand	3
UK	2008	Vietnam	1

In France, between 1997 to 2009, *Xcc* was officially diagnosed from 36 consignments mainly originating from Asia (Thailand, Malaysia, Vietnam, Sri Lanka, China, Bangladesh) and also from Argentina (LNPV, unpublished data). In Spain, secondary inspections done by local authorities in markets, supermarkets, packinghouses… have also identified additional diseased consignments (Lopez, M.M. personal communication). Nineteen interceptions (origin South America) have been done during the period 2003-2005 (Ministry of Agriculture, Spain).

Approximately 90 % of the reported interceptions have been done by UK only. This suggests (i) a lack of consistent reporting from some UE countries and/or (ii) inspection efforts that may be country-dependent. Most of the origins from which interceptions have been made are minor exporting countries. Among these, the most significant citrus exporter to the EU27 is Pakistan (small citrus 3 ktons, half of which is sent to UK). In contrast, huge volumes that should be more extensively surveyed originate primarily from Argentina (lemon 268 ktons, orange 96 ktons, grapefruit 24 ktons, small citrus 33 ktons), Uruguay (lemon 10 ktons, orange 58 ktons, small citrus 29 ktons) and China (pummelo/grapefruit 68 ktons) (Eurostat, 2008). No interception has been reported from Brazil although huge volumes are imported. This can likely be explained by the fact that imported citrus primarily originate from Sao Paulo state, which undergoes an eradication strategy for *Xcc*.

3.4: Likelihood pest will not be detected at port of entry

Rating	**Description** (likelihood of no detection is ....)	**Probability Assignment** [1]
High	> 10% (greater than one in ten will not be detected)	50%
Medium	Between 0.1% - 10% (between one in one thousand to one in ten will not be detected)	50%
Low	< 0.1% (less than one in one thousand will not be detected)	0%
	Check sum =	100%

[1] spread your judgment according to your belief / evidence

3.5 Imported or moved subsequently to an area with an environment suitable for survival

Consider the geographic location of likely markets and the proportion of the commodity that is likely to move to locations suitable for pest survival. Even if infested commodities enter the EU, perhaps not all final destinations will have suitable climatic conditions for pest survival.

Information / evidence: Provide reasoning then give judgment

Fresh fruit citrus are imported all year long in the EU (see examples in the Table in section 3.1) (Eurostat, 2008). Typically, volumes decrease in relation to the beginning of EU harvesting season but imported fruit volumes are high during late spring, summer and beginning of autumn (Eurostat, 2008).
Important quantities of fresh citrus fruits are re-exported through the EU by many member countries (i.e. Netherlands, Belgium, Germany, France, UK…). In 2008, the Netherlands imported from third countries around 390 ktons of sweet orange (one sixth of which originated from countries where *Xcc* has established) and 150 ktons of grapefruit (one third of which originated from countries where *Xcc* has established) and re-exported approximately 180 ktons of sweet orange and 120 ktons of grapefruit to other EU countries, including citrus producing countries (Eurostat, 2008). Examples of re-exportation of major species from France and Netherlands to citrus-producing countries is given below.

Citrus fruits (tons) imported from third countries in France and the Netherlands and redistributed to EU citrus-producing countries source: Eurostat (2008)

	Grapefruit	Orange	Lemon
Imported in France from third countries (tons)	41,510	60,479	12,764
% imported from countries where *Xcc* is established	6	2	67

Re-exported from France to : Greece Italy Portugal Spain	 62 1,104 214 623	 113 1,876 25 1,516	 189 2,063 63 1,473
Imported in the Netherlands from third countries (tons)	153,853	391,037	97,766
% imported from countries where *Xcc* is established	32	14	67
Re-exported from the Netherlands to : Greece Italy Portugal Spain	 575 3,277 41 5,428	 161 6,741 124 11,839	 725 1,459 45 2,079

The citrus fruit produce waste is the peel and it is this part of the fruit that is infected; therefore the inoculum is not destroyed but fated for waste. The main intended use of the commodity is consumption. However, some of the fruits that are imported from third countries are used for transformation as juice. Stockhouses for trade and processing plants in Spain, Italy and Greece are located in citrus producing areas (Baker et al., 2008; questionnaire sent to NPPOs within the Primaphacie project). Data from season 2003-2004 indicated that approximately 2.5 M tons of citrus (62 % of sweet orange) were transformed in the UE primarily for juice production.
Moreover, some alternative uses of citrus fruit are industrial (pectin extraction, cosmetics…). No waste treatment is considered by the EU-based industries, as the import of citrus fruits in the EU is only allowed under requirements that theoretically should allow only the import of *Xcc*-free fruits.

Citrus fruits produced and transformed in the EU (Tons) source: Contrat cadre n° 30-CE-0035027/00-3, évaluation OCM Fruits & Légumes

	Sweet orange	Lemon	Small fruit	Total
Italy	782,000	298,000	192,000	1,272,000
Spain	491,000	197,000	249,000	937,000
Greece	254,000	1,660	1,100	256,760
Total	1,530,000	497,000	442,000	2,465,760

Depending on species/cultivars, citrus fruit production periods in the EU is primarily over approximately half a year. At least in Spain, plants process fruits from third countries during the remaining months (source IVIA). Precise amounts are not known.

Citrus fruits imported by citrus-producing EU countries (tons) source: Eurostat, 2008. Values in brackets indicate volumes imported from countries where citrus canker has established (most of it originating from Argentina and Uruguay).

