

## SCIENTIFIC OPINION

### Scientific Opinion on the risk of *Phyllosticta citricarpa* (*Guignardia citricarpa*) for the EU territory with identification and evaluation of risk reduction options<sup>1</sup>

EFSA Panel on Plant Health (PLH)<sup>2,3</sup>

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#### ABSTRACT

The Panel conducted a risk assessment of *Phyllosticta citricarpa* for the EU. *P. citricarpa* causes citrus black spot (CBS) and is absent from the EU. Under the scenario of absence of specific risk reduction options against *P. citricarpa*, the risk of entry of *P. citricarpa* was rated as likely for citrus plants for planting and citrus fruit with leaves, moderately likely for citrus fruit without leaves, unlikely for citrus leaves for cooking and very unlikely for Tahiti lime fruit without leaves. Establishment was rated as moderately likely because susceptible hosts are widely available and environmental conditions in many EU citrus-growing areas are suitable (with high uncertainty) for *P. citricarpa* ascospore production, dispersal and infection. Current fungicide treatments will not prevent establishment. Environmental favourability is increased by the use of sprinkler and micro-sprinkler irrigation in some EU citrus-growing locations. Spread with trade was rated as moderately likely. Model results indicate that CBS epidemics are most likely to develop in EU citrus-growing areas in late summer to early autumn and in some locations also in late spring to early summer. CBS is expected to affect mainly lemons and late-maturing sweet orange and mandarin varieties, with moderate negative consequences for the production of fresh fruit, but with environmental impact of additional fungicide treatments. Negative consequences would be minor for early-maturing citrus varieties and minimal for citrus for processing. Uncertainty concerning the consequences is high, mainly because of the lack of data on critical climate response parameters for the pathogen but also because information on impact in areas at the limits of the current distribution is scarce. Since eradication and containment are difficult, phytosanitary measures should focus on preventing entry. Current phytosanitary measures are evaluated to be effective, with the exception of pest-free production sites.

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#### KEY WORDS

*Phyllosticta citricarpa*, *Guignardia citricarpa*, citrus black spot, European Union, pest risk assessment, risk reduction options

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## SUMMARY

The European Commission requested EFSA to prepare a pest risk assessment of the citrus black spot (CBS) fungus *Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*), to identify risk reduction options and to evaluate their effectiveness in reducing the risk to plant health posed by this organism in the EU territory.<sup>4</sup> EFSA was also requested to carry out an evaluation of the effectiveness of the present EU requirements<sup>5</sup> for *Guignardia citricarpa* in reducing the risk of introduction of this harmful organism into the EU. Furthermore, EFSA was requested to assess the risk associated with *Citrus latifolia* plants, including fruit, for the entry of this organism into the EU.

Following a request from the European Commission, the EFSA's Scientific Panel on Plant Health (PLH) had undertaken in the summer 2013 a public consultation on the draft Scientific Opinion on the risk to plant health of *Phyllosticta citricarpa* (*Guignardia citricarpa*) for the EU territory. The comments received during the public consultation were taken into account and the Scientific Opinion was revised accordingly.

The Panel on Plant Health (PLH) conducted the risk assessment following its guidance documents on a harmonised framework for pest risk assessment (EFSA PLH Panel, 2010) and on evaluation of risk reduction options (EFSA PLH Panel, 2012).

The Panel conducted the risk assessment in the absence of current and potential new risk reduction measures in place. The risk assessment therefore expresses the full risk posed by *P. citricarpa* to the EU territory corresponding to a situation in which all current EU citrus requirements listed in Council Directive 2000/29/EC (in Annexes II, III, IV and V) and Commission Decisions 2004/416/EC and 2006/473/EC are lifted without being replaced by any other risk reduction measures. The Panel undertook a simplified quantitative pathway analysis exercise for the trade of commercial citrus fruit in order to examine with further detail the various steps involved in a potential pathogen entry process and to support the qualitative ratings.

The risk assessment covers *Guignardia citricarpa* Kiely, which has since been renamed *Phyllosticta citricarpa* (McAlpine) Van der Aa. Other *Phyllosticta* species associated with citrus are not included.

After consideration of the evidence, the Panel reached the following conclusions:

### **With regard to the pest categorisation:**

*P. citricarpa* is absent from the EU and has a potential for establishment and spread and for causing consequences in the risk assessment area.

### **With regard to the assessment of the risk to plant health for the EU territory:**

Under the scenario in which all current EU requirements listed in Council Directive 2000/29/EC and Commission Decisions 2004/416/EC and 2006/473/EC are lifted, the conclusions of the pest risk assessment are as follows:

#### Entry

The probability of entry is rated as:

- moderately likely for the citrus fruit trade pathway (medium uncertainty)
- very unlikely for the Tahiti lime (*Citrus latifolia*) fruit trade pathway (high uncertainty)

<sup>4</sup> The request was made pursuant to Article 29(1) and Article 22(5) of Regulation (EC) No 178/2002.

<sup>5</sup> The current requirements are listed in Annexes III, IV and V of Council Directive 2000/29/EC, as well as in Commission Decisions 2004/416/EC and 2006/473/EC.

- unlikely for citrus fruit import by passengers traffic pathway (medium uncertainty)
- likely for the citrus fruit with leaves trade pathway (medium uncertainty)
- likely for the citrus plants for planting trade pathway (low uncertainty)
- likely for the Tahiti lime (*Citrus latifolia*) plants for planting trade pathway (high uncertainty)
- likely for the citrus plants for planting import by passengers traffic (medium uncertainty)
- unlikely for the citrus leaves (medium uncertainty).

### Establishment

The probability of establishment is rated as moderately likely because of:

- the widespread availability of susceptible hosts (no uncertainty)
- the climate suitability for ascospores maturation, dispersal and infection of many EU citrus-growing areas in late summer and early autumn and for specific location also in late spring and early summer (high uncertainty)
- cultural practices (fungicides) not preventing establishment (low uncertainty)
- sprinkle and micro-sprinkle irrigation (still used in part of the EU citrus-growing areas) favouring establishment (low uncertainty)

Overall, the uncertainty on the probability of establishment is rated as high, mainly because of lack of knowledge of how *P. citricarpa* will respond under the EU climatic conditions. Although it is known which environmental factors are important to the organism in the various stages of the life cycle, there is insufficient scientific evidence to determine the exact thresholds of these factors required by the organism, e.g. temperature and wetness levels and durations. Further validation of the models applied by the Panel, especially for marginal areas within the current distribution of the citrus black spot disease, would be needed to reduce the uncertainty on the probability of establishment of *P. citricarpa* in the EU.

### Spread

Natural spread of *P. citricarpa* is known to mainly happen by dispersal of airborne ascospores. There is little evidence about the dispersal distances of the pathogen by natural means, The pathogen is very likely to spread with human assistance along the commercial fruit and plants for planting pathways. However, because spread is defined as the expansion of the geographical distribution of a pest within an area, the rate of spread depends not only on the rapidity of movement and the number of spread pathways but also on the likelihood of finding a suitable environment for establishment. When the proportion of the citrus-growing areas identified as potentially suitable for *P. citricarpa* is taken into account, the Panel considered that a rating of moderately likely is most appropriate for spread.

Although there is uncertainty about the potential natural spread of ascospores carried by wind over long distances, this uncertainty does not concern the two main pathways of spread (intra-European trade of commercial fruit and plants for planting).

### Endangered area

The risk assessment has identified parts of the EU where host plants are present and where, based on simulation results, the climatic conditions are suitable for ascospore maturation and release followed by infection.

Conclusions from simulations of the release of ascospores based on gridded interpolated climate data of the EU citrus-producing areas show that, in almost all years, ascospore release in the EU citrus growing areas will start early enough to coincide with climatic conditions that are conducive to infection in September and October. However, the simulations indicate that the onset of ascospore release in most areas will start too late to coincide with the climatic conditions conducive to infection in spring. Therefore, early-maturing citrus varieties might generally be infected in late summer and early autumn, which is when the availability of inoculum coincides with suitable conditions for infection. Owing to the long incubation time, fruits from these early varieties will be harvested before symptoms appear. The late-maturing oranges varieties and lemons are expected under such scenario to show CBS symptoms

There are some areas, however, such as locations in Portugal, southern Italy, Cyprus, the Greek islands, Malta and southern Spain, where development of ascospores is expected also in late spring and early summer months in part of the years simulated. In those years, it is expected that symptoms can develop on the fruit before harvest, and therefore have an impact on the fruit quality.

The uncertainty is high as indicated in the establishment section

### Consequences

The results from the simulation of ascospore maturation, release and infection show that citrus black spot will develop and express symptoms mainly in late-maturing sweet orange varieties and lemons grown within the endangered area. The expected consequences will be moderate for fresh fruit of late-maturing citrus varieties and lemons. There would a potential for reduction in disease incidence by chemical treatments, but this would cause environmental impacts because in most EU citrus-growing areas fungicides are not widely applied and the most effective fungicide products are not currently registered for use in citrus in the EU MSs. In addition, to export citrus fruit to areas where CBS is regulated, additional fungicide treatments in the orchards, official inspections, quality controls in packing houses and/or establishment of pest-free areas might be needed to meet the phytosanitary requirements of these countries.

The consequences for fresh fruit of early-maturing citrus varieties are assessed as minor. The impact on early-maturing varieties would be sporadic in time and space, limited to years with rainy springs and summers and/or to specific locations. However, the impact could be higher in areas where late spring and early summer infection, based on simulation results, is expected to be more frequent...

The consequences would be minimal for citrus for processing, as external lesions or spots on citrus fruit are not a quality issue for citrus for processing.

As for establishment, the uncertainties about consequences are high owing to the lack of information on key parameters in the epidemiological models and on the incubation period; the lack of knowledge about the rate of disease build-up for this pathogen; and the limited information available about the impact of the disease and the programmes of fungicide treatments in semi-arid areas within the current CBS area of distribution, e.g. Eastern Cape, where environmental conditions are more similar to those in the pest risk assessment area.

**With regard to risk reduction options**, the Panel notes that, for the reduction of the probability of entry of *P. citricarpa*, prohibition and import from pest-free areas have overall a high to very high effectiveness with moderate to high feasibility for all pathways. Prohibition of parts of the host also has high effectiveness and very high feasibility with regard to the prohibition of citrus fruit with leaves and peduncles. For the fruit pathway, systems approaches as well as the induction of precocious symptoms expression in latent infections also have high effectiveness and feasibility. For plants for planting, certification and pre- and post-entry quarantine systems were also found to have high effectiveness and feasibility.

For reduction of the probability of establishment and spread, the application of strict waste processing measures would be highly effective in reducing the potential transfer of *P. citricarpa* from infected citrus fruit, both for entry and spread, although with low feasibility. The effectiveness of eradication, as well as of containment, is assessed as low. The application of drip irrigation practices will moderately reduce the probability of establishment.

The effectiveness of current EU phytosanitary measures to reduce the risk of *P. citricarpa* introduction ranges from moderate to high, except for the pest free production site, for which the effectiveness is rated as low.

After establishment, *P. citricarpa* has not been eradicated anywhere and is reported to be very difficult to contain. Therefore risk reduction options to prevent the entry of the pathogen are evaluated as most effective. Should the disease be reported from the risk assessment area, limited options are available to reduce the risk of establishment and spread.

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## BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION

The current European Union plant health regime is established by Council Directive 2000/29/EC on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community (OJ L 169, 10.7.2000, p.1).

The Directive lays down, amongst others, the technical phytosanitary provisions to be met by plants and plant products and the control checks to be carried out at the place of origin on plants and plant products destined for the Union or to be moved within the Union, the list of harmful organisms whose introduction into or spread within the Union is prohibited and the control measures to be carried out at the outer border of the Union on arrival of plants and plant products.

Citrus black spot is a serious disease of cultivated citrus plants caused by strains pathogenic to *Citrus* of the fungus *Guignardia citricarpa* Kiely. It is mainly a fruit disease and the unsightly lesions that develop on fruits do not cause post-harvest decay but render the fruits unmarketable. This pathogen is not known to occur in the EU.

*Guignardia citricarpa* (all strains pathogenic to *Citrus*) is a regulated harmful organism in the EU, listed in Annex IIAI of Council Directive 2000/29/EU. Annexes III, IVAI and VB of this Directive list requirements for the introduction into the EU of citrus plants, including fruits, which could be a pathway for the entry of this pathogen. In addition, temporary emergency measures are in place which impose additional requirements for the import of certain citrus fruits from Brazil in connection with *Guignardia citricarpa* (all strains pathogenic to *Citrus*) (Commission Decision 2004/416/EC; OJ L 151, 30.4.2004, p. 76). Certain third countries, as well as certain areas of third countries, are recognised as being free from *Guignardia citricarpa* (all strains pathogenic to *Citrus*) by Commission Decision 2006/473/EC (OJ L 187, 8.7.2006, p. 35).

In spite of the present import requirements against *Guignardia citricarpa* (all strains pathogenic to *Citrus*), infested citrus fruit are often intercepted during import inspections. In order to carry out an evaluation of the present EU requirements against *Guignardia citricarpa* (all strains pathogenic to *Citrus*), a pest risk analysis covering the whole territory of the EU is needed, which takes into account the latest scientific and technical knowledge for this organism, including the work on citrus black spot funded by EFSA in the context of the recent Prima Phacie project ('Pest risk assessment for the European Community plant health: A comparative approach with case studies'). EFSA has already worked on *Guignardia citricarpa* in the past, when it prepared a scientific opinion on a pest risk analysis and additional documentation on *Guignardia citricarpa* provided by South Africa (Question number: EFSA-Q-2008-299; doi:10.2903/j.efsa.2009.925). A recently published scientific paper (Yonow T, Hattingh V and de Villiers M, 2013. CLIMEX modelling of the potential global distribution of *Guignardia citricarpa* and the risk posed to Europe. *Crop Protection*, 44, 18–28) has modelled the potential global distribution of *Guignardia citricarpa* with the CLIMEX software also discussing the conclusions of the above-mentioned EFSA scientific opinion (2008).

It is also important that the risk assessment provides clarity regarding the risk posed by *Citrus latifolia* plants, including fruit, for the introduction of *Guignardia citricarpa* into the Union. The Brazilian Phytosanitary Authorities have recently informed the Commission that they consider that *Citrus latifolia* is not a host of this fungus in field conditions, and that therefore the trade of *Citrus latifolia* fruit poses only a low risk for the introduction of *Guignardia citricarpa*. The Brazilian Phytosanitary Authorities have indicated that the following three documents, which are made available to EFSA for information, support their position:

- Pathogenicity, colony morphology and diversity of isolates of *Guignardia citricarpa* and *G. mangiferae* isolated from *Citrus* spp.. R. Baldassari et al., *Eur J Plant Pathol* (2008) 120:103–110

- Patogenicidade, morfologia de colônias e diversidade de isolados de *Guignardia citricarpa* e *G. mangiferae* obtidos de *Citrus* spp.. R. Baldassaeri, Doctoral thesis, June 2005
- Reporte sobre la evaluación de riesgos de *Guignardia citricarpa* Kiely en frutos cítricos; COSAVE 2004

#### **TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION**

EFSA is requested, pursuant to Article 29(1) and Article 22(5) of Regulation (EC) No 178/2002, to provide a pest risk assessment of *Guignardia citricarpa* (all strains pathogenic to *Citrus*), to identify risk reduction options and to evaluate their effectiveness in reducing the risk to plant health posed by this harmful organism. The area to be covered by the requested pest risk assessment is the EU territory. In the risk assessment EFSA is also requested to provide an opinion on the effectiveness of the present EU requirements against *Guignardia citricarpa* (all strains pathogenic to *Citrus*), which are listed in Annex III, IV and V of Council Directive 2000/29/EC, as well as in Commission Decision 2004/416/EC and Commission Decision 2006/473/EC, in reducing the risk of introduction of this pest into the EU territory. In its scientific opinion EFSA is requested to indicate what is the risk posed by *Citrus latifolia* plants, including fruit, for the introduction of this organism into the Union.

## ASSESSMENT

### 1. Introduction

#### 1.1. Purpose

This document presents a pest risk assessment prepared by the EFSA Scientific Panel on Plant Health (hereinafter referred to as the Panel) for *Phyllosticta citricarpa* (synonym *Guignardia citricarpa*) in response to a request from the European Commission. The opinion includes the identification and evaluation of risk reduction options in terms of their effectiveness in reducing the risks posed by this organism.

Following a request from the European Commission, a public consultation was undertaken in the summer 2013 on the draft Scientific Opinion on the risk to plant health of *Phyllosticta citricarpa* for the EU territory. The comments received during the public consultation were taken into account by the Panel and the Scientific Opinion was revised.

#### 1.2. Scope

This risk assessment is for *Phyllosticta citricarpa* (McAlpine) Van der Aa, which was previously named *Guignardia citricarpa* Kiely (see section 3.1.1.1).

The species *Phyllosticta citriasiana* Wulandari, Crous & Gruyter, which has recently been associated with tan spot on pomelo (*Citrus maxima* (Burm.) Merr.) fruit, and *Phyllosticta capitalensis* Henn., which is not pathogenic to citrus, as well as other citrus-associated *Phyllosticta* species, are not included in this pest risk assessment (see section 3.1.1).

The pest risk assessment area is the territory of the European Union (hereinafter referred to as the EU) with 28 Member States (hereinafter referred to as EU MSs),<sup>6</sup> restricted to the area of application of Council Directive 2000/29/EC, which excludes Ceuta and Melilla, the Canary Islands and the French overseas departments.

### 2. Methodology and data

#### 2.1. Methodology

##### 2.1.1. The guidance documents

In order to maximise transparency and consistency, the risk assessment has been conducted in line with the principles described in the document 'Guidance on a harmonised framework for pest risk assessment and the identification and evaluation of pest risk management options' (EFSA PLH Panel, 2010). The evaluation of risk reduction options (also referred as risk management options) has been conducted in line with the principles described in the above-mentioned guidance (EFSA PLH Panel, 2010), as well as with the 'Guidance on methodology for evaluation of the effectiveness of options to reduce the risk of introduction and spread of organisms harmful to plant health in the EU territory' (EFSA PLH Panel, 2012).

Harmonised rating descriptors used in this opinion that follow the EFSA guidance documents are presented in Appendix A.

When expert judgement and/or personal communication have been used, justifications and evidence are provided to support the statements. Personal communications have been considered only when in written form and supported by evidence, and when other sources of information have not been publicly available.