	Sweet orange	Lemon	Small fruit	Total
Italy	47,688 (9,233)	70,024 (61,966)	3,105 (1,837)	142,773 (74,947)
Spain	132,350 (54,850)	74,016 (57,248)	2,623 (1,404)	205,706 (116,701)
Greece	7,634 (534)	41,123 (28,407)	366 (0)	49,981 (29,213)
Portugal	28,676 (14,898)	3,828 (2,935)	3,392 (2,965)	38,508 (21,801)

The primary source of inoculum of many local outbreaks in countries where citrus canker is under surveillance (e.g. Sao Paulo state, Australia, Florida) has not been determined. Waste derived from industrial activity (transformation and trade of fruits originating from third countries in EU-based shipping centres) may not always be managed so that it prevents the escape of pathogens to the environment (Baker *et al.*, 2008). It cannot be excluded that this material be transferred in the vicinity of citrus plants. *Xcc* may survive up to 120 days on decomposing plant litter, including fruits (Civerolo, 1984; Graham *et al.*, 1987; Leite and Mohan, 1990).

3.5: Likelihood commodity that will be moved to suitable environment for pest survival (same as % of commodity moved to suitable environment)		
Rating	**Description** (likelihood, or amount moved to suitable environment is ....)	**Probability Assignment** [1]
High	> 10% (greater than one in ten)	100%
Medium	Between 0.1% - 10% (between one in one thousand to one in ten)	0%
Low	< 0.1% (less than one in one thousand)	0%
	Check sum =	100%

[1] spread your judgment according to your belief / evidence

3.6 Come into contact with host material suitable for reproduction

Even if the final destinations of infested commodities are suitable for pest survival, suitable hosts must be available in order for the pest to survive. Consider the complete host range of the pest species.

Information / evidence: Provide reasoning then give judgment
Xcc could likely come to contact with host species in citrus producing countries of the EU (major producers are Spain, Italy, Portugal and Greece) and could be dispersed through natural or human-assisted spread as reported worldwide (e.g. Gottwald *et al.*, 2002). It is very likely that *Xcc* could arrive in the PRA area during the months of the year most appropriate for establishment. *Xcc* may survive for ca. 120 days on decomposing plant litter, including fruit (Civerolo, 1984; Graham *et al.*, 1987; Leite and Mohan, 1990). The probability of transfer of *Xcc* from infected (and contaminated) fruits to citrus trees remains uncertain due to the paucity of literature. One study based on three experiments conducted in Florida and one in Argentina concluded on the lack of transmission from cull piles of fruit to surrounding trap plants unless environmental conditions highly conducive to spread were applied (Gottwald *et al.*, 2009). Therefore these results show that such dispersal is possible, although with a very low efficiency, and consistent with previous data collected in Japan.

Goto *et al*. (1978) observed some canker leaf lesions on *Citrus natsudaidai* from splash dispersal (produced by a rainfall simulator) of rice straw contaminated with *Xcc* at concentrations as low as 10^2 *Xcc* per gram of straw. Moreover, results by Gottwald *et al*. (2009) are difficult to transpose to situations where the lower branches of adult citrus trees grown commercially can be very close to the soil surface with a putative presence of symptomatic fruit or fruit peel. Another study involved the highly resistant Satsuma mandarin for which low *Xcc* population sizes are recorded in lesions (Shiotani *et al*., 2009), making the data impossible to transpose to susceptible cultivars. Indeed, the transfer of *Xcc* from imported infected fruits to citrus hosts is theoretically possible, although with a low likelihood. There is no authenticated record of this having happened (Das, 2003) but, importantly, there is a general lack of knowledge on the origin of inoculum associated with new outbreaks in countries where the pathogen is not widely established. For example, all recent outbreaks in Australia had the origin of inoculum unexplained (Broadbent *et al*., 1992; Gambley *et al*., 2009). The Florida outbreak of 1986-1994 started on backyard trees in the Tampa area, but the source of inoculum is unknown, although likely not a resurgence from outbreaks that occurred decades earlier (Schubert *et al*., 2001). Similarly, the huge outbreak known as the 'Miami outbreak' that was reported in 1995 and failed to be eradicated a decade later started from backyard trees but the precise origin of the inoculum is unknown (Gottwald *et al*., 1997; Schubert *et al*., 2001).

3.6: Likelihood pest will transfer to host material where it can reproduce		
Rating	**Description** (likelihood of pest transfer is ....)	**Probability Assignment** [1]
High	> 10% (greater than one in ten)	0%
Medium	Between 0.1% - 10% (between one in one thousand to one in ten)	0%
Low	< 0.1% (less than one in one thousand)	100%
	Check sum =	100%

[1] spread your judgment according to your belief / evidence

If there are multiple pathways, repeat steps 3.1 to 3.6 for each pathway.

- *Enter your scores for likelihoods into the Excel spreadsheet "Method 4 input table.xls" and send to Willem Roelofs (willem.roelofs@fera.gsi.gov.uk).*
- *Willem will return the results of combining scores to you indicating the overall introduction potential for inclusion in the risk assessment document.*

Pathway 2: Trade of ornamental rutaceous plants or plant parts......................................

3.1 Quantity of commodity imported annually

Quantity of commodity imported annually: The likelihood that an exotic pest will be introduced depends on the amount of the potentially-infested commodity that is imported. For qualitative pest risk assessments, the amount of commodity imported is estimated in units of tonnes, or other metric such as standard 40 foot long shipping containers.

Information / evidence: Provide reasoning then give judgment

Among the reported host species and based on data from ONPV France and Netherlands, only species of *Murraya* (M. *koenigii*, *M. paniculata* [syn. *M. exotica*], *Murraya* sp.) are regularly imported in the EU as ornamental plant or foliage. Thus this pathway is only evaluated on the risk represented by genus *Murraya*.

In areas of origin where citrus canker occurs, it is possible that *Murraya* plants could get diseased, but their susceptibility is not clearly established. Depending on commercial opportunities and EU consumers taste, other species such as *Severiana*, *Eremocitrus*, *Microcitrus* could be imported in the future.