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<sup>6</sup> When the data utilised do not yet include Croatia (which joined the EU in July 2013), it is specified that they refer only to the EU-27.

### 2.1.2. Methods used for conducting the risk assessment

The Panel conducted the risk assessment considering the absence of current requirements listed in Annexes II, III, IV and V of Council Directive 2000/29/EC and in Commission Decisions 2004/416/EC and 2006/473/EC, but under the assumption of a citrus disease management in the country of origin to comply with fruit quality standards. However, all the data on imports and interceptions presented in this document were obtained under the regulations currently in place in the EU. These data should be interpreted with caution because quantities of imported products will probably change if the regulations are removed and because interception numbers depend on the import control procedure currently in place at the EU borders.

The conclusions for entry, establishment, spread and impact are presented separately. The descriptors for qualitative ratings given for the probabilities of entry and establishment and for the assessment of impact are shown in Appendix A.

The Panel undertook a simplified quantitative pathway analysis exercise shown in Appendix E in order to examine with further detail the various steps involved in a potential pathogen entry process and to support the qualitative ratings given.

### 2.1.3. Methods used for evaluating the risk reduction options

The Panel identifies potential risk reduction options and evaluates them with respect to their effectiveness and technical feasibility, i.e. consideration of technical aspects which influence their practical application. The evaluation of efficiency of risk reduction options in terms of the potential cost-effectiveness of measures and their implementation is not within the scope of the Panel evaluation.

The descriptors for qualitative ratings given for the evaluation of the effectiveness and technical feasibility of risk reduction options are shown in Appendix A.

### 2.1.4. Level of uncertainty

For the risk assessment conclusions on entry, establishment, spread and impact and for the evaluation of the effectiveness of the risk reduction options, the levels of uncertainty have been rated separately.

The descriptors for qualitative ratings given for the level of uncertainty are shown in Appendix A.

## 2.2. Data

### 2.2.1. Data collection

#### 2.2.1.1. Data on cultivation areas and trade

Data on cultivation areas and trade (imports or/and exports) were collected from Eurostat and extracted from January to May 2013. In detail:

- Data on cultivation areas were collected from the apro\_cpp database.<sup>7</sup>
- Trade data were collected from the Comext database<sup>8</sup> for data since 1988 (Table 1) and from the Nimexe database for data from 1976 to 1987 (Table 2).

<sup>7</sup> <http://epp.eurostat.ec.europa.eu/portal/page/portal/agriculture/data/database>

<sup>8</sup> <http://epp.eurostat.ec.europa.eu/newxtweb/mainxtnet.do>

**Table 1:** Trade commodities of the HS and CN classifications used for trade data since 1988

| Code     | Name   |
|----------|--|
| 0805     | Citrus fruit, fresh or dried   |
| 080510   | Fresh or dried oranges   |
| 080520   | Fresh or dried mandarins including tangerines and satsumas, clementines, wilkings and similar citrus hybrids |
| 080540   | Fresh or dried grapefruit  |
| 08055010 | Fresh or dried lemons: “citrus limon, citrus limonum”  |
| 08055090 | Fresh or dried limes: “citrus aurantifolia, citrus latifolia”  |
| 080590   | Fresh or dried citrus fruit (excluding oranges, lemons “citrus limon, citrus limonum” and citrus hybrids)    |

**Table 2:** Trade commodities used for trade data during the period 1976–1987

| Code   | Name  |
|--------|---|
| 802    | Citrus fruit, fresh or dried  |
| 80,202 | Fresh sanguines and semi-sanguines from 1 April to 30 April   |
| 80,203 | Fresh navels, navelines, navelates, salustianas, vernas, valencia lates, maltese, shamoutis, ovalis, trovita and hamllins from 1 April to 30 April, other than sanguines and semi-sanguines                   |
| 80,205 | Other fresh, sweet oranges from 1 April to 30 April except navels, navelines, navelates, salustianas, vernas, valencia lates, maltese, shamoutis, ovalis, trovita, hamllins, sanguines and semi-sanguines     |
| 80,206 | Fresh sanguines and semi-sanguines from 1 May to 15 May   |
| 80,207 | Fresh navels, navelines, navelates, salustianas, vernas, valencia lates, maltese, shamoutis, ovalis,trovita and hamllins from 1 May to 15 May other than sanguines and semi-sanguines                         |
| 80,209 | Other fresh, sweet oranges from 1 May to 15 May except navels, navelines, navelates, salustianas, vernas,valencia lates maltese, shamoutis, ovalis, trovita, hamllins and sanguines and semi-sanguines        |
| 80,212 | Fresh sanguines and semi-sanguines from 16 May to 15 October  |
| 80,213 | Fresh navels, navelines, navelates, salustianas, vernas, valencia lates, maltese, shamoutis, ovalis, trovita and hamllins except sanguines and semi-sanguines from 16 May to 15 October                       |
| 80,215 | Other fresh, sweet oranges from 16 May to 15 October except navels, navelines, navelates, salustianas, vernas, valencia lates, maltese, shamoutis, ovalis, trovita, hamllins and sanguines and semi-sanguines |
| 80,216 | Fresh sanguines and semi-sanguines from 16 October to 31 March  |
| 80,217 | Fresh navels, navelines, navelates, salustianas, vernas, valencia lates, maltese, shamoutis, ovalis,trovita and hamllins except sanguines and semi-sanguines from 16 October to 31 March                      |
| 80,219 | Other fresh, sweet oranges from 16 October to 31 March except navels,navelines, navelates, salustianas, vernas,valencia lates, maltese, shamoutis, ovalis, trovita, hamllins and sanguines and semi-sanguines |
| 80,224 | Oranges, other than sweet, fresh oranges from 1 April to 15 October   |
| 80,227 | Oranges, other than sweet, fresh oranges from 16 October to 31 March  |

| Code   | Name   |
|--------|--|
| 80,228 | Clementines  |
| 80,229 | Monreales and satsumas   |
| 80,231 | Mandarins and wilkings   |
| 80,232 | Clementines  |
| 80,234 | Tangerines   |
| 80,237 | Other similar citrus hybrids except monreales, satsumas, mandarins, wilkings, clementines      |
| 80,250 | Lemons   |
| 80,270 | Grapefruit   |
| 80,290 | Citrus fruit, fresh or dried, other than oranges, mandarins and hybrids, lemons and grapefruit |

#### 2.2.1.2. Interception data

The extraction of the citrus interceptions data due to *P. citricarpa* from the Europhyt database was conducted on 19 April 2013. Interceptions data on pomelo (*Citrus maxima*) were not included in the analysis by the Panel, as earlier CBS interceptions on *C. maxima* predated knowledge on the new *Phyllosticta* species affecting pomelo (section 3.1.1.1).

#### 2.2.1.3. Climate and weather data

Weather data from agrometeorological stations and interpolated weather data from the Joint Research Centre (JRC) were used for simulations (EFSA, 2008; JRC, 2012; JRC, 2013). For details see section 1 in Appendix F of this scientific opinion.

The interpolated climate data grids of CRU CL 1.0 (New et al., 1999) and CRU CL 2.0 (New et al., 2002) as well as climate data grids covering the EU produced by JRC were used for CLIMEX simulations (EFSA, 2008; JRC, 2012).

### 2.2.2. Literature search

The literature on CBS and *P. citricarpa* up to April 2013 was searched using the following search engines: Web of Science, CAB Abstracts and Google Scholar. The keywords used were “*Phyllosticta citricarpa*”, “*Guignardia citricarpa*”, “Citrus Black Spot” and “citricarpa”. For the meta-analysis of published treatment experiments against *P. citricarpa*, the last two keywords were first combined with “fungicide”, and then with “trial”. The literature cited in the papers retrieved was inspected and papers citing retrieved papers were examined. The Panel took advantage of the extensive bibliographic collection on CBS already gathered for the scientific opinion of the EFSA Panel on Plant Health (PLH) in 2008 and focused the literature search on publications that have appeared since then. All the scientific and technical papers available were evaluated and data for the following variables were extracted: country, location, year, citrus species, cultivar, age of the trees, spray volume, experimental design, size of experimental unit, number of replicates, sample size, fungicides, number of sprays and disease incidence expressed as the proportion of CBS-affected fruit. Fungicide treatments were classified according to the chemical groups of the products evaluated and their combinations (FRAC, 2013). Field trials without untreated control trees were discarded. Most of the papers included CBS incidence as the only disease intensity metric. Therefore, in the cases where disease severity was the only variable available, data were transformed to disease incidence when possible or otherwise discarded.

### 3. Pest risk assessment

#### 3.1. Pest categorisation

##### 3.1.1. Identity of pest

###### 3.1.1.1. Taxonomic position

Citrus black spot disease (CBS) was first described in Australia (Cobb, 1897; Kiely, 1948). The causal agent of CBS was identified as *Guignardia citricarpa* Kiely (anamorph *Phyllosticta citricarpa* (McAlpine) Van der Aa), which was also detected on asymptomatic citrus trees as well as on other hosts in Australia and South Africa (Kiely, 1948; Wager, 1952). For many years, the coexistence of pathogenic and non-pathogenic strains of *G. citricarpa* was assumed. However, based on pathogenicity tests, McOnie (1964a) demonstrated that the non-pathogenic strains belonged to other *Guignardia* species that did not play a role in the causation of CBS. More recently, based on morphological, molecular and physiological analyses, Baayen et al. (2002) identified isolates obtained from CBS-affected fruits as *G. citricarpa* and isolates from asymptomatic citrus and other hosts as *G. mangiferae* A.J. Roy (anamorph *P. capitalensis* P. Hennings). Baldassari et al. (2008) demonstrated by means of field inoculations that only those isolates identified as *G. citricarpa* were pathogenic to sweet orange (*C. sinensis* Osbeck) fruit, whereas *G. mangiferae* isolates did not induce symptoms and did not sporulate on inoculated fruit. In this study, isolates of *G. citricarpa* were also obtained from asymptomatic Tahiti lime (*C. latifolia* Tanaka) fruit, which had previously been considered to be resistant to CBS but is apparently an asymptomatic host.

In 2011, a new code for fungal nomenclature was approved by the International Botanical Congress in Melbourne. The current 'Melbourne Code' abolishes the dual nomenclature for fungi, and gives priority to the oldest name irrespective of whether it is teleomorphic (sexual reproduction) or anamorphic (asexual reproduction) (Norvell, 2011). In the case of the CBS pathogen, the anamorph name *P. citricarpa* has priority over the teleomorph name *G. citricarpa*, and it should be now used as the only identifier of this species. Since the original type material of *P. citricarpa* has been lost, Glienke et al. (2011) designated a new type specimen for this species (epitype). An epitype for the non-pathogenic *P. capitalensis* was also designated, but it was defined as a different species to *G. mangiferae*, which was previously considered to be its teleomorph, and it is currently regarded as a pathogen of mango and not associated with citrus (Glienke et al., 2011; Wikee et al., 2011, 2013a, b). Glienke et al. (2011) defined a new species, *P. citribraziliensis* C. Glienke & Crous, sp. nov., based on three isolates obtained from asymptomatic citrus leaves (*Citrus* sp.) in Brazil. Another new species, *P. citriasiana* Wulandari, Crous & Gruyter, was detected in diseased fruits of pomelo (*Citrus maxima* (Burm.) Merr.) in intercepted consignments imported into the EU from Asia. This fungus was associated with a disease known as citrus tan spot, but confirmatory pathogenicity test and re-isolation have not been published so far (Wulandari et al., 2009). A new species, *Phyllosticta citrimaxima* Wikee, Crous, K.D. Hyde & McKenzie, has been recently isolated from tan spots in fruits of *C. maxima*, but pathogenicity tests and re-isolation are not available. In extensive surveys conducted in China, Wang et al. (2012) found a new species on citrus, *P. citrichinaensis* X.H. Wang, K.D. Hyde & H.Y. Li, that was associated with leaf and fruit spots of citrus, but confirmatory pathogenicity tests and re-isolation were not provided in this study. Thus, while new knowledge on the *Phyllosticta* species associated with citrus is continuously emerging, the current knowledge supports the conclusion that only *P. citricarpa* has proven to be pathogenic to citrus and a potential threat to citrus cultivation in regions that are suitable for this pathogen.

###### 3.1.1.2. Biology and life cycles

The primary infection cycle of the CBS pathogen is driven by ascospores formed into sexual fruiting bodies (pseudothecia) in the leaf litter. Citrus leaves drop all year around and mature pseudothecia are formed between 23 and 180 days after leaf fall depending on the temperature and humidity (Lee and Huang, 1973; Kotzé, 1981). Ascospores of *P. citricarpa* in spore traps are morphologically indistinguishable from those of the non-pathogenic species *P. capitalensis*, which is widespread in

CBS-affected areas. Thus, most data on ascospore dynamics available in the literature are based on mixed populations with unknown proportions of both species and should be interpreted with caution. Studies from South Africa and Taiwan indicated that maturation of ascospores occurs practically simultaneously in early summer on infected leaves abscised during late autumn, winter and early spring (Kotzé, 1963; McOnie, 1964b; Lee and Huang, 1973). Once mature, ascospores are mainly released during rain events. Studies conducted in the Mpumalanga province in South Africa indicated that at least 3 mm of precipitation are required for a significant release of ascospores (McOnie 1964b). The presence of frequent dews was associated with ascospore production in Australia, but the role of dews in ascospore release was not confirmed (Kiely, 1948). Irrigation might also trigger ascospore release, but all the studies available were conducted in regions where citrus is seldom irrigated during the time of ascospore production.

In Sao Paulo, Brazil, comparatively low to moderate numbers of ascospores were produced from October to March with peak production in January and February (Reis et al., 2003). In the Limpopo province of South Africa, ascospore release occurred mainly from November to March with the highest numbers from December to January (McOnie 1964b, c). Recent studies in the Limpopo province in South Africa indicated a similar period of ascospore availability from October to March. Degree-day models have been developed based on these data to predict the onset and duration of ascospore release in this region in South Africa (Fourie et al., 2013) and as a function of temperature and wetness in Misiones in Argentina (Dummel et al., 2012). Once released, ascospores are disseminated by air currents and infect susceptible leaves and fruit. Under artificial inoculation conditions, leaves of lemon (*C. limon* (L.) Osbeck) cv. Eureka were susceptible for at least 10 months and sweet orange (*C. sinensis*) cv. Valencia for up to 8 months (Truter et al., 2004; Truter, 2010). In South Africa, fungicide sprays are stopped four months after fruit set because sweet oranges are then considered resistant to CBS (McOnie, 1964b, c; Kotzé, 1981). However, ontogenic resistance was not demonstrated experimentally and the lack of fungicide sprays may be associated with the low inoculum levels and unfavourable weather conditions coinciding with the later stages of fruit development. Studies conducted in Brazil and Ghana indicated a susceptibility period of six and seven months after fruit set, respectively (Reis et al., 2003; Baldassari et al., 2006; Brentu et al., 2012), although longer periods were not evaluated.

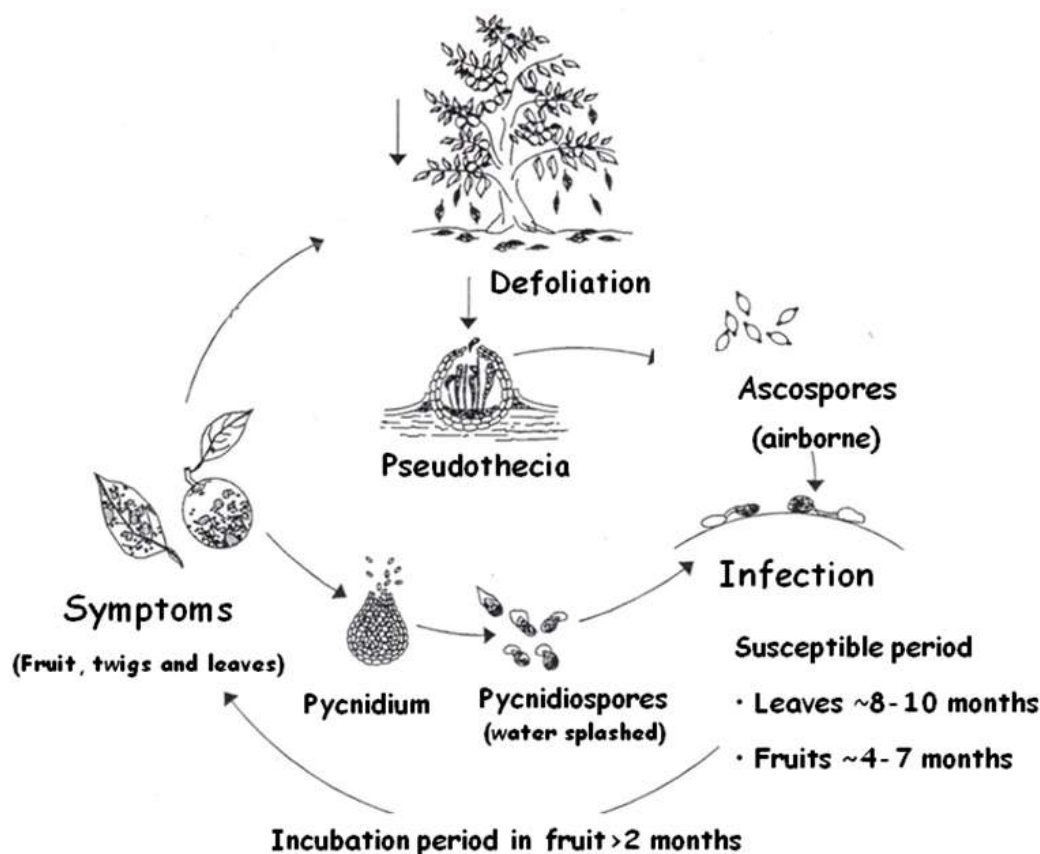
CBS occurs mainly in subtropical citrus-growing regions characterised by a summer rainfall pattern (Kotzé, 1981, 2000) and high annual precipitation. However, the disease is also present in semi-arid areas such as the Eastern Cape province in South Africa (Paul et al., 2005) with an annual rainfall of about 400 mm. The full range of temperatures and humidities suitable for ascospore infection have not been determined experimentally, and only ascospore germination rates and field infection data are available in the literature. According to Kotzé (1963), the conditions required for ascospore germination varied from 15 to 29.5 °C and from 15 to 38 hours of wetness. McOnie (1967) found that ascospores were able to infect with at least 15 hours of continuous wetness. In field studies conducted in Sao Paulo, Brazil, sweet orange fruit were infected with nearly 14 hours of wetness per day and 22 to 25 °C, but temperatures outside this range were not evaluated (Reis et al., 2006).

The secondary infection cycle of *P. citricarpa* is caused by pycnidiospores (conidia) formed into asexual fruiting bodies (pycnidia) on lesions in fruit, twigs and leaf litter. Pycnidia and pycnidiospores are produced mostly in dead twigs (Whitesite, 1967; Spósito et al., 2011) and on fruit lesions during the latest stages of fruit development (Kotzé 1981). Pycnidiospores are splash dispersed or washed off by rain to relatively short distances, infecting susceptible leaves and fruit. Under *in vitro* conditions, pycnidiospores of *P. citricarpa* can germinate and form appressoria between 10 and 40 °C and 12–48 hours of wetness (Noronha, 2002). Although the role of pycnidiospores in CBS epidemics was recognised in pioneering works in Australia and Zimbabwe (Kiely, 1948; Whiteside, 1967), pycnidiospores were later considered insignificant as a source of inoculum in South Africa (Kotzé, 2000). Pycnidiospores produced in fruit lesions were indicated as a potential source of inoculum only where out-of-season fruit or late-hanging fruit with lesions remain on the trees after blossoming and fruit set (Kotzé, 1981). In a study conducted in Brazil, it was observed that pycnidiospores of *P. citricarpa* that had formed on the lesions of sweet orange fruit from the previous harvest did not



significantly increase the severity of disease on the fruits of the subsequent harvest period (Baldassari et al., 2006). However, epidemiological studies conducted in Sao Paulo, Brazil, found an aggregated spatial pattern of CBS-affected trees in the orchard as well as diseased fruit in the canopy, suggesting that splash-dispersed conidia have an important role in this region (Spósito et al., 2007, 2008). Further experiments demonstrated that CBS-affected fruit and dead twigs on the tree were able to spread the disease in the canopy, supporting the importance of rain-dispersed pycnidiospores in field epidemics (Spósito et al., 2011).

The disease is characterised by a relatively long incubation period, and fruit symptoms become visible several months after infection. During this latent stage, the pathogen develops between the cuticle and epidermis without significant host injury (McOnie, 1967; Marques et al., 2012). Subsequent fungal growth and lesion formation are driven by phenological and environmental factors (Timmer, 1999; Spósito et al., 2004; Sousa and de Goes, 2010). In general, high temperatures and increased exposure to sunlight reduce the duration of the incubation period and augment disease severity. The disease is more severe on old and drought-stressed trees than in young and vigorous trees (Kotzé, 1963; Brodrick and Rabie, 1970; Kotzé, 1971; 1981; Ninin et al., 2012). The incubation period is also affected by the growth stage in which the fruit was infected. In artificial inoculations conducted under greenhouse conditions, the incubation period ranged from over 200 days for 3-cm-diameter sweet orange fruit to about 50 days for 7-cm-diameter fruit (Aguilar et al., 2012). Foliar lesions of CBS appear as small sunken necrotic spots surrounded by a dark-brown ring. However, they are rare and present in only lemons or trees in poor condition (Kotzé, 1981, 2000). One feature that has been observed for CBS is that the pathogen may be present for many years in a region before the disease reaches epidemic proportions. In Mpumalanga province in South Africa, symptoms were present for over three decades before control measures became necessary (Kotzé, 1981). Whilst the existence of a lag phase following an initial introduction to a new area is a general feature of a pathogen's epidemiology at various scales, this process has not been studied in detail for *P. citricarpa*. Another general feature of pathogens is that they can be sporadic in time and space. During a lag phase, inoculum is built up through multiplication in small scale epidemics where the fungus is present mostly as latent mycelia in asymptomatic citrus fruit and leaves. When sufficient inoculum has been built up and if weather conditions become suitable at a specific location, epidemics can develop and cause severe disease impact. Therefore, estimates of disease progression, even in semi-arid regions, such as the Eastern Cape in South Africa, where CBS emerged more recently (McOnie, 1964 d; Paul et al., 2005), should be interpreted with caution.



**Figure 1:** Life cycle of *Phyllosticta citricarpa* (adapted from a drawing by D. Drouillard in Timmer (1999) © American Phytopathological Society and modified according to Aguiar et al. (2012), Brentu et al. (2012), Reis et al. (2003) and Truter (2010))

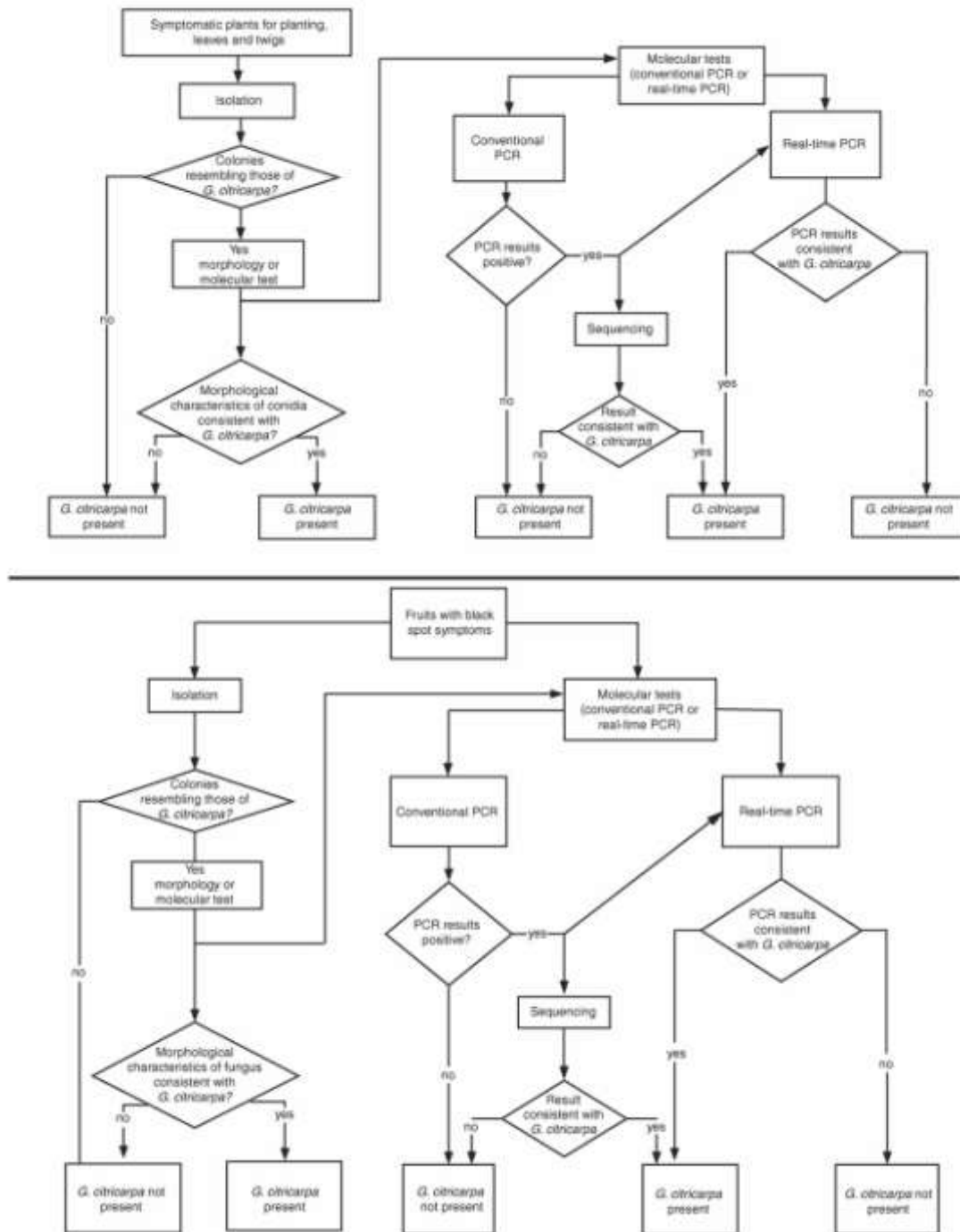
### 3.1.1.3. Detection and identification

Symptoms of CBS can be detected by visual examination of fruit by trained inspectors and with the aid of multispectral imaging (Bulanon et al., 2013; Stegmayer et al., 2013). The formation of CBS lesions in affected asymptomatic fruit can be induced by treatment with ethephon and storage under continuous light and warm temperatures (Baldassari et al., 2007). Truter (2010) proposed a method of artificial wilting of symptomless green citrus leaves to enhance detection of *P. citricarpa*. The pathogen can be isolated from fruit lesions and leaves by plating fragments of affected tissues in agar media. Pycnidia of *P. citricarpa* can be found in CBS lesions in fruit, but they are also induced by maintaining fruit under high-humidity conditions (EPPO, 2003). Among the six *Phyllosticta* species currently described in citrus, *P. capitalensis* and *P. citribraziliensis* are endophytes, so they do not cause lesions or sporulate on fruit (Baldassari et al., 2008; Glienke et al., 2011). Moreover, *P. citribraziliensis* has been detected only in healthy citrus leaves and it is not known to be present on fruit (Glienke et al., 2011). In the case of *P. citrichinaensis*, which is present only in China, Wang et al. (2012) reported that pycnidia have never been found on lesions associated with this species. Pycnidia of *P. citriasiana* and *P. citrimaxima* can be present in fruit lesions, but these two species are present only in *C. maxima* in China, Thailand and Vietnam (Wulandari et al., 2009; Wikee et al., 2013b). Therefore, pycnidia of *P. citricarpa* present in CBS lesions in sweet orange, mandarin, grapefruit and lemon fruits are not likely to be confused with any of the other *Phyllosticta* species described in citrus. Indeed, pycnidia of *P. citricarpa* were observed in 52–95 % of the positive interceptions of citrus fruit at border inspections in the EU (Table 5).

The coexistence of *P. citricarpa* with non-pathogenic strains of *Phyllosticta* (formerly *Guignardia* sp.) in CBS-affected fruit has been recognised since the early work of McOnie (1964a). Differential morphological and physiological characteristics, such as higher colony growth rate, presence of lobate

margin and production of infertile perithecia, were recognised in *P. citricarpa* even before other *Phyllosticta* species were formally described in citrus (McOnie, 1964a; Whiteside, 1967; Lee, 1969). These features were further supported by other distinctive characteristics such as pycnidiospores with barely visible mucoid sheaths, and the formation of a yellow pigment on oatmeal agar (Baayen et al., 2002; Wang et al., 2012).

In general, the use of molecular procedures is required for an accurate identification of the pathogen (Figure 2). Several specific polymerase chain reaction (PCR) methods are available for *P. citricarpa* (Bonants et al., 2003; Meyer et al., 2006; Peres et al., 2007; van Gent-Pelzer et al., 2007; Stringari et al., 2009) plus the standard ITS sequencing (White et al., 1990). In contrast to isolations in culture media, PCR methods cannot differentiate between living and dead stages of the pathogen. However, any of the existing field or postharvest treatments is able to eradicate or suppress the pathogen from tissues affected in the fruit rind (Seberry et al., 1967; Korf et al., 2001; Agostini et al., 2006). Thus, the prevalence of dead stages of *P. citricarpa* into CBS lesions is a rather unlikely. The method by Bonants et al. (2003) is not specific enough to differentiate between *P. citricarpa* and *P. citriasiana* (EPPO, 2009; Wulandari et al., 2009) and the method by Peres et al. (2007) does not differentiate between *P. citricarpa* and *P. citrichinaensis* (Wang et al., 2012). However, *P. citrichinaensis* is described only in China and *P. citriasiana* is present only in *C. maxima* in China, Thailand and Vietnam. Thus, problems of specificity with these molecular methods are restricted only to some specific situations and do not affect the interceptions of citrus fruit imported from America or Africa, where neither *P. citrichinaensis* nor *P. citriasiana* have been described. New molecular methods have recently been developed (Wang et al., 2012; Stammler et al., 2013; Tomlinson et al., 2013; Hu et al., 2014). Nevertheless, further testing will be required to determine how they will perform with newly emerging *Phyllosticta* species associated with citrus (Wulandari et al., 2009; Glienke et al., 2011; Wang et al., 2012; Wikee et al., 2013b).



**Figure 2:** Flow diagram for the identification of *Phyllosticta citricarpa* in the PM 7/17 standard protocol of the European and Mediterranean Plant Protection Organization (EPPO/OEPP) © EPPO (2009)

### 3.1.1.4. Citrus taxonomy and host range of *P. citricarpa*

The vast majority of citrus species and their wild relatives are native to south-eastern Asia, the East Indian Archipelago, New Guinea, Melanesia, New Caledonia and Australia; another group occurs in tropical Africa. The commonly cultivated citrus species belong to three genera: *Citrus*, *Fortunella* and *Poncirus* that are all closely related and belong to the subtribe Citrinae, the tribe Citreae, the orange subfamily Aurantioideae and the plant family Rutaceae. All the genera have persistent unifoliolate or simple leaves except the monotypic genus *Poncirus*, which has trifoliolate, deciduous leaves.

The genus *Fortunella* (kumquat) includes species of small trees and shrubs. All species have small leaves and orange-coloured fruits of small size.

The genus *Poncirus* includes a single species, *P. trifoliata*, with trees of small size and trifoliolate leaves. It differs from all the other true citrus fruit trees, which are found only in tropical or subtropical regions. Having penetrated far into the temperate zone in north-eastern Asia, it has become a deciduous tree with small leaf buds and larger scale-covered flower buds (formed in early summer) that pass the winter on the leafless terminal twigs and open before (and sometimes with) the leaves early in the following spring. *Poncirus* hybridises freely with *Citrus*. Such hybrids, called citranges, are often used as rootstocks.

The genus *Citrus* is divided into two very distinct subgenera, *Citrus* and *Papeda*, that are easily distinguished by leaf, flower and fruit characteristics. The subgenus *Citrus* includes all the commonly cultivated species of citrus, all of which have fruit with pulp-vesicles filled with juice free, or almost free, from droplets of oil, which are located in the rind. Species from the genus *Citrus* are the most important from an agronomical point of view. The botanical classification within this genus is not unique. Nowadays, the classification in common use is that established by Swingle (1967).

**Table 3:** Main citrus species cultivated worldwide

| Botanical name                                | Common English name |
|---|---------------------|
| <i>Fortunella</i> spp.                        | Kumquat             |
| <i>Poncirus trifoliata</i> (L.) Raf.          | Trifoliolate orange |
| <i>Citrus medica</i> L.                       | Citron              |
| <i>Citrus limon</i> (L.) Burm.f.              | Lemon               |
| <i>Citrus aurantifolia</i> (Christm.) Swingle | Key lime            |
| <i>Citrus latifolia</i> Tanaka                | Tahiti lime         |
| <i>Citrus limettioides</i> Tanaka             | Sweet lime          |
| <i>Citrus hystrix</i> DC                      | Kaffir lime         |
| <i>Citrus aurantium</i> L.                    | Sour orange         |
| <i>Citrus sinensis</i> Osbeck                 | Sweet orange        |
| <i>Citrus reticulata</i> Blanco               | Mandarin            |
| <i>Citrus unshiu</i> (Swingle) Marcow.        | Satsuma mandarin    |
| <i>Citrus maxima</i> (Burm.) Merr.            | Pomelo              |
| <i>Citrus paradisi</i> Macfad.                | Grapefruit          |

All commercial citrus species and cultivars are considered to be susceptible to CBS, except for sour orange (*C. aurantium*) (Kotzé, 1981) and Tahiti lime (*C. latifolia*) (Baldassari et al., 2008). In the case of sour orange, *P. citricarpa* was isolated from asymptomatic leaves in Brazil (Wickert et al., 2009). Isolates obtained in this country from CBS lesions and other fruit blemishes were reported by several studies (Baayen et al., 2002; Wulandari et al., 2009; Glienke et al., 2011), although no evidence of reproduction on this citrus species was found. Tahiti lime is reported not to exhibit CBS symptoms under field conditions, even in areas with high inoculum pressure. However, *P. citricarpa* was isolated in Sao Paulo, Brazil, from asymptomatic fruit and leaves of Tahiti lime (Baldassari et al., 2008; Wickert et al., 2009). Although there is no documented evidence of *P. citricarpa* reproduction on

Tahiti lime fruit, it can colonise and form viable ascospores in Tahiti lime leaves, suggesting that this citrus species may well play a role in CBS epidemiology (Baldassari et al., 2008).

Lemon (*C. limon*) is considered to be the citrus species that is most susceptible to CBS, and it has been stated that the first disease outbreaks in a region always occurred in lemon orchards and later spread to adjacent citrus orchards (Kotzé, 1981). However, CBS emerged recently in Florida (USA) directly in sweet orange orchards (Schubert et al., 2012). Late-maturing cultivars of sweet orange were considered more susceptible than early-maturing ones (Timmer, 1999). However, cultivar field trials conducted in Brazil as well as studies comparing the rate of disease progress indicated that cultivar reaction to the disease is more linked to the interaction of environmental factors with the dynamics of fruit maturation (Spósito et al., 2004; Sousa and de Goes, 2010).

In Australia, Miles et al. (2013) failed to detect CBS symptoms in pomelo (*C. maxima*). Surveys were conducted in two commercial orchards, citrus arboretums and fruit markets in areas of the Northern Territory, Queensland and New South Wales, where CBS is prevalent. However, the same study indicated that only 22 ha of pomelo is commercially cultivated in Australia, which is a rather limited sampling area. Recent surveys conducted in China also indicated that pomelo (*C. maxima*) is not affected by *P. citricarpa* (Wang et al., 2012). However, more data from other geographic regions as well as proper pathogenicity tests are needed to completely exclude this citrus species as a potential host of *P. citricarpa*.

With regard to kumquat (*Fortunella* spp.), this species was recorded by Kiely (1948) in Australia as moderately susceptible to CBS under conditions of natural infection, but no further experimental information is available.

No definitive information has been found on the susceptibility of *Poncirus* Raf. (trifoliate orange) to *P. citricarpa*.