Based on statistics from France and Netherlands (ONPV), we can hypothesize that this pathway concerns small quantities but represent high value of plant material, such as bonsai. The origin of these plants is not readily available, but based on date from ONPV France and Netherlands and unchecked information from the internet, the main origin is Asia, which is an area of high prevalence of *Xcc*. Quite a lot of bonsai lots are coming from China where *Xcc* is widespread and foliage lots were shown to originate from other tropical countries where *Xcc* is present (Thailand, India) or absent (Burundi, Dominican republic…). These low volumes are submitted to fluctuations depending on EU consumer demands; imported *Murraya* plants are primarily bonsai but they can be used as hedges in public or private gardens.

3.1: Quantity of annual imports (Examples provided for tonnes and containers, other units can be used)			
Rating	**Tonnes imported into PRA area (per year)**	**Number of containers (per year)**	**Probability Assignment** [1]
High	> 1,000,000	>100 containers	0%
Medium	100 -1,000,000	10 - 100 containers	0%
Low	< 100	< 10 containers	100%
		Check sum =	100%

[1] spread your judgment according to your belief / evidence

3.2 Survive postharvest treatment:

For this sub-element, postharvest treatment refers to any manipulation, handling or specific phytosanitary treatment to which the commodity is subjected. Examples of postharvest treatments include culling, washing, chemical treatment, cold storage, etc. If there is no postharvest treatment, estimate the likelihood of this sub-element as High.

Information / evidence: *Provide reasoning then give judgment*

No postharvest method is known to supress or markedly affect *Xcc* populations in canker lesions or in latently infected tissues. Sorting of apparently healthy plants within a contaminated lot or pruning of diseased twigs can sometimes be achieved before shipment but they do not guarantee a complete elimination of inoculum. In the case when plants in the consignment bear juvenile organs (leaves, twigs), high population sizes of *Xcc* can be present as latent infections and these are visually undetectable.

3.2: Likelihood of surviving post harvest treatments		
Rating	**Description** (likelihood of survival is ....)	**Probability Assignment** [1]
High	> 10% (greater than one in ten survive)	100%
Medium	Between 0.1% - 10% (between one in one thousand to one in ten survive)	0%

Low	< 0.1% (less than one in one thousand survive)	0%
	Check sum =	100%

[1] spread your judgment according to your belief / evidence

3.3 Survive shipment

Estimate survival during shipment; assume standard shipping conditions.

Information / evidence: Provide reasoning then give judgment
Propagation material, grafted plants and foliage are transported and stored under conditions that favour the survival of the plant itself (air transport in cool boxes). Such conditions have no negative effect on the survival of *Xcc* (Goto, 1962). It is thus very likely that *Xcc* survives the transport.

3.3: Likelihood of surviving during shipping		
Rating	**Description** (likelihood of survival is ....)	**Probability Assignment** [1]
High	> 10% (greater than one in ten survive)	100%
Medium	Between 0.1% - 10% (between one in one thousand to one in ten survive)	0%
Low	0.1% (less than one in one thousand survive)	0%
	Check sum =	100%

[1] spread your judgment according to your belief / evidence

3.4 Not be detected at the port of entry

Unless specific protocols are in place for special inspection of the commodity in question, assume standard inspection protocols for like commodities. If no inspection is planned, estimate this sub-element as high.

Information / evidence: Provide reasoning then give judgment
Canker symptoms could be confused with abiotic stresses (shocks, physiological disorders…) or to other diseases (scab, back spot) by inexperienced inspectors. Survival of *Xcc* in association with wooden boxes has been reported (Das, 2003).
All ornamental plants or foliage imported in EU from third countries are submitted to phytosanitary procedures according to the council directive 2000/29/CE:
- in reference to Annex V, part B any plants must be accompanied by a phytosanitary certificate which gives an evidence that plants have been controlled at the origin,
- in reference to Annexe IV, part A planting material must answer to specific requirements , chap I, art.39 any planting material must free of fruits and flowers, grown in nurseries, and visually inspected before exportation and attested free of bacterial, virus and virus-like symptoms.
- foliage are controlled by reference to Annex I, to ensure that quarantine pests and diseases listed in this annex are not present on imported plants: in that particular case foliage controls are done to ensure that pests are nor present.

Special requirements exist for the genus *Murraya* (Annex II, Part A) for the Asian citrus psyllid (*Diaphorina citri* Kuway) but not for *Xcc*.

3.4: Likelihood pest will not be detected at port of entry		
Rating	**Description** (likelihood of no detection is ....)	**Probability Assignment** [1]
High	> 10% (greater than one in ten will not be detected)	50%
Medium	Between 0.1% - 10% (between one in one thousand to one in ten will not be detected)	50%
Low	< 0.1% (less than one in one thousand will not be detected)	0%
	Check sum =	100%

[1] spread your judgment according to your belief / evidence

3.5 Imported or moved subsequently to an area with an environment suitable for survival

Consider the geographic location of likely markets and the proportion of the commodity that is likely to move to locations suitable for pest survival. Even if infested commodities enter the EU, perhaps not all final destinations will have suitable climatic conditions for pest survival.

Information / evidence: Provide reasoning then give judgment
Ornamental plants could be redistributed within the EU, but the volumes are very low. Part of this material could be used as propagative material but most likely at very small scales. Imports likely occur year-round. Diseased ornamental rutaceous species could be settled in the vicinity of more susceptible host species in private gardens for example.

3.5: Likelihood commodity that will be moved to suitable environment for pest survival (same as % of commodity moved to suitable environment)		
Rating	**Description** (likelihood, or amount moved to suitable environment is ....)	**Probability Assignment** [1]
High	> 10% (greater than one in ten)	0%
Medium	Between 0.1% - 10% (between one in one thousand to one in ten)	10%
Low	< 0.1% (less than one in one thousand)	90%
	Check sum =	100%

[1] spread your judgment according to your belief / evidence

3.6 Come into contact with host material suitable for reproduction

Even if the final destinations of infested commodities are suitable for pest survival, suitable hosts must be available in order for the pest to survive. Consider the complete host range of the pest species.

Information / evidence: Provide reasoning then give judgment
If the imported plants are used as mother plants for propagation in nurseries, then the risk of transfer is very high. Diseased or contaminated ornemental plants could act as a source of inoculum if present in a citrus producing area. Diseased ornamental rutaceous species could be settled in the vicinity of more susceptible host species in private gardens for example.

3.6: Likelihood pest will transfer to host material where it can reproduce		
Rating	**Description** (likelihood of pest transfer is ....)	**Probability Assignment** [1]
High	> 10% (greater than one in ten)	10%
Medium	Between 0.1% - 10% (between one in one thousand to one in ten)	80%
Low	< 0.1% (less than one in one thousand)	10%
	Check sum =	100%

[1] spread your judgment according to your belief / evidence

If there are multiple pathways, repeat steps 3.1 to 3.6 for each pathway.

- *Enter your scores for likelihoods into the Excel spreadsheet "Method 4 input table.xls" and send to Willem Roelofs (willem.roelofs@fera.gsi.gov.uk).*
- *Willem will return the results of combining scores to you indicating the overall introduction potential for inclusion in the risk assessment document.*

Pathway 3: Illegal entry of plant propagative material...

3.1 Quantity of commodity imported annually

Quantity of commodity imported annually: The likelihood that an exotic pest will be introduced depends on the amount of the potentially-infested commodity that is imported. For qualitative pest risk assessments, the amount of commodity imported is estimated in units of tonnes, or other metric such as standard 40 foot long shipping containers.

Information / evidence: Provide reasoning then give judgment

The pathway is composed of illegally imported material, which is by definition uncontrolled. Precise volumes are therefore unknown, although likely low. Although the frequency of import may be low, the consequences can be very high. For example, the introduction in Spain of Toxoptera citricida, the citrus brown aphid (Hermoso de Mendoza *et a*l., 2008), and of severe *Citrus Tristeza Virus* strains (Cambra *et al*., 1992) is most likely the result of the illegal importation of citrus plant propagative material (Lopez, M.M., personal communication). Tonnage cannot apply here, so the estimate in the Table below is a number of introductions.

3.1: Quantity of annual imports (Examples provided for tonnes and containers, other units can be used)			
Rating	**Tonnes imported into PRA area (per year)**	**Number of containers (per year)**	**Probability Assignment** [1]
High	> 1,000,000	>100 containers	0%
Medium	100 -1,000,000	10 - 100 containers	0%
Low	< 100	< 10 containers	100%
		Check sum =	100%

[1] spread your judgment according to your belief / evidence

3.2 Survive postharvest treatment:

For this sub-element, postharvest treatment refers to any manipulation, handling or specific phytosanitary treatment to which the commodity is subjected. Examples of postharvest treatments include culling, washing, chemical treatment, cold storage, etc. If there is no postharvest treatment, estimate the likelihood of this sub-element as High.

Information / evidence: Provide reasoning then give judgment
The pathway is composed of illegally imported material, so the only management practice applied to it is based on the destruction of plant material when detected.

3.2: Likelihood of surviving post harvest treatments		
Rating	**Description** (likelihood of survival is ....)	**Probability Assignment** [1]
High	> 10% (greater than one in ten survive)	100%
Medium	Between 0.1% - 10% (between one in one thousand to one in ten survive)	0%
Low	< 0.1% (less than one in one thousand survive)	0%
	Check sum =	100%

[1] spread your judgment according to your belief / evidence

3.3 Survive shipment

Estimate survival during shipment; assume standard shipping conditions.

Information / evidence: Provide reasoning then give judgment
Budwood or grafted plants are transported and stored under conditions that favour the survival of the plant itself. Such conditions have no negative effect on the survival of *Xcc* (Goto, 1962). It is thus very likely that *Xcc* survives the transport.

3.3: Likelihood of surviving during shipping		
Rating	**Description** (likelihood of survival is ....)	**Probability Assignment** [1]
High	> 10% (greater than one in ten survive)	100%
Medium	Between 0.1% - 10% (between one in one thousand to one in ten survive)	0%
Low	0.1% (less than one in one thousand survive)	0%
	Check sum =	100%

[1] spread your judgment according to your belief / evidence

3.4 Not be detected at the port of entry

Unless specific protocols are in place for special inspection of the commodity in question, assume standard inspection protocols for like commodities. If no inspection is planned, estimate this sub-element as high.

Information / evidence: Provide reasoning then give judgment
The pathway is composed of illegally imported material which would primarily enter through mail or courier services, travelers luggage, and would therefore very likely enter the PRA area undetected.

3.4: Likelihood pest will not be detected at port of entry		
Rating	**Description** (likelihood of no detection is ....)	**Probability Assignment** [1]
High	> 10% (greater than one in ten will not be detected)	100%
Medium	Between 0.1% - 10% (between one in one thousand to one in ten will not be detected)	0%
Low	< 0.1% (less than one in one thousand will not be detected)	0%
	Check sum =	100%

[1] spread your judgment according to your belief / evidence

3.5 Imported or moved subsequently to an area with an environment suitable for survival

Consider the geographic location of likely markets and the proportion of the commodity that is likely to move to locations suitable for pest survival. Even if infested commodities enter the EU, perhaps not all final destinations will have suitable climatic conditions for pest survival.

Information / evidence: Provide reasoning then give judgment
Illegally imported propagative material would most likely be planted locally and not massively redistributed within the EU. Imports likely occur most often from spring to autumn (at a time most suitable for grafting). However, because the material is plant for planting, the risk that the material be distributed cannot be excluded, even if it is not over wide scales.