#### 3.1.1.5. Reports of impact in the area of current distributions

In most of the area of its current distribution, *P. citricarpa* is reported to cause severe quality and yield losses to citrus fruit production. The apparent absence of severe impact at specific locations, e.g. in Addo, Eastern Cape, South Africa, where the pathogen is reported to “*persist but not flourish*” (Yonow et al., 2013), could be due to the relatively recent emergence of the disease as well as to the fungicide schedules currently in place. However, this province in South Africa is not officially recognised among the low-pest prevalence areas for CBS (2008 Amendment of Act No 36 of 1983). Several types of CBS symptoms including hard spot, virulent spot, and false melanose occur on the rind of affected fruit (Figure 3), reducing its commercial value for the fresh market (Kotzé, 2000). Premature fruit drop due to CBS causes significant yield loss in Brazil, and probably in other citrus regions of the world (Reis et al., 2006; Araújo et al., 2013). Leaf lesions are seldom seen in well-managed sweet orange orchards and they appear more commonly on lemons (Kotzé, 2000). In order to obtain more information about disease impacts, the Panel undertook a meta-analysis of recorded disease incidence in untreated and fungicide-treated plots from published field trials for the control of CBS. The results from this meta-analysis are described in section 3.6.1.1.



**Figure 3:** Left: fruits of sweet orange with symptoms of citrus black spot caused by *Phyllosticta citricarpa*; right: lesions of citrus black spot in a lemon fruit with pycnidia of *P. citricarpa*

### 3.1.2. Current distribution

Reports of *P. citricarpa*, from EPPO PQR (EPPO, 2013a), from scientific and technical literature and from interception records by EU MSs, are given in Table 4 below. When analysing the interception data, interceptions of consignments of *C. maxima* (pumelo) were not included as new *Phyllosticta* species have been recently described on pomelo (see section 3.1.1.1)

*P. citricarpa* (as *G. citricarpa* (all strains pathogenic to citrus) is listed in the EU Directive 2000/29/EC as not known to occur in the EU and is reported in the EPPO PQR as absent from all the citrus producing EU MSs (Croatia, Cyprus, France, Greece, Italy, Malta, Portugal and Spain).

**Table 4:** Reports of *Phyllosticta citricarpa* from the EPPO PQR (EPPO, 2013a), interception records (Europhyt, online; interceptions on *C. maxima* not included), scientific and technical literature

| Country       | State/region  | Reports   | Source                                   |
|---------------|---------------|---|--|
| <b>Africa</b> |               |   |  |
| Benin         |               | Detected in two consignments exported to France in 1999 and in 2005                             | EPPO, 2013a; Europhyt (online)           |
| Cameroon      |               | Detected in a <i>C. sinensis</i> consignment exported to United Kingdom in 2006                 | EPPO, 2013a; Europhyt (online)           |
| Ghana         | Eastern       | Widely distributed  | Brentu et al., 2012                      |
| Ghana         | Ashanti       | Widely distributed  | Brentu et al., 2012                      |
| Guinea        |               | Detected in a <i>C. sinensis</i> consignment exported to France in 2000                         | EPPO, 2013a; Europhyt (online)           |
| Kenya         |               | Present, no details   | EPPO, 2013a                              |
| Mozambique    |               | Present, no details   | EPPO, 2013a                              |
| South Africa  |               | Present, restricted distribution  | EPPO, 2013a                              |
| South Africa  | KwaZulu-Natal | Present   | Paul et al., 2005; Carstens et al., 2012 |
| South Africa  | Mpumalanga    | Present   | Paul et al., 2005; Carstens et al., 2012 |
| South Africa  | Limpopo       | Present   | Paul et al., 2005; Carstens et al., 2012 |
| South Africa  | North West    | Present   | Paul et al., 2005; Carstens et al., 2012 |
| South Africa  | Eastern Cape  | Present   | Paul et al., 2005; Carstens et al., 2012 |
| Swaziland     |               | Reported in literature. Detected in several <i>C. sinensis</i> consignments exported to EU MSs. | Stammler et al, 2013, Europhyt (online)  |

| Country                  | State/region              | Reports   | Source                                |
|--------------------------|---------------------------|---|---------------------------------------|
| Uganda                   |                           | Present, few occurrences  | EPPO, 2013a                           |
| Zambia                   |                           | Present, no details   | EPPO, 2013a                           |
| Zimbabwe                 |                           | Present, no details   | EPPO, 2013a                           |
| <b>America</b>           |                           |   |                                       |
| Argentina                |                           | Present, restricted distribution  | EPPO, 2013a                           |
| Brazil                   |                           | Present, restricted distribution  | EPPO, 2013a                           |
| Brazil                   | Rio Grande do Sul         | Present, no details   | EPPO, 2013a                           |
| Brazil                   | Rio de Janeiro            | Present, no details   | EPPO, 2013a                           |
| Brazil                   | Sao Paulo                 | Present, no details   | EPPO, 2013a                           |
| Cuba                     |                           | Present, no details   | EPPO, 2013a; Hidalgo and Pérez, 2010  |
| United States of America |                           | Present, few occurrences  | EPPO, 2013a                           |
| United States of America | Florida                   | Present, few occurrences  | EPPO, 2013a                           |
| Uruguay                  |                           | Present, no details.<br>Detected in 3 consignments exported to EU MSs from 2001 to 2010 (2 on <i>C. sinensis</i> , 1 on <i>C. reticulata</i> ). | USDA APHIS, 2012a; Europhyt (online)  |
| <b>Asia</b>              |                           |   |                                       |
| Bangladesh               |                           | Detected in several consignments exported to United Kingdom (in <i>C. sinensis</i> , <i>C. aurantifolia</i> and <i>Citrus</i> spp.)             | Europhyt (online)                     |
| Bhutan                   |                           | Present, no details   | EPPO, 2013a                           |
| China                    |                           | Present, restricted distribution  | EPPO, 2013a                           |
| China                    | Fujian                    | Present, no details   | EPPO, 2013a                           |
| China                    | Guangdong                 | Present, no details   | EPPO, 2013a                           |
| China                    | Sichuan                   | Present, no details   | EPPO, 2013a                           |
| China                    | Xianggang (Hong Kong)     | Present, no details   | EPPO, 2013a                           |
| China                    | Yunnan                    | Present, no details   | EPPO, 2013a                           |
| China                    | Zhejiang                  | Present, no details   | EPPO, 2013a                           |
| Indonesia                |                           | Present, no details   | EPPO, 2013a                           |
| Indonesia                | Java                      | Present, no details   | EPPO, 2013a                           |
| Philippines              |                           | Present, no details   | EPPO, 2013a                           |
| Taiwan                   |                           | Present, no details   | EPPO, 2013a                           |
| Thailand                 |                           | Detected in a <i>C. sinensis</i> consignment exported to the Netherlands in 2006  | Europhyt (online)                     |
| Vietnam                  |                           | Detected in eight consignments to the Netherlands on <i>C. maxima</i>   | Europhyt (online)                     |
| <b>Oceania</b>           |                           |   |                                       |
| Australia                |                           | Present, restricted distribution  | EPPO, 2013a                           |
| Australia                | (coastal) New South Wales | Present, no details   | EPPO, 2013a; Miles et al., 2013       |
| Australia                | Queensland                | Present, no details   | EPPO, 2013a                           |
| Australia                | Northern Territory        | Present, no details   | Paul et al., 2005; Miles et al., 2013 |
| Australia                | Victoria                  | Present, no details   | EPPO, 2013a                           |
| Vanuatu                  |                           | Present, no details   | EPPO, 2013a                           |



### 3.1.3. Regulatory status in the EU

#### 3.1.3.1. History of regulatory status in the citrus producing EU MSs

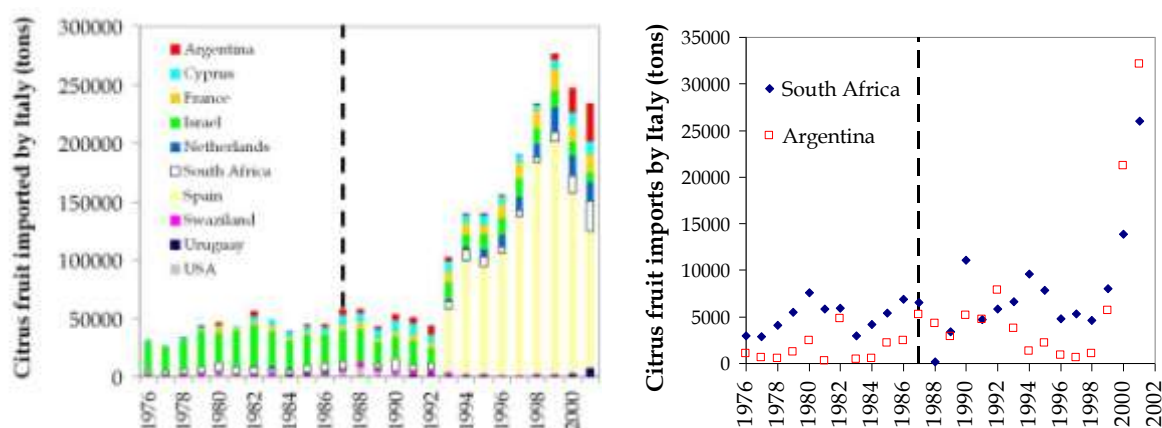
In most EU MSs growing citrus, the import of citrus plants and plant parts, including fruit, has been historically forbidden following national plant quarantine rules, until, after joining the European Community/EU, common EC/EU phytosanitary measures introducing also particular requirements for citrus fruit and *P. citricarpa* were implemented.

In Spain, the import of fresh fruit, live plants and plant parts of citrus and other woody fruit species from Japan, USA, Canada and New Zealand has been prohibited since 1929 (Real Orden No 976, Gaceta de Madrid 114: 464–465). In 1934, this prohibition was expanded to plant material imported from Portugal, Argentina, Brazil, Mexico and South Africa (Orden, Gaceta de Madrid 228: 1526). These regulations were derogated in 1987 (Orden 7366, BOE 71:8395–8411) when the European Directive 77/93/CEE was implemented in Spain. This new regulation prohibited the import of all kind of citrus material from any country. The import of citrus fruit in Spain was first allowed in 1993, but with specific provisions to avoid the introduction of *P. citricarpa* and other harmful organisms, when European Directive 77/93/CEE was implemented (Real Decreto 2071/1993, BOE 300:35603–35603) and later the Directive 2000/29/CE (Real Decreto 58/2005, BOE 19:2583–2665).

Similarly, in Italy the import of fresh fruit (with the exception of grapefruit), live plants and plant parts of citrus have been forbidden since the 1930s by national law (L. 18 June 1931, no 987). After implementation of European Directive 77/93/EC (Ministerial Decree D.M. 31 January 1996), the import of citrus fruit was still forbidden as Italy was recognised as protected zone. Only in 1999 was protected zone status for Italy removed (D.M. 8 July 1999) and, since then, the import of citrus fruit from third countries has been allowed provided that the requirements of Directive 77/93/CEE and later Directive 2000/29/EC have been met.

#### 3.1.3.2. History of the citrus fruit trade in the citrus-producing EU MSs

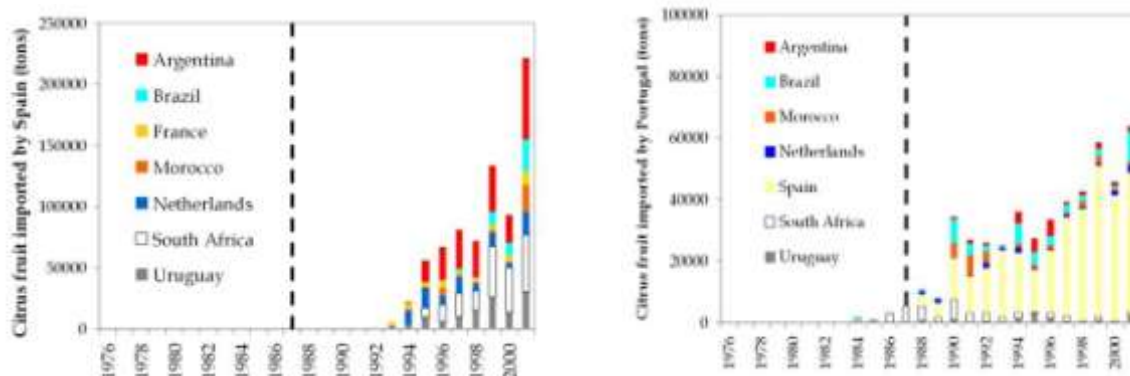
Citrus fruit trade into the citrus-producing EU MS was limited until the 1990s. For example, Italy imported less than 50 000 tonnes of citrus fruit until 1992, with about a five-fold increase over the following decade, mostly due to imports from Spain (Figure 4). Focusing on imports of citrus fruit from South Africa and Argentina to Italy, it is clear that historically there has been little import of this commodity from these two countries (Figure 4). It should be also noted that, until 1998, Italian imports of citrus fruit from third countries such as Argentina and South Africa were only of grapefruit. The same applies to other non European countries where *P. citricarpa* is present, such as Uruguay.



**Figure 4:** Annual citrus fruit imports in Italy (1976–2001) from the 10 major exporter countries (left) and from South Africa and Argentina (right-) (Eurostat, online). Until 1998, all citrus fruit imports by Italy from South Africa and Argentina were of grapefruit

It is important to note that trade data for the periods 1976–1987 and 1988–2001 come from two different datasets and so might not be entirely comparable. However, the jump in the imports between 1992 and 1993 in the left-hand panel of Figure 4 coincided not with the changeover between the two datasets, but with Spain and Portugal joining the EU.

A similar process can be observed for Spain and Portugal, two other major citrus-growing EU countries (Figure 5). Spain moved from a situation of importing no citrus fruit from third Countries at the beginning of the 1990s to importing more than 200 000 tonnes of citrus fruit imports in 2001, mainly from Argentina, Brazil, Morocco, the Netherlands, South Africa and Uruguay (originating from Non-EU countries). As far as Portugal is concerned, most citrus fruit imports have traditionally come from Spain (Figure 5)

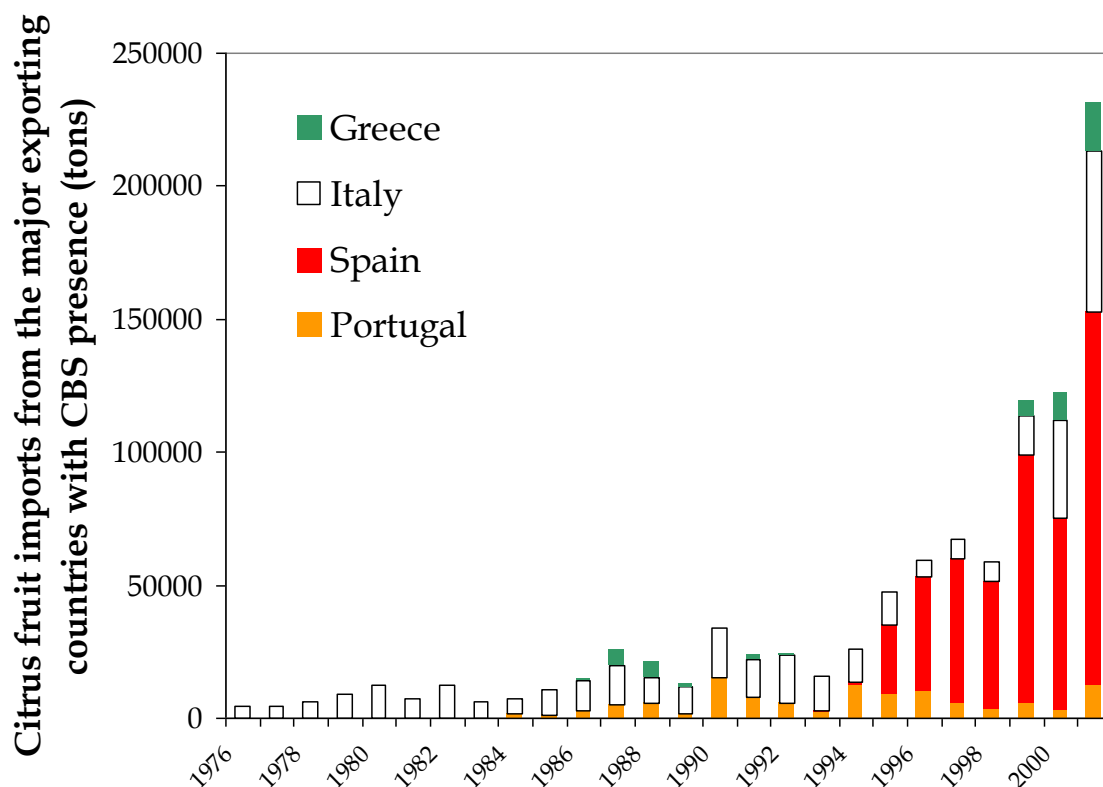


**Figure 5:** Annual citrus fruit imports from third countries (1976–2001) in Spain (left) and Portugal (right) (Eurostat, online)

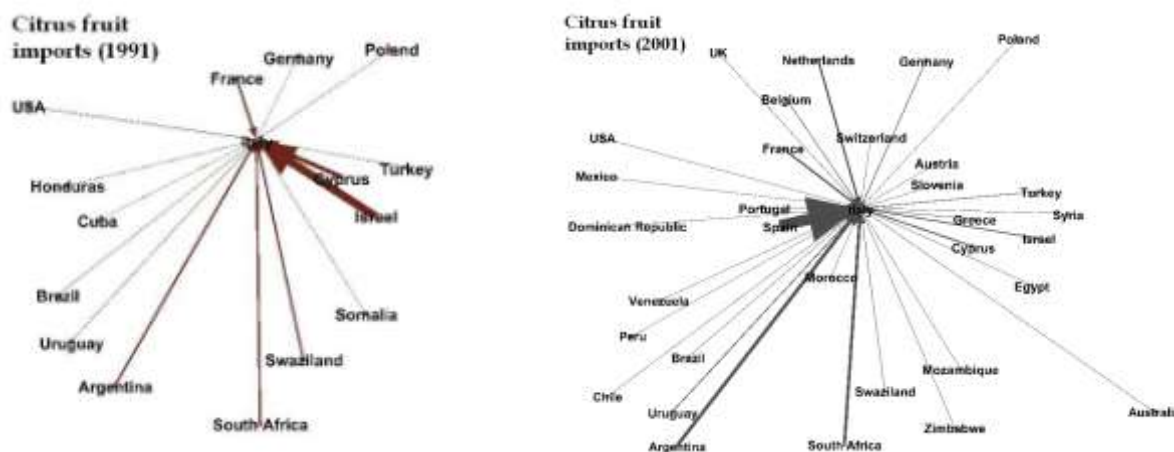
Historically, imports of citrus fruit by the major EU citrus-growing countries (Greece, Italy, Portugal, Spain) from the major exporting third countries where CBS is present (Argentina, Australia, Brazil, South Africa and Swaziland) were very limited until the mid-1990s. Indeed, Spain only started to import citrus fruit from third countries in the 1990s (Figure 6).