3.5: Likelihood commodity that will be moved to suitable environment for pest survival

(same as % of commodity moved to suitable environment)		
Rating	**Description** (likelihood, or amount moved to suitable environment is ....)	**Probability Assignment** [1]
High	> 10% (greater than one in ten)	0%
Medium	Between 0.1% - 10% (between one in one thousand to one in ten)	50%
Low	< 0.1% (less than one in one thousand)	50%
	Check sum =	100%

[1] spread your judgment according to your belief / evidence

3.6 Come into contact with host material suitable for reproduction

Even if the final destinations of infested commodities are suitable for pest survival, suitable hosts must be available in order for the pest to survive. Consider the complete host range of the pest species.

Information / evidence: Provide reasoning then give judgment

Budwood could likely be grafted in a citrus producing region of the PRA area and be established in the vicinity of citrus plants in orchards or private gardens. Although much less likely because of the awareness of nurserymen, such illegally imported budwood could be used in nurseries.

The source(s) of inoculum of outbreaks in areas where *Xcc* had been absent or not widely distributed is most often not precisely known. However when documented, there is evidence or strong suspicion of citrus propagative material (legally or illegally introduced) being the source of the related outbreaks. For example, the 1912 outbreak in Northern Territory (Australia) was caused by the importation of citrus plants from China and Japan (Broadbent *et al*., 1992). The outbreak recorded in 1981 in the Cocos Islands likely originated from backyard trees but the precise context of this introduction remains unreported (Broadbent *et al*., 1992). The 1991 and 2004-2005 outbreaks in Northern Territory and Queensland, respectively, have not been elucidated but it is hypothesized that the former one has been the result of illegal budwood importation (Broadbent *et al*., 1992; Gambley *et al*., 2009). In Florida, the 1910 outbreak was caused by the introduction of trifoliate rootstock from Japan (Schubert *et al*., 2001). The outbreak of 1986-1994 started on backyard trees in the Tampa area, but the source of inoculum is unknown, although likely a new introduction (Schubert *et al*., 2001). An illegal movement of contaminated material was suspected as the cause of an isolated outbreak in South Florida in 1990, but its precise nature has been impossible to determine (Gottwald *et al*., 1992). Similarly, the major outbreak known as the ‘Miami outbreak’ that was reported in 1995 and failed to be eradicated a decade later started from backyard trees but the precise origin of the inoculum is unknown (Gottwald *et al*., 1997; Schubert *et al*., 2001). The importation of the A^w strain was linked to the illegal importation of propagative material from India by an Indian-origin citizen (Schubert *et al*., 2001; Sun *et al*., 2004). In Brazil, the history of introductions has been poorly documented. The initial outbreak in Sao Paulo state (Presidente Prudente) in 1957 was reported to have occurred first in a small nursery owned by a manager of Japanese origin (Rossetti, 1977). In Spain, the introduction of *Toxoptera citricida*, the citrus brown aphid (Hermoso de Mendoza et al., 2008), and of severe *Citrus tristeza virus* strains (Cambra et al., 1992) is most likely the result of the illegal importation of citrus plant propagative material (Lopez, M.M., personal communication).

3.6: Likelihood pest will transfer to host material where it can reproduce		
Rating	**Description** (likelihood of pest transfer is ....)	**Probability Assignment** [1]
High	> 10% (greater than one in ten)	100%
Medium	Between 0.1% - 10% (between one in one thousand to one in ten)	0%
Low	< 0.1% (less than one in one thousand)	0%
	Check sum =	100%

[1] spread your judgment according to your belief / evidence

If there are multiple pathways, repeat steps 3.1 to 3.6 for each pathway.

- *Enter your scores for likelihoods into the Excel spreadsheet "Method 4 input table.xls" and send to Willem Roelofs (willem.roelofs@fera.gsi.gov.uk).*
- *Willem will return the results of combining scores to you indicating the overall introduction potential for inclusion in the risk assessment document.*

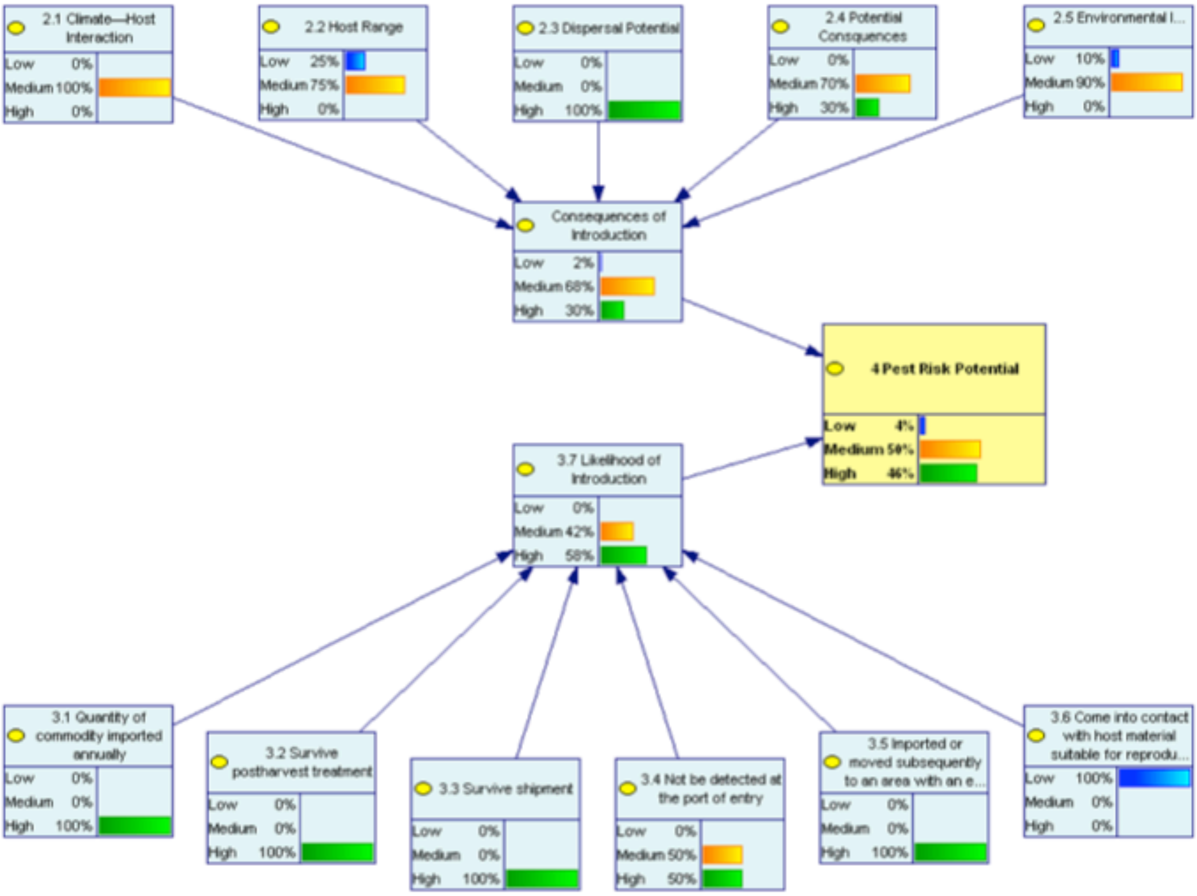
3.7 Potential for introduction via individual pathways

(Include results from Willem)

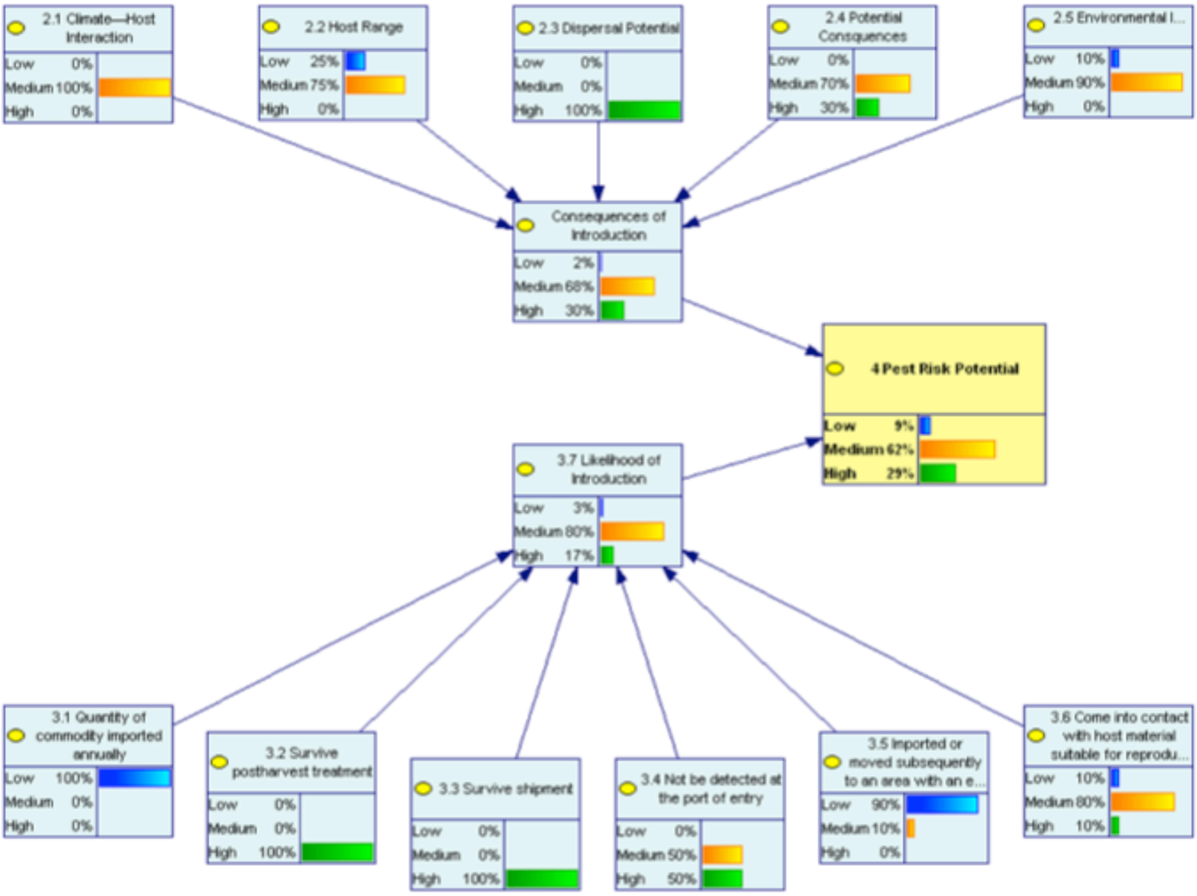
3.8 Overall potential for introduction

(Include results from Willem)