Therefore, the argument that European citrus-growing areas are not suitable for the introduction of CBS because there have been plenty of opportunities for introduction during decades of massive import of citrus fruit into such areas from CBS-affected regions (Kotzé, 2000) is not supported by the trade data. The analysis of historical trade statistics shows that the import of significant amounts of citrus fruit from CBS-affected countries into the EU citrus-growing areas started only recently (in the mid-1990s), i.e. after the integration of the Mediterranean countries into the EU. However, all these imports of citrus fruits met the current European phytosanitary regulations on *P. citricarpa* (section 3.1.3.3), implemented by the Mediterranean countries after their integration into the EU.

Structural change during the 1990s in the citrus fruit trade into EU MSs can be observed also in the increase in the number of exporting countries. Again taking Italy as an example, citrus fruit was imported from 15 countries in 1991, but from 32 countries in 2001 (Figure 7). Similar recent structural changes in the trade of plant commodities have also been documented for other horticultural sectors in Europe (Dehnen-Schmutz et al., 2010).



**Figure 6:** Volume of citrus fruit imported during the period 1976–2001 by the major EU citrus-growing countries (Greece, Italy, Spain and Portugal) from the five major exporters where CBS is present (Argentina, Australia, Brazil, Swaziland and South Africa) (Eurostat, online). Note that, until 1998, imports to Italy of citrus fruit from these third countries were exclusively of grapefruit



**Figure 7:** Countries exporting citrus to Italy in 1991 (left) and 2001 (right). The imported quantities increased approximately five-fold. The size of arrows relates to the traded volumes

### 3.1.3.3. Current EU regulatory status

*Guignardia citricarpa* (all strains pathogenic to *Citrus*) is listed in the EU Council Directive 2000/29/EC in Annex II, Part A, Section I. This is the list of organisms harmful to plants and plant products that are not known to occur in the EU and are relevant for the entire EU, whose introduction

into, and spread within, all EU MSs is banned if they are present on certain plants or plant products. In particular, *Guignardia citricarpa* (all strains pathogenic to *Citrus*) is banned if present on “plants<sup>9</sup> of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, other than seeds”, i.e. if this pathogen is present on living plants or part of plants, as fruit, branches with foliage or flowers, plant tissue culture.

Annex IV, Part A. Section I, paragraph 16.4, of the Council Directive 2000/29/EC describes the special requirements related to *Guignardia citricarpa* for the introduction into the Community of fruits originating in third countries of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, other than fruits of *Citrus aurantium* L. According to these requirements, the imported fruit should be accompanied by an official statement that:

- the fruit originate in a country recognised as being free<sup>10</sup> from *Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*); or
- the fruit originate in an area recognised as being free<sup>11</sup> from *Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*); or
- no symptoms of *Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*) have been observed in the field of production and in its immediate vicinity since the beginning of the last cycle of vegetation, and none of the fruits harvested in the field of production has shown, in appropriate official examination, symptoms of this organism; or
- the fruit originate in a field of production subjected to appropriate treatments against *Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*), and none of the fruits harvested in the field of production has shown in appropriate official examination, symptoms of this organism.

In addition, temporary emergency measures are in place that impose additional requirements for the import of certain citrus fruits from Brazil in connection with *Guignardia citricarpa* (all strains pathogenic to *Citrus*) (Commission Decision 2004/416/EC; OJ L 151, 30.4.2004, p. 76).

Commission Decision 2006/473/EC (OJ L 187, 8.7.2006, p. 35) lists the third countries, as well as certain areas of third countries, recognised as being free from *Guignardia citricarpa* (all strains pathogenic to *Citrus*).

In Council Directive 2000/29/EC, other requirements are listed for citrus plants and fruit that are not specific to *P. citricarpa*.

Annex III, Part A, (16) prohibits the introduction of plants of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, other than fruit and seeds from third countries into all MSs. This prohibition therefore covers living plants, branches with foliage or cut flowers and plant tissue culture. However, citrus plants for research or breeding programmes can still be introduced by following the conditions listed in Commission Directive 95/44/EC.

Annex IV, Part A, Section I, point 16.1, states that fruit of *Citrus* L., *Poncirus* Raf. and *Fortunella* Swingle as well as their hybrids originating in third countries shall be free from peduncles and leaves and the packaging shall bear an appropriate origin mark.

<sup>9</sup> In Article 2 of Council Directive 2000/29/EC, “plants” are considered to mean: living plants and living parts thereof, including seeds; living parts of plants are considered to include: fruit (in the botanical sense, other than preserved by deep freezing), vegetables (other than preserved by deep freezing), tubers, corms, bulbs, rhizomes, cut flowers, branches with foliage, cut trees retaining foliage, plant tissue cultures.

<sup>10</sup> In accordance with the procedure laid down in Article 18 of Council Directive 2000/29/EC.

<sup>11</sup> In accordance with the procedure laid down in Article 18, and mentioned on the certificates referred to in Articles 7 and 8 of this Directive.

- Annex V, Part B, point 3, states that fruits of *Citrus* L., *Poncirus* Raf. and *Fortunella* Swingle and their hybrids originating outside the EU shall be subjected to a plant health inspection in the country of origin or the consignor country, before being permitted to enter the Community.

*Guignardia citricarpa* is in the A1 List of the European and Mediterranean Plant Protection Organization (EPPO, 2013a).

#### 3.1.4. Regulatory status in third countries

Outside the EU, according to the EPPO PQR database (EPPO, 2013a), *P. citricarpa* is in the A1 List of the Caribbean Plant Protection Commission (CPPC) and in the A2 Lists of the Asia and Pacific Plant Protection Commission (APPC), Comité de Sanidad Vegetal del Cono Sur (COSAVE), the Interafrican Phytosanitary Council (IAPSC) and the Pacific Plant Protection Organisation (PPPO). In America, it is a quarantine pest in the United States and is in the A1 List of Chile, Paraguay and Uruguay. In Asia and Europe, it is in the A1 List in Turkey and is a quarantine pest in Israel and Jordan. In Oceania, it is a quarantine pest in New Zealand

#### 3.1.5. Potential for establishment and spread in the pest risk assessment area

Host plants of *P. citricarpa* are widely grown in orchards of the southern EU MSs (see Table 6). In a previous scientific opinion (2008), the EFSA PLH Panel did not agree with the model-based evidence and conclusion by Paul et al. (2005) that the climate of the EU is unsuitable for the establishment of *P. citricarpa*. Currently, the Panel is still of the opinion that there is a potential for establishment and spread in the risk assessment area that should be evaluated.

#### 3.1.6. Potential for consequences in the pest risk assessment area

The pathogen causes different degrees of yield and quality losses in citrus orchards in the area of its current distribution (see sections 3.1.1.5 and 3.6.1.1). Therefore, the Panel concludes that there is a potential for consequences in the risk assessment area that should be evaluated.

#### 3.1.7. Conclusion of pest categorisation

*P. citricarpa* is absent from the EU and has a potential for establishment and spread and for causing consequences in the risk assessment area. For this reason, a risk assessment for *P. citricarpa* is needed for the EU territory.

### 3.2. Probability of entry

As stated above (section 2.1.2), the Panel conducted the risk assessment considering the absence of current requirements listed in Annexes II, III, IV and V of Council Directive 2000/29/EC and in Commission Decisions 2004/416/EC and 2006/473/EC, but under the assumption of common citrus disease management in the country of origin to comply with fruit quality standards. However, all the data on imports and interceptions presented in this document were obtained under the regulations currently in place in the EU. These data should be interpreted with caution because quantities of imported products will probably change if the regulations are removed and because interception numbers depend on the procedure of import control currently in place at the EU borders.

#### 3.2.1. Identification of pathways

The Panel identified the following pathways for entry of *P. citricarpa* into the EU:

- Citrus fruit commercial trade
- Tahiti lime fruit (*Citrus latifolia*) commercial trade
- Citrus fruit import by passenger traffic
- Citrus fruit with leaves and peduncles commercial trade

- v. Citrus plants for planting
- vi. Tahiti lime (*Citrus latifolia*) plants for planting
- vii. Citrus plants for planting import by passenger traffic
- viii. Citrus plants and plant parts not for planting, excluding fruit

Seeds have not been considered as a pathway for *P. citricarpa* in this opinion. According to current knowledge, *P. citricarpa* infections are limited to the rind (exocarp and mesocarp) of citrus fruit (Kotzé, 1981). Seeds are located in the internal juice sacs (endocarp), which are not colonised by the pathogen. Seeds could hypothetically be affected by *P. citricarpa* if extensive rotting occurred in harvested fruit, but this has not been reported.

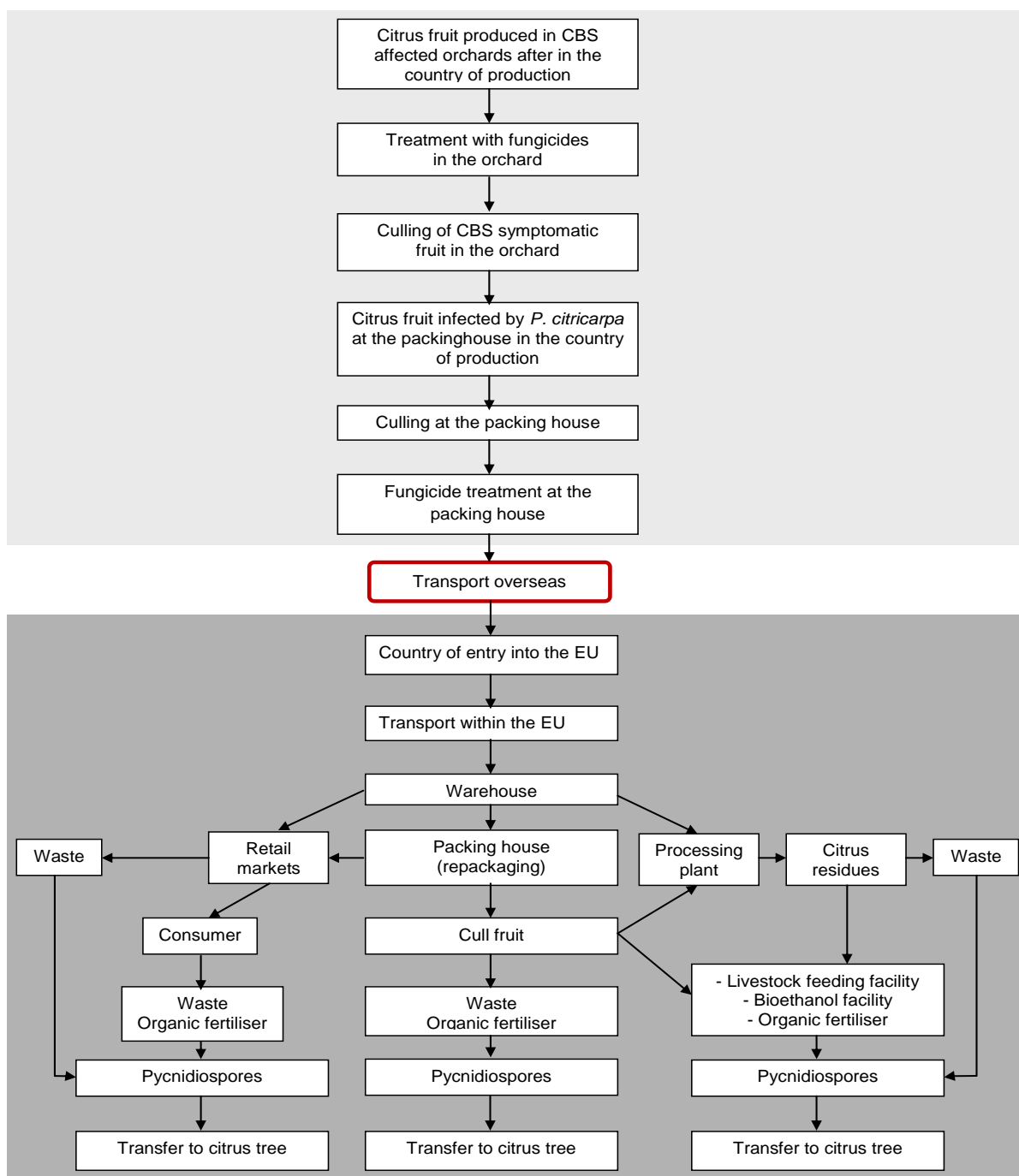
Citrus flowers are not known to be infected or colonised by *P. citricarpa*, so they are also not considered as a potential pathway in this opinion. Citrus branches with flowers and/or leaves for ornamental purposes are a theoretical entry pathway; however, as the Panel could not find any information or data regarding such a trade, this pathway is not dealt with in this opinion. Ornamental citrus grown in pots are instead included in pathway V regarding citrus plants for planting.

Infected citrus twigs are known to be a source of inoculum of *P. citricarpa* (Spósito et al., 2011) but there are no reports of infection or colonisation of lignified wood tissues such as large branches. In fact, severe pruning to leave only a framework of branches has been proposed as an alternative eradication method to the removal of entire trees (Whiteside, 1967) (section 3.4.4). Citrus wood has therefore not been evaluated as a potential pathway for *P. citricarpa* in this opinion.

One comment received, during the public consultation on the draft of this scientific opinion during the summer 2013, highlighted the existence of an international trade of citrus peel and discarded fruit imported into the EU for cattle feed. However the Panel could not find information on such trade nor on the treatment or processing of such material before shipping. Therefore the import of citrus peel and/or waste intended for animal feed is not further analysed here, but it is recommended to conduct further investigation on this pathway.

### 3.2.2. Entry pathway I: citrus fruit commercial trade

This pathway (graphically illustrated in Figure 8) concerns the importation of fruit without leaves and peduncles of citrus species from third countries where *P. citricarpa* is present (see Table 1), into the EU. With the exclusion of Tahiti lime (*C. latifolia*), that is dealt as a separate pathway in section 3.2.4, and of sour orange (*C. aurantium*), all other species and varieties of citrus species are considered in this pathway including sweet oranges (*C. sinensis*), mandarins and clementines (*C. reticulata*), lemons, other limes (*C. aurantifolia* and *C. limettioides*), satsumas (*C. unshiu*) and grapefruit (*C. paradisi*). Recent surveys conducted in China indicated that the pomelo (*C. maxima*) is not affected by *P. citricarpa* (Wang et al., 2012) but more data from other regions and proper pathogenicity tests would be needed to completely exclude this citrus species as a potential host (see section 3.1.1.4 on host range).



**Figure 8:** Graphical pathway model illustrating steps in the entry pathway of *Phyllosticta citricarpa* with commercial trade of citrus fruit, with the exclusion of Tahiti lime and sour orange fruit. The pathway starts in CBS-affected orchards in a country of origin outside the EU and ends with the transfer of spores of the pathogen to a susceptible host within the EU. The scheme is illustrative and departures from the depicted sequence may apply in some countries of origin and certain EU MSs, depending upon local characteristics of citrus production, trade and processing. For instance, in current practice (with the EU legislation in place), there is import inspection, but, in the scenarios considered in this opinion, there is no inspection specifically targeted at CBS

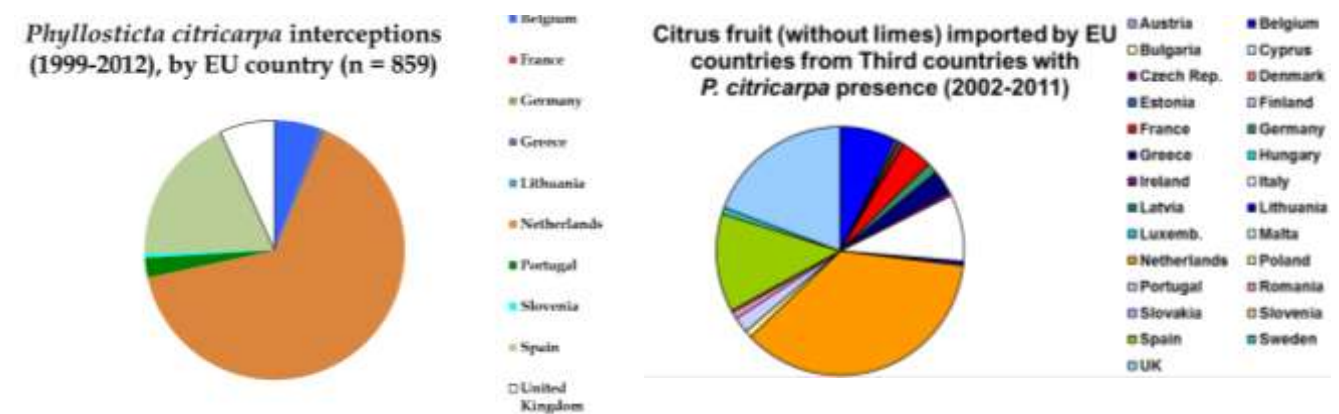
The pathway of *P. citricarpa* entry with imported citrus fruit has previously been analysed in pest risk assessment documents made by the Republic of South Africa (Hattingh et al., 2000), the Southern Cone Plant Health Committee (COSAVE) (Cortese et al., 2004), the EFSA (EFSA, 2008), the United States Department of Agriculture Animal and Plant Health Inspection Service (USDA APHIS, 2010a)

and the EFSA cooperation project on “Pest risk assessment for the European Community plant health: a comparative approach with case studies” (project acronym Prima phacie) (MacLeod et al., 2012). The evidence cited in these documents has been considered by the Panel and where there are differences with the conclusions of these documents these are discussed below.

The reader should bear in mind that, as stated above, the Panel assessed the probability of entry in the absence of current EU regulations.

Living stages of *P. citricarpa* are frequently found on imported citrus fruit during border inspections at the EU points of entry (see Figure 9 and Table 5). This shows that *P. citricarpa* is associated with the citrus fruit pathway and is able to survive transport and storage as well as existing pest management procedures. During 1999–2012 there were 859 interceptions of *P. citricarpa* on citrus fruit consignments from third countries to the EU. There were also three interceptions of *P. citricarpa* on plants for planting (including one bonsai), 67 interceptions of *Phyllosticta* spp. (without identification of the species; mostly from China), two interceptions of *P. citriasiana* (both from China) and 29 interceptions on *Citrus* sp. (without identification of the *Citrus* species). These interceptions were not included in the following graphs for the fruit pathway. Also interceptions on pomelo (*C. maxima*) were not included in the analysis as new *Phyllosticta* species have been recently described on pomelo (see section 3.1.1.1). On average, about 60 interceptions were reported per year, with a minimum of 19 (2000) and a maximum of 137 interceptions (2006). Most interceptions were made by the Netherlands (65 %), but approximately 18 % (160) were from Spain, and a few interceptions were made by France, Greece and Portugal, three other EU citrus-growing countries.