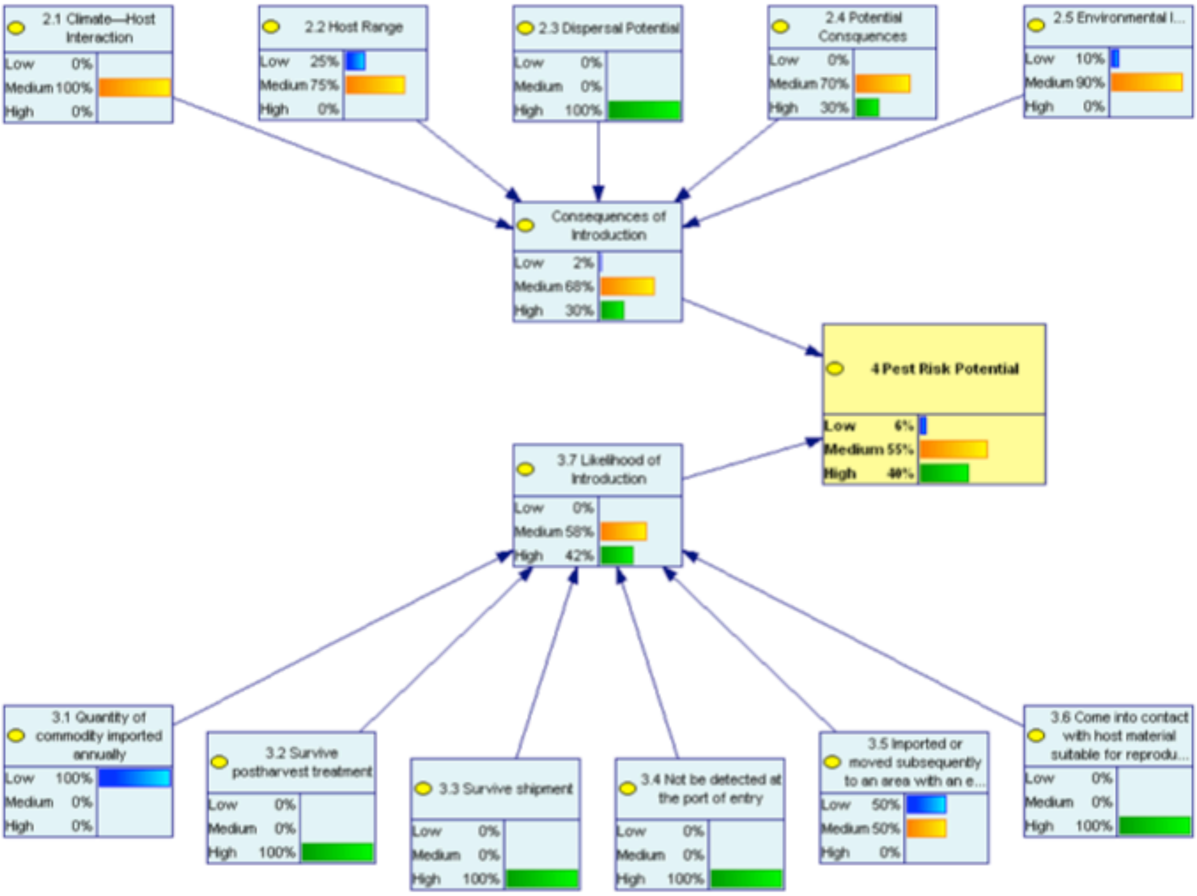
Pathway 1: trade of fresh fruits



Pathway 2: trade of ornamental rutaceous plants and plant parts



Pathway 3: illegal entry of plant propagative material



4.0 Overall pest risk

A combination of Consequences of introduction (2.1 to 2.5) with potential for introduction (3.0 to 3.8) (Include results from Willem)

Based on the expert opinion of the assessors involved in this assessment, the overall risk presented by *Xcc* to its hosts in the EU is high. The BBN output suggests that overall risk (pest risk potential) is mostly split between the classes described as ‘medium’ and ‘high’. Sensitivity analyses should be conducted, as some of the results somewhat differ from those derived from other methods. For example, method 4 estimates that the most important risk is associated with pathway 1 “trade of fresh fruits”, somewhat inconsistent with methods 1, 2 and 5 which suggest that the risk for this pathway is lower than the one for pathway 3 ‘illegal entry of plant propagative material’. The risk evaluation for methods 1, 2 and 5 therefore better reflected the expert judgement.

5.0 Conclusion

Xcc is not reported to be present in the PRA area (EU27) where it is considered as a quarantine organism for the PRA area under the council directive 2000/29/EC. The directive 2000/29/EC lists in the Annex II part A, section 1 b, “*Xanthomonas campestris* (all strains pathogenic to *Citrus*)”, which includes *Xcc*, banning its introduction into EU if present on plants of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, other than seeds. Annex III part A prohibits the introduction into all EU member states of plants of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, other than fruit and seeds. Special requirements, regarding "*Xanthomonas campestris* (all strains pathogenic to *Citrus*)", are specified in Annex IV part A , section I point 16.2, for the introduction and movement into and within all EU member states of fruit of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, originating in third countries. Citrus canker has been a destructive and costly disease in many areas. The probability of introduction is high, given the numerous interceptions reported on fruit consignments in the EU and the reports from several areas (e.g. USA, Brazil, Australia) stating on several introductions of *Xcc* in putative association with illegal entries of citrus propagative material. From the three pathways that have been documented herein, a single pathway (trade of fresh fruits) represents massive volumes at high frequencies, but the probability of transfer and establishment by inoculum brought up through this pathway is likely low, although poorly documented. Although likely consisting of low volumes, the pathway that is at very high risk for establishment corresponds to the illegal entry in the EU27 of plant propagative material, consistent with several reports from other areas worldwide.

Although primarily used for pests and weeds, climex-based analyses have also been used for agricultural plant pathogens (Baker et al., 2000; Paul et al., 2005; Pivonia & Yang, 2004; Shaw & Osbourne, 2011; Yonow et al., 2004). However, the estimation of where a pathogen might live, but does not, relies on numerous parameters that may not all be taken into account in climatic mapping-based models. For example, the lack of rain during the summer months would limit the presence of the canopy wetness necessary for infection, but it was shown from Spanish data that rainfall and rain days are not good indicators of the citrus canopy wetness because of the frequent presence of dew during summer nights with temperatures over 15 and even 20°C (Vicent & Garcia-Jimenez, 2008). Based on a climex map for *Xcc*, Spain (Valencia province, Baleares), Greece, Italy (Basilicata, Calabria, Campania, Lazio, Puglia, Sicilia and Toscana regions) and France (PACA region) have some of their citrus growing areas with an ecoclimatic index (EI) over 30 that could likely allow establishment of *Xcc*. Other areas with an ecoclimatic index between 10 and 30 still considered at risk for establishment include other citrus-growing areas in the above-mentioned countries as well as those in Portugal, Greece, Cyprus, France (Corsica), Italy (Liguria and Sardegna regions) and Malta.

The climatic conditions of Southern Europe, the presence of approximately 0.5 M ha of *Citrus* host plants in groves, nurseries, smallholdings, private gardens…, the global warning, the biology of *Xcc* and the absence of natural antagonists or enemies, the cultural practices and the poorly efficient IPM measures are consistent with a possible establishment of *Xcc* in the endangered areas of the PRA area listed above. The probability of spread may be considered likely. Natural dispersal would primarily be by rain and wind-driven rain and would spread *Xcc* at small to medium scales. Some weather events such as summer storms, which can be quite frequent in Southern Europe, have the ability to spread *Xcc* at larger distances (i.e. approximately at up to a kilometre scale). Human activities would undoubtedly favour spread of *Xcc* whatever the considered scale. Long distance spread would primarily be through human activities (e.g. movement of contaminated or exposed plant material and through machinery, clothes, etc. polluted by *Xcc* during grove or nursery maintenance operations) and the importance of which would be largely dependant on the deployment of prompt and strict quarantine measures for isolating outbreaks. Human-driven unintentional spread could also be increased due to the massive presence of citrus trees in streets, private and public gardens. It is unknown how likely intentional movement of *Xcc* by persons in the PRA area could be achieved. Although likely not well suited as a bio-terrorism agent, *Xcc* is also listed as 'dual use technology and organism' (council regulation EC 394/2006).

Susceptible *Citrus* cultivars are widely grown in the PRA area. Should *Xcc* become established in some parts of the PRA area, direct damage (yield loss, tree defoliation, alteration of fruit external quality, abandon of groves, higher soil erosion in areas where citrus are grown in terraces, higher soil erosion in areas where citrus are grown in terraces) would be likely high. Furthermore, the establishment of *Xcc* would threaten internationally major resources of citrus germplasm that are present in several citrus-producing areas in the EU27 (e.g. Spain, Corsica) and supply pest-free propagative material worldwide. In the absence of efficient IPM measures, costs of inspection, quarantine, eradication of trees in infected areas and certification of plants would be very high. Indirect damage would also include a possible loss of export markets, social consequences and moderate environmental consequences (e.g. soil pollution by copper, loss of biodiversity, degradation of the ability of citrus-planted surfaces to decrease atmospheric CO_2).

Reported uncertainty, such as for example the one reported on the potential role of minor susceptible rutaceous host species in the introduction and spread of *Xcc* (see the 'Degree of uncertainty' section above), should be addressed through research efforts. A comprehensive evaluation of costs linked to the establishment of *Xcc* in endangered areas of the EU27, as done in other countries (e.g. Australia, Brazil, USA – Alam & Rolfe, 2006; Jetter *et al*., 2000; Spreen *et al*., 2003; Bassanezi *et al*., 2008) should be undertaken.

Topics with medium to high uncertainty are listed below:

- The rate of infection of citrus fruits imported from countries where *Xcc* is present and the concentration of *Xcc* in consignments: This rate is difficult to assess, as it is dependent on the technologies implemented by exporting countries in the field and in packinghouses, the percentage of consignments subjected to a strict respect of phytosanitary measures, but it is also highly dependent on environmental conditions, which are by definition variable. The numerous interceptions in the EU of consignments containing diseased fruits suggest a lack of total reliability of the integrated measures that are taken in a systems approach for eliminating the risk of exporting contaminated and/or diseased fruits.
- The number of interceptions from the fresh fruit pathway: Although rather high (> 100 over the last 5 years),it is likely uncorrect and markedly underestimated (90% of the EPPO reports done by UK; major citrus importers from countries where *Xcc* is prevalent do not report; inspection efforts upon arrival may be quite variable among EU countries)
- The extent of trade of *Murraya* plants (and to a lesser extent of other ornamental rutaceous species) in the EU27 and their use as mother propagative material
- The susceptibility of *Murraya* and other ornamental rutaceous species to the strains and pathotypes of *Xcc* reported worldwide and the associated symptomatology.
- No studies have been made to investigate the possibility of latent infection and/or endophytic and/or epiphytic presence of Xcc in *Murraya* plants.
- The extent of illegal importation of citrus material
- The role of infected citrus fruit/peel present in the vicinity of susceptible plants as a source of primary inoculum allowing establishment: The two published papers on this issue (Gottwald *et al*., 2009; Shiotani *et al*., 2009) are insufficient for fully addressing this question, which deserves the production of much more experimental data. Both studies have also been published in a context of countries willing to have fruit from contaminated places of production being exported to *Xcc*-free countries and this would justify that extensive EU-conducted trials be performed. Moreover, there is globally a lack of knowledge on sources of primary inoculum associated with outbreaks in areas where *Xcc* is not endemic. The likelihood of diseased citrus to act as a source of primary inoculum is low but cannot presently be completely ruled out.

- An important role of biofilm formation in survival of *Xcc* was suggested recently (Rigano et al., 2007; Cubero *et al*., 2011) but its biological significance remains poorly understood.

- A reversible viable but not culturable (VBNC) state has been suggested for *Xcc* in response to copper ions (Del Campo *et al.*, 2009; Lopez, M.M., personal communication) These results highlight the need of research on asymptomatic survival to evaluate the long-term survival and epidemiological significance of these VBNC populations.
- Searches in databases reviewed in WP1 of Pratique and other searches did not allow to estimate precisely the risk of natural spread by wind-driven rains in citrus production areas of the EU.

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