All trade data shown for this pathway correspond to the Eurostat category “Citrus fruit, fresh or dried” detracted of the Eurostat category “Fresh or dried limes ‘citrus aurantifolia, citrus latifolia’” (see section 2.2.1.1). Because the import of Tahiti lime is not reported alone in Eurostat, the trade data of this species were detracted using the overall category for the limes group (both lime species). It was not possible to detract the import of sour orange (*C. aurantium*) from these figures owing to the lack of statistical data, however the import of sour orange fruit (that is only used for marmalade) is considered as very little.



**Figure 9:** Distribution by EU country of (left) the 859 *Phyllosticta citricarpa* EU interceptions on citrus fruit consignments imported from third countries where *P. citricarpa* is present (1999–2012) (excluded pomelo) and (right) citrus fruit imported from third countries with *P. citricarpa* presence (2002–2011) excluded limes (*C. latifolia* and *C. aurantifolia*).

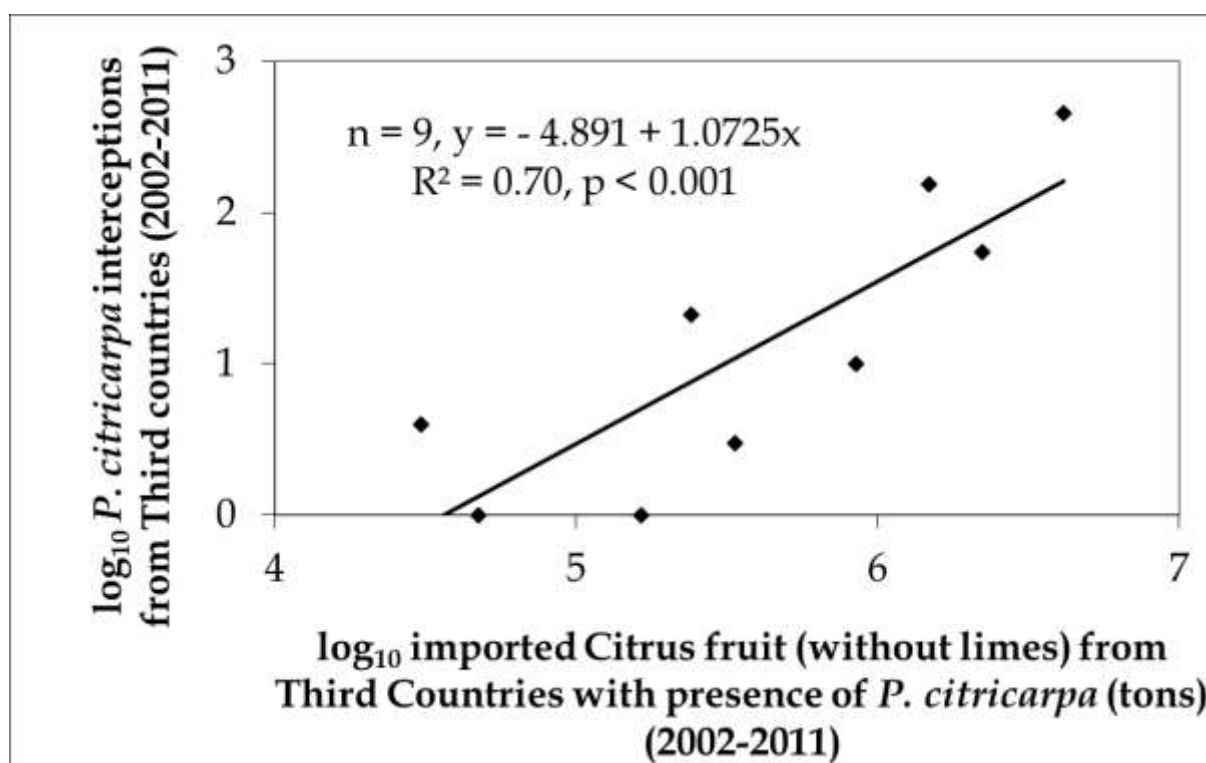


**Table 5:** Proportion of positive diagnoses in imported citrus fruit in The Netherlands and United Kingdom where pycnidia of *Phyllosticta citricarpa* were detected

| Year | Positive diagnoses of <i>P. citricarpa</i> |                          |                |                          |
|------|--|--------------------------|----------------|--------------------------|
|      | The Netherlands                            |                          | United Kingdom |                          |
|      | Total No                                   | Proportion with pycnidia | Total No       | Proportion with pycnidia |
| 2004 | 21   | 95.2                     | —*             | —*                       |
| 2005 | 82   | 93.9                     | —*             | —*                       |
| 2006 | 124  | 87.9                     | 12             | —*                       |
| 2007 | 75   | 80.0                     | 9              | —*                       |
| 2008 | 111  | 85.6                     | 12             | —*                       |
| 2009 | 36   | 63.9                     | 14             | —*                       |
| 2010 | 21   | 61.9                     | 15             | —*                       |
| 2011 | 89   | 79.8                     | 1              | —*                       |
| 2012 | 40   | 80.0                     | 15             | 66.7                     |
| 2013 | 66   | 86.4                     | 27             | 51.9                     |

Source: Europhyt (online), J. Meffert (NFCPSA, The Netherlands) and R. McIntosh (FERA, UK), personal communications.  
 Legend: \*= no data available

For the EU MSs which intercepted *P. citricarpa* over the period 2002-2011, with the exclusion of interceptions on pomelo, there is a strong correlation between the number of *P. citricarpa* interceptions and the volume of citrus fruit, excluded limes, imported by the same EU MS from third countries with reported presence of *P. citricarpa* (Figure 10).



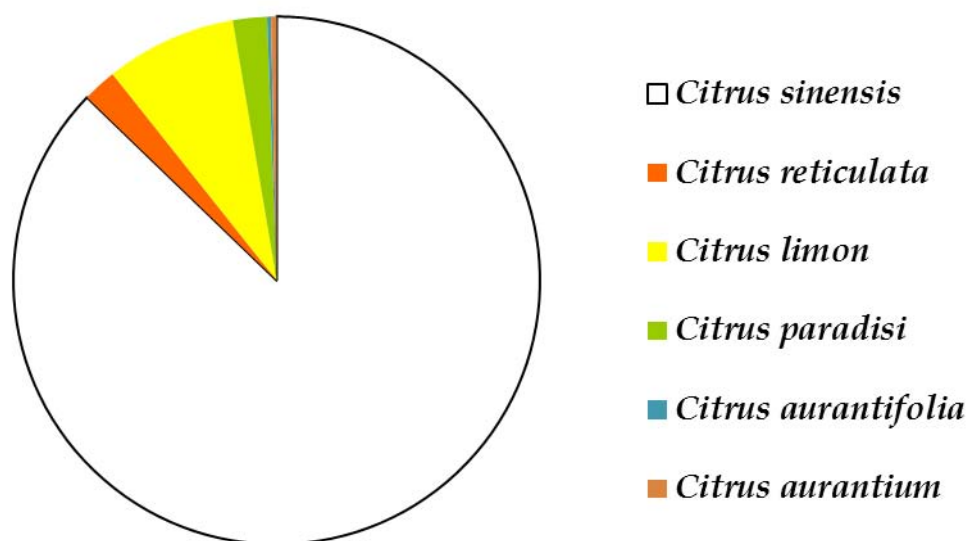
**Figure 10:** Log–log correlation of number of *Phyllosticta citricarpa* interceptions made by EU MSs (2002–2011), excluded interceptions on pomelo, and imported volumes of citrus fruit from third countries with reports of *P. citricarpa* (2002–2011), excluded limes, for the EU MSs which intercepted *P. citricarpa* at their borders. This figure covers the period 2002-2011, however the correlation was found to be just as strong when using all interception data (1999–2012) and when not log-transforming the data

### 3.2.2.1. Probability of association with the pathway at origin

The association of the pathogen with the citrus pathway varies with the citrus species: lemons and late-maturing sweet orange cultivars are generally considered to be more susceptible (Kotzé, 1981), mostly because they hang on the tree for a longer period and are therefore more exposed to pathogen inoculum during periods when environmental factors are suitable for disease development and have more time for symptom development. Early-maturing sweet orange cultivars are considered less susceptible as they are harvested earlier (Timmer, 1999; Spósito et al., 2004; Sousa and de Goes, 2010). Results from the meta-analysis (section 3.6.1) indicate that, under field trial conditions, disease incidences when using the best fungicide programmes ranged from 0.6 % to 7 % of CBS-affected fruit and from 7 % to 32 % with the least effective fungicides. Data from Sao Paulo state, Brazil, indicated that the incidence of CBS disease in fruits from commercial orchards intended for export was less than 2 % on arrival at the packing house, whereas in fruits harvested from orchards intended for domestic markets the disease incidence ranged from 19.3 % to 64.1 % (Fisher et al., 2008).

Most (approximately 87 %) *P. citricarpa* interceptions on citrus fruit consignments imported into the EU from third countries were made on shipments of sweet orange. About 8 % (70) of interceptions were made on shipments of lemon (Figure 11), the citrus species most susceptible to *P. citricarpa*, of which more than half (43) originated from South Africa.

## *Phyllosticta citricarpa* EU interceptions (1999-2012), by *Citrus* spp (n = 859)



**Figure 11:** Distribution by citrus species of the 859 *Phyllosticta citricarpa* EU interceptions on citrus fruit consignments, excluded pomelo (*C. maxima*), imported from third countries between 1999 and 2012

In the countries where *P. citricarpa* is present, fungicide treatments and some cultural practices are currently applied for the management of CBS (Kotzé, 1981; Timmer, 1999, Miranda-Bellote et al., 2013). However, in Brazil, cultural practices such as the early harvest of symptomatic citrus fruit and the removal of leaf litter from the orchard floor have been shown to be incapable of reducing CBS incidence and severity to satisfactory levels (Spósito et al., 2011). Pre-harvest applications of fungicides reduce CBS incidence or delay symptom development in citrus fruit in storage, but they do not seem to eradicate quiescent infections completely (Seberry et al., 1967; Andrade et al., 2001; Agostini et al., 2006). A meta-analysis of available data from fungicide trials against CBS (see section 3.6.1) shows that fungicide treatments are unable to reduce the level of infection of citrus fruit to

negligible levels if disease pressure is high, as is usually the case in orchards in which fungicide trials are carried out.

The efficacy of culling fruit in the field and/or in the packing house is limited owing to the presence of latent infections in asymptomatic fruit that may develop symptoms after harvest during transport and storage (Kotzé, 1981; Agostini et al., 2006; Baldassari et al., 2007). In addition, symptoms on fruit are variable and unspecific, with the exception of hard spot with pycnidia. Some lesions are very small (1–3 mm in diameter) and may therefore be confused with those caused by other citrus pathogens, as well as with those caused by mechanical or insect damage (Snowdon, 1990; Kotzé, 2000).

Low storage temperatures (8 °C), waxing or hot water treatments of fruit may reduce or delay the post-harvest development of CBS symptoms, but they are unlikely to eliminate the pathogen (Seberry et al., 1967; Korf et al., 2001; Agostini et al., 2006). Agostini et al. (2006) also showed that post-harvest fungicide dips and waxes were ineffective in controlling CBS. The same authors also reported that, once quiescent infections are present in the fruit, it appears to be difficult to prevent the development of CBS symptoms after harvest. Washing and brushing of fruit are procedures approved by APHIS for citrus packing houses located in CBS quarantined areas in Florida (APHIS, 2012). Although these measures will most probably remove any pycnidiospores present on its surface, they are unlikely to affect the latent mycelium inside the fruit peel or the pycnidiospores embedded within pycnidia.

In conclusion, cultural practices and pre- and/or post-harvest treatments applied in the current area of *P. citricarpa* distribution may reduce the incidence and severity of CBS infection in citrus fruit imported into the pest risk assessment (PRA) area, but they will not completely eliminate the pathogen.

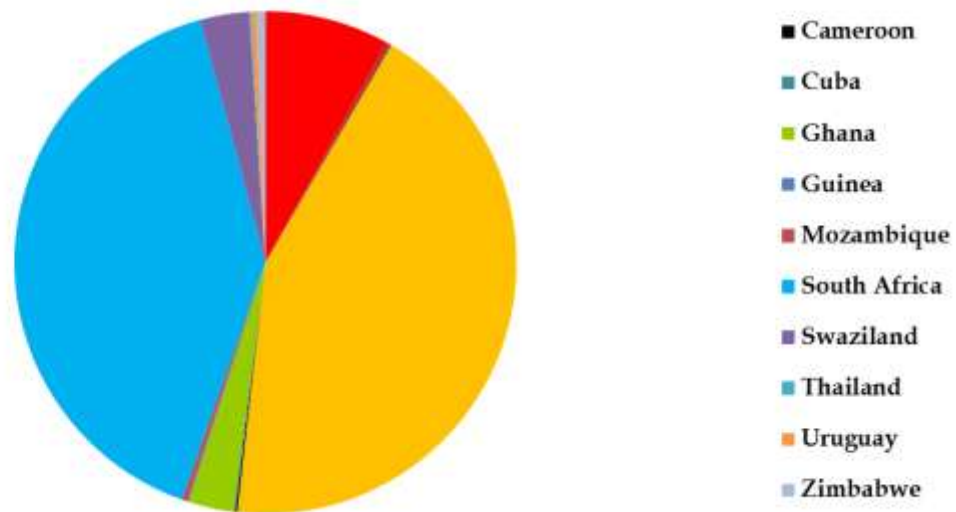
Based on the above, and in agreement with MacLeod et al. (2012), the Panel rates the probability of association with the pathway at origin for *P. citricarpa* on fresh citrus fruit imported from infested areas into the pest risk assessment area is assessed as likely, with a **medium** uncertainty.

#### *Volume of the movement along the pathway*

Every year a large volume of citrus fruit is imported into the EU from third countries where *P. citricarpa* is present. Data for each exporting third country for the period 2002–2011 are shown in Table 31 in the Appendix B. The main exporters of citrus fruit into the EU are Argentina, Brazil, China, the United States, Uruguay, South Africa and Zimbabwe. Minor imports originate from Australia, Cuba, Ghana, Mozambique and New Zealand. Very small quantities of citrus fruit have been imported into the EU from Kenya, the Philippines, Taiwan, Uganda and Zambia.

Most EU interceptions of *P. citricarpa* on citrus fruit consignments imported from third countries over the period 1999–2012 originated from Brazil and South Africa (Figure 12). The number of countries from which interceptions originated (13) provides evidence that citrus fruit can be considered as a major potential pathway of entry for the pathogen.

***Phyllosticta citricarpa* EU interceptions on citrus fruit (1999-2012), by country of origin (n = 859)**



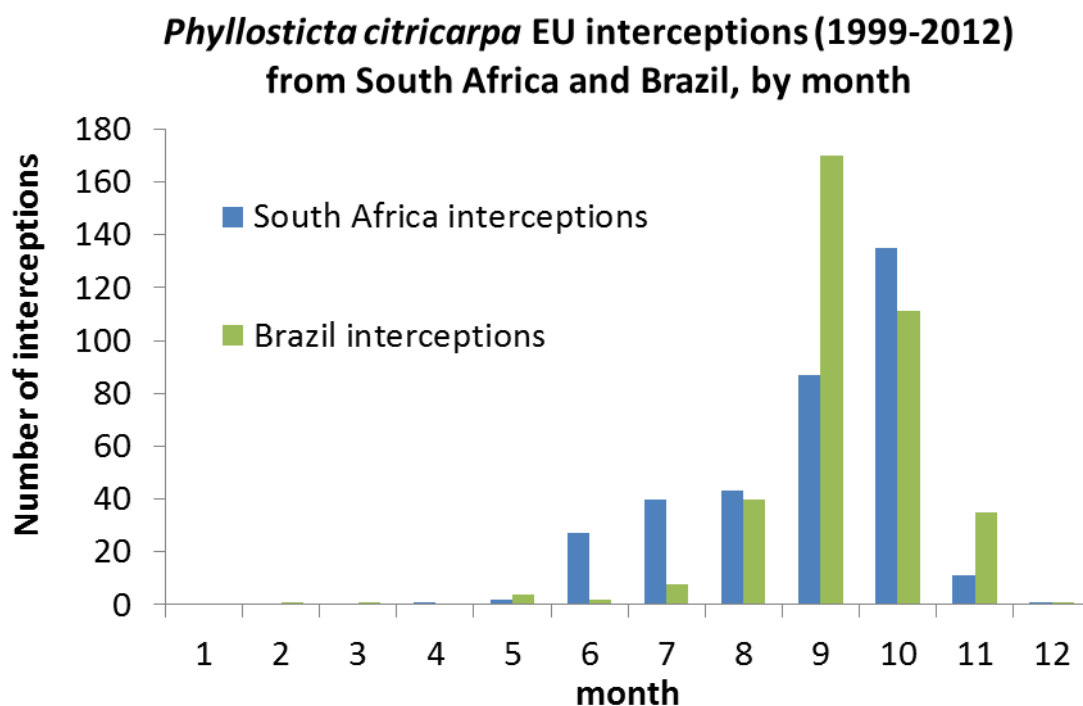
**Figure 12:** Distribution by country of origin of the 859 *Phyllosticta citricarpa* EU interceptions on citrus fruit consignments, excluded pomelo (*C. maxima*), imported from Third Countries between 1999 and 2012

Based on the above, and in agreement with MacLeod et al. (2012), it is clear that the volume of citrus fruit imported into the pest risk assessment area from third countries where the pest is present is **massive**, with **low** uncertainty.

*Frequency of the movement along the pathway*

The frequency of imports of citrus fruit into the pest risk assessment area varies between different years, citrus species, exporting countries and the importing EU MSs (Eurostat, online). Generally, citrus fruit consignments from third countries where *P. citricarpa* is present are imported into the EU throughout the whole year, with the main import period between March and November (Eurostat, online) and volumes decreasing once the EU harvest season has begun (MacLeod et al., 2012).

Most *P. citricarpa* interceptions on citrus fruit consignments imported from third countries into the EU were made during the late summer and autumn in Europe, mainly in September and October, as exemplified by data from South Africa and Brazil (all years, all receiving EU countries, all *Citrus* spp.) (Figure 13). This timing has implications for the probability of transfer of the pathogen to a suitable host (see below), which would be much lower if affected consignments were imported during the European winter, particularly for shipments going directly to EU citrus-growing countries, but also in case of re-exported consignments from, for example, the Netherlands to Spain and other Mediterranean EU countries.

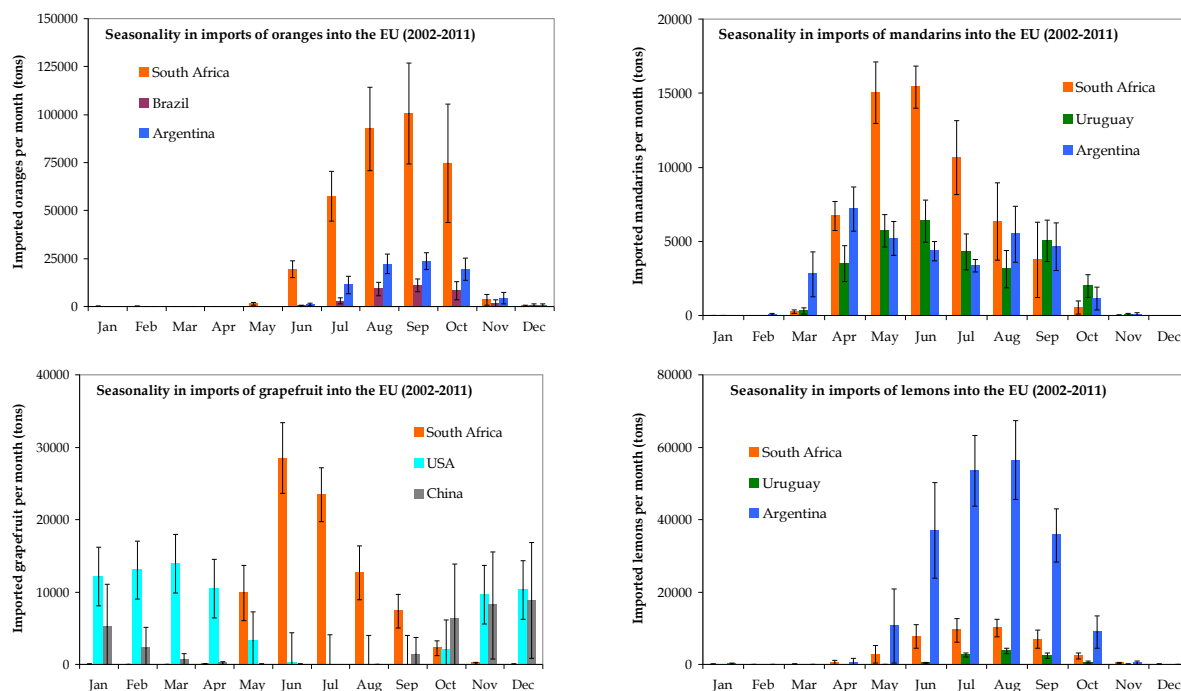


**Figure 13:** Distribution by month of the *Phyllosticta citricarpa* EU interceptions on citrus fruit consignments, excluded pomelo, imported from Brazil ( $n = 373$ ) and South Africa ( $n = 347$ ) between 1999 and 2012.

The seasonality in imports appears to be consistent across different years, as shown by the relatively small error bars in the frequency distribution of imports of sweet oranges from the three major exporting third countries (South Africa, Brazil and Argentina) to the EU (these monthly data do not include Croatia) over the period 2002–2011 (Figure 14). A similar pattern is observed for mandarins. The pattern is different for grapefruit, because two of the major exporters to the EU (the USA and China) are located in the northern hemisphere. The major exporter of lemons to the EU is Argentina, but the seasonality of lemon imports into the EU from the three major exporters (South Africa, Brazil and Argentina) is similar to that for sweet oranges (Figure 14).

In agreement with MacLeod et al. (2012), and based on the data above, the Panel conclude that citrus fruit are imported **very often** into the risk assessment area from third countries where the pest is present, with **low** uncertainty.

Based on the above ratings, the Panel concludes that, overall, the probability of association of *P. citricarpa* with the commercial fruit pathway at origin is rated as likely, with **medium** uncertainty. The medium rating is mainly due to different incidence and severity of CBS in affected citrus fruit from different locations and years and to the difficulties in ensuring that fruit is disease free if it originates from countries where the disease is endemic, owing to the limited efficacy of fungicides, as indicated by the meta-analysis of control trials presented later, in section 3.6.1 of this opinion.



**Figure 14:** Quantity of sweet oranges, mandarins, grapefruit and lemons imported monthly into the EU-27 MSs from South Africa, Argentina and Brazil, the three major exporters of oranges into the EU, during the period 2002–2011 (Eurostat, online). Error bars are standard deviations

### 3.2.2.2. Probability of survival during transport or storage

In general, the transport of commercial citrus fruit takes place under cool conditions (Wills et al., 1998). Whilst sweet oranges and mandarins are typically shipped at 1 °C and 4 °C, respectively, lemons and limes are usually shipped at 10 °C, because of their sensitivity to chilling injury. Depending on the time of the year, the conditions of harvested trees, and fruit conditions, grapefruit is shipped at 10 or 15 °C (Wardowski, 1981). These data are in agreement with EPPO (2013b), which indicates that citrus consignments are transported by ship, mostly with fruit pulp temperature of 4–10 °C with shipping times ranging from 12 to 18 days. Nevertheless, some consignments may be shipped at lower (2.5 °C) or higher (16 °C) temperatures (Transport Information Service [http://www.tis-gdv.de/tis\\_e/ware/inhaltx.htm#6](http://www.tis-gdv.de/tis_e/ware/inhaltx.htm#6); R. Robinson, South African Perishable Products Export Control Board, personal communication 2011; and M. Brook, Citrus Growers Association of Southern Africa, personal communication, 2011, cited in EPPO, 2013b). Such low temperatures during transport and storage are likely to prolong the survival of *P. citricarpa* pycnidia and pycnidiospores on CBS lesions. Mature pycnidiospores of *P. citricarpa* produced on infected citrus fruit were shown to be still viable after three weeks' storage of the fruit at 4.5 or 10 °C, but apparently lost their viability at 25 °C (Korf et al., 2001). Similarly, the viability of freshly exuded pycnidiospores of *P. citricarpa* incubated at 25 °C was reduced by 60 % after four days and by 100 % after three months, respectively (Kiely, 1948).

In addition, the survival of latent *P. citricarpa* mycelium is not affected by the low temperatures typically used when transporting and storing citrus fruit: CBS symptoms develop rapidly when fruit with quiescent infections encounters higher temperatures (Kotzé, 1981; Agostini et al., 2006; Baldassari et al., 2007; Er et al., 2013). *P. citricarpa* was successfully isolated from CBS lesions in citrus fruit kept for more than 40 days under various moisture conditions at 8 °C or ambient temperatures of 15–25 °C (Agostini et al., 2006). The pathogen was isolated from CBS lesions in more than 85% of the fruits maintained for three weeks at 4.5 °C, 10 °C or 25 °C (Korf et al., 2001). Recently, Er et al. (2013) demonstrated that lesions and pycnidia developed in asymptomatic, latently infected fruit even when maintained at 4 °C. These findings imply that the pathogen is likely to remain

viable long after fruit stored in such conditions have become unmarketable. This has important implications for the likelihood of transfer to suitable hosts (see below).

Based on the above, and in agreement with MacLeod et al. (2012), the Panel concludes that, in terms of duration and conditions of transport and storage, *P. citricarpa* in the form of (i) pycnidiospores within pycnidia in fruit lesions and/or (ii) latent mycelium present in asymptomatic fruit is **very likely** to survive transport and storage conditions (with **low** uncertainty).

In 2008/2009, the volume of citrus fruit imported by the EU from Argentina, Brazil, China, South Africa and Uruguay by sea was three orders of magnitude greater than the volume of air imports (MacLeod et al., 2012). Time required for citrus fruit to be shipped from other continents to Europe depends on the transport routes, with shipping times ranging from 12 to 18 days (EPPO, 2013b): three weeks or longer is reported for shipments from South Africa (Terblanche, 1999). Although they would be very valuable, no data are available on the incidence and severity of CBS infection in citrus fruit consignments (proportion of infected fruit and number of lesions per fruit) imported by EU countries, because consignments including CBS-affected fruit are rejected without further evaluation.

#### *Likelihood of the pest multiplying/increasing in prevalence during transport/storage*

Er et al. (2013) indicated that CBS lesions and pycnidia of *P. citricarpa* developed in asymptomatic, latently infected fruit maintained at 4 °C, 12 °C or 22 °C, but new infections during transport or storage were not demonstrated. Since the optimal temperature for the hyphal growth of *P. citricarpa* in synthetic medium is 25–2 °C (Chiu, 1955; Kotzé, 1981) and the pathogen remains virtually inactive at temperatures lower than 15 °C (Chiu, 1955), the Panel considers, in agreement with MacLeod et al. (2012), that it is **very unlikely** (with **low** uncertainty) that the pathogen will multiply or increase in prevalence during transport/storage of infected citrus fruit, which normally occurs at low temperatures.

#### 3.2.2.3. Probability of surviving existing pest management procedures

The management of CBS in its current area of distribution (cultural practices and chemical treatments applied pre- and post-harvest) can reduce the level of disease in the orchard or delay symptom development in transit and storage but does not eliminate the pathogen, particularly quiescent infections on citrus fruit (Kotzé, 1981). Similarly, physical treatments of citrus fruit in packing houses can reduce or delay the post-harvest development of CBS symptoms but without eliminating the pathogen (Seberry et al., 1967; Korf et al., 2001; Agostini et al., 2006). For instance, Korf et al. (2001) isolated living stages of the pathogen from fruit lesions in a proportion ranging from 12.3 % to 96.6 % in all treatments and temperatures evaluated.

The application of post-harvest fungicides can reduce the viability of *P. citricarpa* pycnidiospores present in fruit lesions before the treatment (Korf et al., 2001). In fact, post-harvest chemical treatments were suggested by EFSA (2008) as a risk mitigation measure for CBS. In Florida, post-harvest treatments with imazalil and thiabendazole are currently compulsory in CBS-affected areas to reduce the risk of disease spread (USDA-APHIS, 2011c). Nevertheless, as indicated above, the pathogen remains viable in the fruit rind so new pycnidia and/or pycnidiospores may be produced after the treatment.

The detection of the pathogen is made difficult by the long incubation period (2–12 months), during which latently infected fruit remains asymptomatic (McOnie, 1967; Kellerman and Kotzé, 1977; Kotzé, 1981; Aguiar et al., 2012). Culling will thus not detect the latently infected fruit, which will also escape any potential border inspection.

Given their variability, CBS symptoms on fruit can be confused with those caused by other citrus pathogens or mechanical or insect damage (Snowdon, 1990; Kotzé, 2000), although living stages of *P. citricarpa* continue to be intercepted on citrus fruit consignments imported into the EU.

Reliable detection and identification of the organism on citrus fruit can be made only after laboratory testing (EPPO, 2003).

Based on the above, and in agreement with MacLeod et al. (2012), the Panel concludes that *P. citricarpa* is **very likely** (with **low** uncertainty) to survive and remain undetected during existing pest management procedures, particularly on latently infected (asymptomatic) fruit and fruit with low disease incidence and severity.

#### 3.2.2.4. Probability of transfer to a suitable host

Large quantities of citrus fruit are imported every year from CBS-affected countries into all the EU MSs, including the citrus-growing EU MSs (i.e. Spain, Italy, Greece, Cyprus, France and Portugal) (see Table 31 and Figure 57 in Appendix B) (Eurostat, online). In addition, some EU MSs (e.g. Belgium and the Netherlands) redistribute within the EU large quantities of fresh citrus fruits imported from CBS-infested countries (see sections 3.4.2 and 3.4.3).

As an example, in 2009 the Netherlands imported approximately 450 000 tons of sweet orange from various CBS-infested countries (including Argentina, Brazil and Uruguay) and redistributed almost 200 000 tons of sweet orange to other EU MSs, including citrus-producing EU MSs (Eurostat, online). Fresh citrus fruit are destined for human consumption or processing. Thus, once fruit consignments enter the pest risk assessment area, they are sent to packing houses, processing plants and wholesale and retail fresh fruit markets before being sold to end users.

Therefore, and in agreement with MacLeod et al. (2012), it is expected that the imported citrus fruit will be **very widely** distributed within the pest risk assessment area (with **low** uncertainty).

The main period of import of citrus fruit from third countries is between March and November (Figure 14), when there is little availability of European fresh citrus fruit (Agustí, 2012). A varying, but significant, proportion of citrus fruit imports to the EU takes place from May to November, the period when weather conditions are potentially favourable for pycnidiospore dispersal and infection (section 3.3.2.5). Moreover, *P. citricarpa* interceptions in imported citrus fruit are mainly concentrated in late summer and autumn (section 3.2.2.1, Figure 13) during the months most suitable for dispersal and infection by pycnidiospores.

Based on the above, citrus fruit consignments imported into the pest risk assessment area from third countries with presence of the pest are **likely** to arrive during a time of the year potentially suitable for disease establishment, with **low** uncertainty.

Most of the citrus fruit consignments imported into the pest risk assessment area arrive at ports because they are transported by sea (Eurostat, online; Europhyt, online). Citrus species susceptible to CBS are widely grown in the southern EU MSs in a variety of locations (commercial orchards, nurseries, smallholdings, private gardens for family consumption, public gardens and along the roadsides in both urban and rural regions). Commercial citrus orchards and nurseries are located mainly in coastal areas, next to rivers (Agustí, 2012), and in some cases in close proximity to ports. Given that fresh citrus fruit imports are destined for human consumption and processing, they will be very widely distributed to packing houses, processing plants and fresh fruit markets in urban and rural regions of all EU MSs.

Most of the sweet oranges and lemons imported by the EU go to non-citrus-producing countries, but the quantities imported by citrus-producing EU countries are not negligible. Mandarins and grapefruit are mostly imported by non-citrus producing EU countries (Table 31 and Figure 58 in Appendix B).

The risk of pathogen transfer associated with citrus fruit imported into the pest risk assessment area from CBS-infested countries is mainly due to discarded unmarketable whole fruit, peel or citrus by-products produced by packing houses, processing plants, fresh fruit markets, households, etc., and their subsequent management. Packing houses and processing plants are usually located within the



citrus-growing regions of the pest risk assessment area and are often in close proximity or even adjacent to commercial citrus orchards (EFSA, 2008; NPPO of Italy, 2010, cited by McLeod et al., 2012).

Citrus pulp is the residue generated by pressing fresh citrus fruit for juice extraction. During this process, 45% to 60 % of their weight remains in the form of peel, rag and seeds. Citrus processing industry by-products can be used to produce high-quality compost as an organic fertiliser (Figure 16; Bernal-Vicente et al., 2008). This compost is also used in citrus orchards. Some scientific research has shown that citrus compost can be as effective as mineral nutrition programmes in offering ecological, agronomic and socioeconomic advantages, allowing the elimination of industrial wastes (Roccuzzo et al., 2010, 2012). In addition, fresh citrus pulp is characterised by a high moisture content, which favours microbial degradation (Cerisuelo et al., 2010). For this reason, ensiled or dried citrus pulp residue is the citrus by-product that is most extensively used for livestock/animal feeding (Bampidis and Robinson, 2006; Caparra et al., 2007; NPPO of Italy, 2010, cited by McLeod et al., 2012). The dried form of citrus pulp is not always economically viable owing to the high costs of artificial dehydration; however, solar dehydration of citrus pulp in open-air facilities is practised in citrus-growing areas with a suitable climate (Kimball, 1991; Caparra et al., 2007). Whole marketable and non-marketable citrus fruit can also be withdrawn from the market and turned into citrus waste (Piquer et al., 2009b) either because it does not meet the requirements for fresh produce (2 %; unmarketable fruits) or to maintain prices (Piquer et al., 2009a). A maximum of 5 % commercialised fruits is withdrawn from the market (EU Regulation 2200/96). Boluda-Aguilar et al. (2010) estimated that 1.5 million tonnes of citrus waste are produced each year in the Mediterranean Basin. The average yearly production of citrus fruit wastes in Spain and Italy has been estimated to be about 500 000 tonnes (Caparra et al., 2007; Boluda-Aguilar et al., 2010). Citrus waste is also used in ethanol production facilities in the EU to obtain biofuel, together with other co-products such as limonene, galacturonic acid and pectin (Boluda-Aguilar et al., 2010; NPPO of Italy, 2010, cited by McLeod et al., 2012; Lanfranchi, 2012). Citrus waste from fruit markets or households may also be discharged in the vicinity of citrus trees either where landfills are located close to commercial or abandoned citrus orchards or where citrus waste is discharged uncontrolled in the vicinity of citrus trees.

Transfer through splash dispersed pycnidiospores should be viewed in the context of entry (first infection event from imported fruit to a citrus tree) not in the context of long-term epidemics (i.e. although of relative minor epidemiological importance compared with ascospores, pycnidiospores are relevant to establish the first outbreak). Symptomatic citrus fruit, peel and citrus by-products can be a source of *P. citricarpa* pycnidiospores, which are produced in pycnidia and may remain viable for a relatively long time (Korf et al., 2001; Agostini et al., 2006). Schutte et al. (2013) indicated that up to 60 % of CBS lesions present in excised sweet orange peel produced pycnidiospores even when exposed directly to sunlight for a continuous period of four hours. In grapefruit peels, pycnidiospores of *P. citricarpa* were observed in 40 % of the lesions after two hours' exposure. In these experiments, excised citrus peel was exposed to temperatures up to 28–32 °C, so they dried out rapidly, resulting in a weight loss of 60–70 % in only 6–10 hours and no further sporulation. However, whole fruits discarded from packing houses or citrus residues from the processing factories, which have a much higher moisture content (~75–85 % water), can withstand harsh conditions for longer periods (Piquer et al., 2009b; Cerisuelo et al., 2010; Lanfranchini, 2012). Moreover, the infectious period of *P. citricarpa* in peels, fruit of other citrus by-products discarded outdoors may be much longer when subjected to mild temperatures together with rains, which substantially reduce dehydration and enhance pycnidiospore production.

Studies conducted in South Africa indicated that pycnidiospores of *P. citricarpa* from pure cultures, symptomatic CBS sweet orange fruit and peelings were not able to colonise lemon leaf litter on the orchard floor (Truter et al., 2007). However, susceptible live tissues (leaves, twigs and fruit on the canopy) of citrus trees in commercial orchards in the risk assessment area are normally very close to the soil, and leaves and fruit very often directly touch the orchard floor (see Figure 16 below and section 3.3.3.3), depending on the cultivation technique and the season. Branches bearing many mature fruits tend to bend closer to the orchard floor before harvest. In addition, during the last few

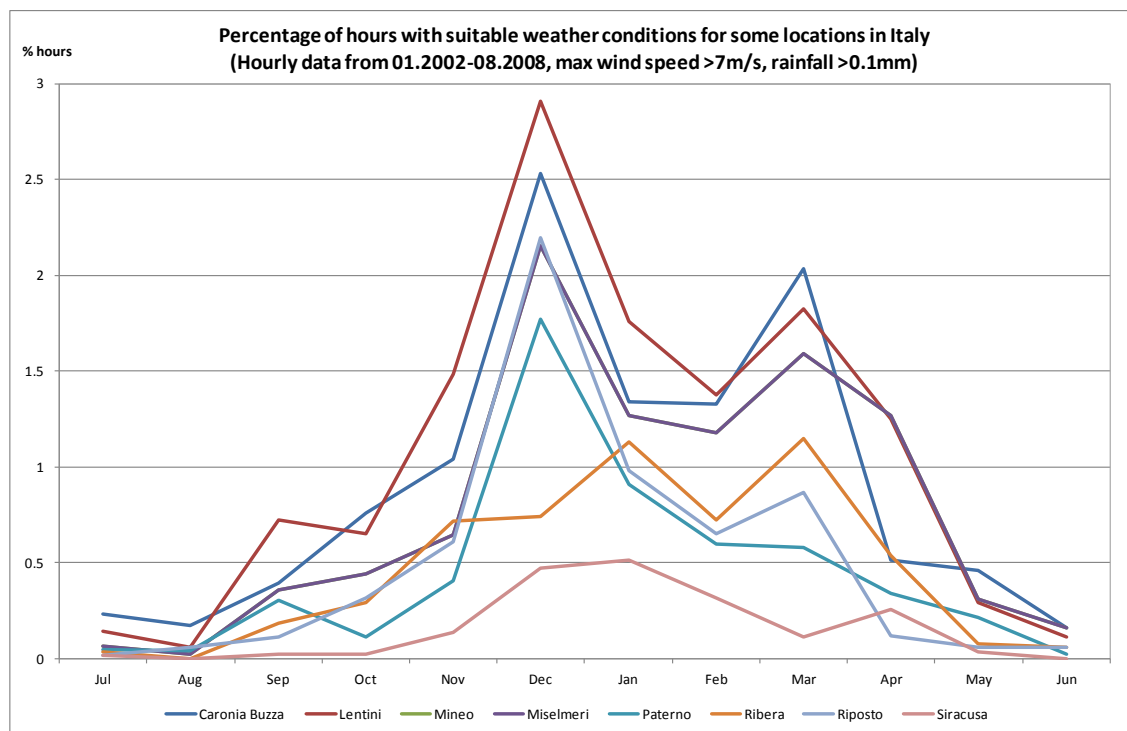
years, there has been a trend towards the cultivation of shorter citrus trees by grafting onto dwarfing rootstocks.

Spósito et al. (2011) evaluated the spread of symptoms from different inoculum sources. No symptoms of the disease were observed in the tree canopy when five sweet orange fruits with more than 10 lesions were placed on the orchard floor 30 cm under the fruits on the canopy. Nevertheless, this study was based solely on visual observations of symptoms, without monitoring rain-dispersed inoculum or confirmatory isolations to detect latent (asymptomatic) infections in leaves and fruit. Therefore, the transfer and subsequent infection of *P. citricarpa* pycnidiospores from CBS-affected fruits in the orchard floor cannot be completely excluded. Moreover, different results might be obtained with a higher number of fruits and lower-hanging leaves and fruits (section 3.3.3.3)

If symptomatic citrus fruit, fruit peel or other citrus by-products with pycnidia are disposed of close to host plants (grown in nurseries, commercial orchards, private and public gardens, roadsides, etc.) in the risk assessment area (Figure 16), the mature pycnidiospores exuded from pycnidia under wet conditions could be splash dispersed by rain (Whiteside, 1967; Spósito et al., 2011) onto the lower parts of the canopy, infecting leaves, twigs and fruit at a susceptible stage.

Perryman and West (2014) studied the splash dispersal potential of *P. citricarpa* pycnidiospores from infected sweet orange fruit. Laboratory experiments showed that fruit misted with water to simulate light rainfall continued to exude *P. citricarpa* pycnidiospores from pycnidia for at least one hour, although longer periods were not evaluated. In the splash dispersal experiments conducted in still air conditions, 99.4 % of the splashes produced by single incident rain drop on the fruit were of less than 2 mm diameter, with an average of 1–21 pycnidiospores. Larger but less frequent splashes of 4–5.5 mm diameter contained an average of 308 pycnidiospores. In these experiments, the maximum horizontal distance of splash was 70 cm and the maximum height was 47.4 cm, reached 20 cm away from the target fruit. However, when multiple incident rain drops were combined, also in still air, splashes were forced higher than occurred in single-drop experiments, over 60 cm. At the greatest horizontal distance evaluated (70 cm), the height of splash was around 40 cm, so larger maximum distances may be expected when multiple rain drops are present.

In other experiment, the combined effect of single incident rain drops and wind was evaluated. Splashes from infected fruit were disseminated up to 2 m downwind from the target fruit at a wind speed of 4 m/s and up to 8 m at 7 m/s, the highest wind speed evaluated, reaching heights up to 75 cm and even higher as a result of fine droplets becoming aerosolised. The height of splash with a wind speed of 7 m/s was still 70 cm at the maximum distance evaluated of 8 m. Thus, greater horizontal dispersal potential would be expected under these conditions. The combination of multiple incident rain drops and wind was not evaluated in this study but, based on the above results, a positive interaction for splash dispersal potential may be expected. As Figure 15 illustrates, wind-driven rains with potential for the dispersal of *P. citricarpa* pycnidiospore are not rare in the PRA area. All this information supports that CBS-affected citrus fruit can provide a source of *P. citricarpa* pycnidiospores with the potential to be splash dispersed to the lower parts of the tree canopy by light rains and sprinkle or micro-sprinkle irrigation and particularly by wind-driven rainfall events



**Figure 15:** Monthly percentage of hours with wind speed > 7 m/s and rainfall > 0.1 mm in some locations in citrus growing area in Italy based on an average from May 2002 to August 2008

Rain splash dispersal followed by periods with temperatures and wetness durations conducive for infection by pycnidiospores could potentially take place from May to November, coinciding with the period of import of citrus fruit from CBS-affected areas into the EU citrus-growing regions (sections 3.2.2.1 and 3.3.2.5). According to the data shown in Figure 13, interceptions of *P. citricarpa* in imported citrus fruit from South Africa and Brazil are recorded mostly from May to November, but with a marked peak in September and October. These two months coincide with the highest values of weather suitability for pycnidiospore splash and infection (Figures 29 and 30).

In addition to rain splash, water drops formed on leaves due to fog, mist or dew occurring during the night in the coastal citrus-growing regions and irrigation (sprinkler or micro-sprinkler) applied during the dry periods may cause drip-splash of *P. citricarpa* pycnidiospores produced on infected fruit, peel of citrus by-products discarded near to citrus plants in the PRA area. Drip-splash can be as efficient as direct rain-splash for the dispersal of mucilaginous conidia (Fitt et al., 1989). Since water drops from micro-sprinklers can be larger than dew drops or rain drops (Montero et al., 2003) and the larger the drop size, the more effective is the dispersal of inoculum by water splash (Fitt et al., 1989), it is conceivable that micro-sprinkler irrigation has the potential to contribute to the dissemination of *P. citricarpa* conidia.

Although not yet investigated, *P. citricarpa* pycnidiospores produced on discarded CBS-affected fruit/peels or citrus by-products might be transported by insects, birds, and other organisms and deposited on susceptible hosts grown at a considerable distance (Kiely, 1948; MacLeod et al., 2012).

Based on the above, together with the information provided by MacLeod et al. (2012), the Panel considers that citrus fruit imported into the EU from infested third countries are very widely distributed in both citrus-growing and non-citrus-growing EU MSs. If CBS-affected citrus fruit, peel or other citrus by-products with pycnidia of *P. citricarpa* are discarded underneath or in close proximity to susceptible citrus trees, the pathogen can be dispersed by natural means to infect susceptible plant tissues.



**Figure 16:** Top left and top right: processing of citrus pulp residues and whole citrus fruit in close proximity to citrus orchards; Middle left: uncontrolled citrus waste discharged in the vicinity of neglected citrus trees; Middle right: sweet orange orchard with low-hanging branches and fruit (Valencia, Spain); Bottom left: citrus waste used for obtaining an organic fertiliser; Bottom right: the organic fertiliser made from citrus waste ready to be applied to the citrus orchards nearby (Catania, Italy)

In agreement with MacLeod et al. (2012), the Panel concludes that the pest is **moderately likely** to transfer from the fruit pathway to a suitable host or habitat, with a **medium** level of uncertainty.

The uncertainties are associated with the frequency and quantity of infected fruit, peel or citrus by-products being discarded in close enough proximity to a host in the citrus-growing regions of the risk assessment area, and the time taken for discarded asymptomatic whole fruit, peel or citrus by-products to produce pycnidiospores before their decomposition (MacLeod et al., 2012).

The import of fresh citrus fruit into the pest risk assessment area occurs mainly during the European late spring, summer and early autumn periods, when there is little if any local production (Agustí, 2012). Because citrus fruit are imported for processing and direct human consumption, it is expected that the commodity will be widely distributed in both urban and rural areas of the EU, in both citrus- and non-citrus-growing regions. The risk of transfer to a suitable host posed by citrus fruit imported from CBS-infested third countries is associated with the discarded fruit, peels, pulp or other citrus fruit by-products derived from packing houses, processing plants, households and fresh fruit markets.

If citrus fruit by-products are discarded in the vicinity of citrus nurseries, commercial or abandoned citrus orchards, and susceptible citrus trees grown in private and public gardens and roadsides, the pathogen is likely to be transferred by natural means (rain or irrigation water, insects, birds, etc.) and infect susceptible plant tissues (leaves, twigs and fruit).

Based on the above, and in agreement with MacLeod et al. (2012), the intended use of the citrus fruit commodity is **moderately likely** to aid transfer of the pathogen to a suitable host, with **medium** uncertainty.

There are uncertainties concerning (i) the prevalence of *P. citricarpa* on infected citrus fruit imported into the pest risk assessment area, (ii) the frequency and quantity of infected citrus fruit by-products being discarded in close proximity to a host in the citrus-growing regions of the pest risk assessment area and (iii) the time taken for discarded asymptomatic whole fruit, peel or citrus by-products to produce pycnidiospores before decomposition by other organisms (MacLeod et al., 2012).

Based on these ratings, the Panel concludes that the transfer of *P. citricarpa* to a suitable host through the commercial fruit pathway is **moderately likely**, with **medium** uncertainty that is mainly owing to the gaps in our knowledge listed above.

### 3.2.3. Quantitative pathway analysis for citrus fruit trade (pathway I)

In order to quantify the amount of citrus fruit material potentially infected by *P. citricarpa* arriving in citrus-growing regions of the EU, a quantitative pathway analysis was carried out (see Appendix E for details). The model was applied to Spain as a case study because it is the main citrus producer country in the EU.

The starting point of the pathway model is the yearly or monthly volume of citrus fruit import into Spain from CBS-affected countries. The end point of the pathway model is where the imported fruit is disposed of in a manner that allows the splash dispersal by rain (ballistic or wind) and subsequent infection of *P. citricarpa* pycnidiospores from infected fruit to susceptible citrus plants.

Five scenarios were considered in order to investigate the effect of the incidence of CBS in the imported fruit on the potential amount of infected fruit or citrus by-products disposed in citrus-growing regions in the EU, and of the effect of phytosanitary inspections and control treatments.

1. Current regulation: imported citrus is free of symptoms of CBS.
2. Current regulation: imported citrus has a low incidence of CBS. For this scenario, the Panel estimates that 2 % of fruit will remain affected by CBS even after application of the most effective fungicide spray programmes, based on the results of the meta-analysis of fungicide control trials (section 3.6.1) and data from commercial citrus production indicated by Fisher et al. (2008). The fruit are then subjected to two stages of phytosanitary inspection, first in the country of origin and secondly at the EU border, both carried out in accordance with International Standards for Phytosanitary Measures (ISPM) guidelines.
3. No regulation: the incidence of CBS in imported citrus is low, as may be achieved in CBS-affected areas using the most effective fungicide spray programmes. For this scenario, the Panel estimates that 2 % of fruit will remain infected after effective treatment, as in scenario 2.

But it is assumed that the fruit will not be subjected to phytosanitary inspections specific for *P. citricarpa*, as such measures are not compulsory in absence of current regulations and represent an added cost.

4. No regulation: the incidence of CBS in imported citrus is medium CBS, as may be achieved in CBS-affected areas with the most affordable, but less effective, fungicide spray programmes. For this scenario, the Panel estimates that 16 % of fruit will remain infected after less effective treatment, based on the meta-analysis and the data from Fisher et al. (2008). As in scenario 3, it is assumed that the fruit will not be subjected to phytosanitary inspections specific for *P. citricarpa*, as it is unlikely these will continue in absence of current regulations owing to the costs involved.
5. No regulation: the incidence of CBS in imported citrus is high, as may be achieved in CBS-affected growing areas in the absence of fungicide sprays. Estimates of the percentage fruit affected by CBS when no treatment is applied range between 46 % and 98 % for different countries based on the meta-analysis and the data from Fisher et al. (2008). The Panel took the midpoint of this range, 72 %, as a simple central estimate for modelling.

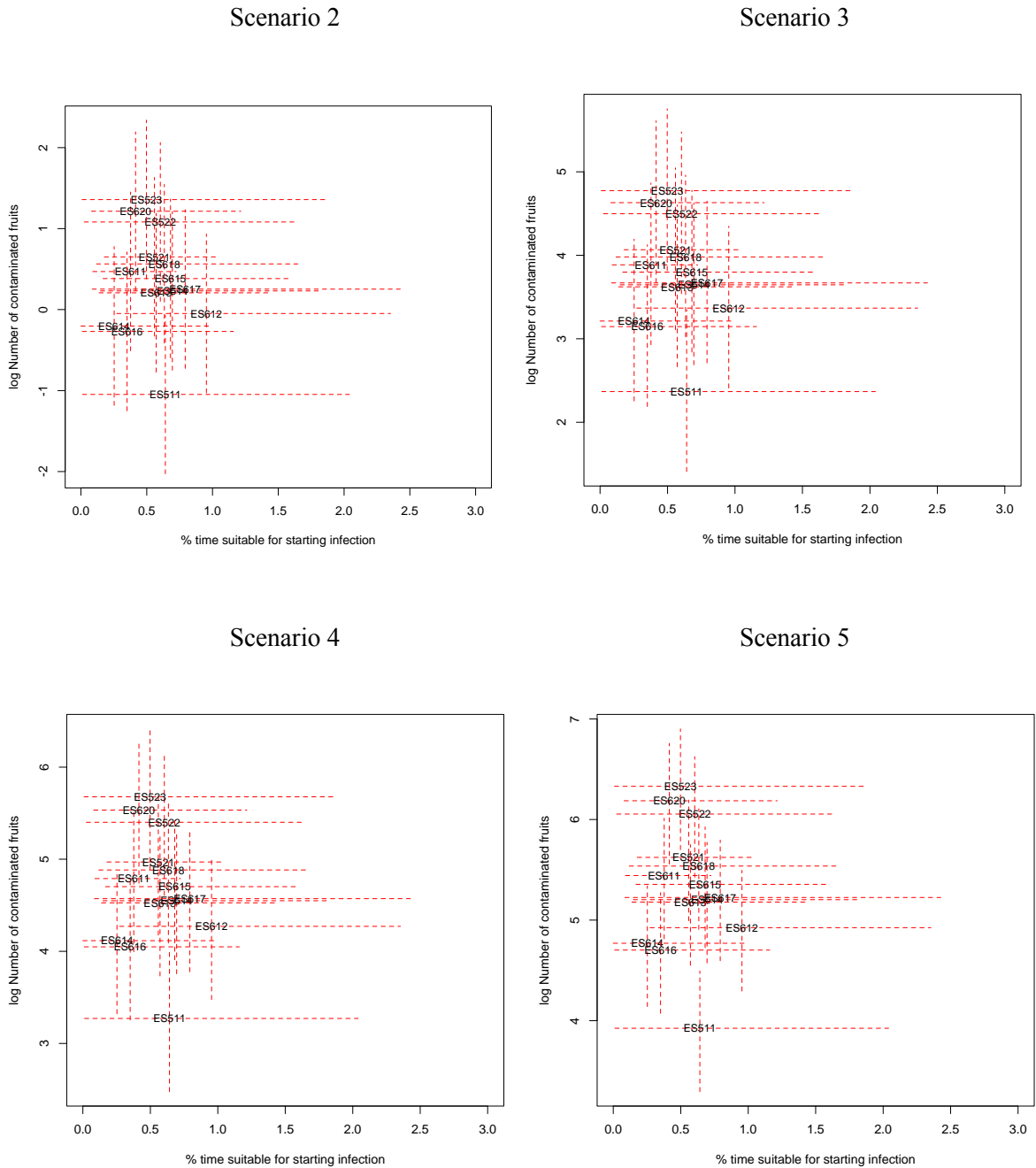
The proportion of imported citrus fruit going to packing houses in Spain is estimated at about 40 %, to retail at 40 % and to food processing (predominantly juice making) at 20 % (Dr M.A. Forner, IVIA, personal communication, December 2013). At the retailer level, there is some direct fruit waste, while the remaining fruit is sold to consumers and then wasted at the consumer level. There are then four alternative pathways for the imported fruit to become a potential source of inoculum of *P. citricarpa*, as follows.

Packing house pathway. Fruit is received and repacked for distribution centres for retail. All packing houses in Spain are in the citrus-growing area because they are associated with the local fruit production. Consequently, imported citrus fruit potentially affected by CBS going to packing houses comes in close proximity to the citrus orchards. The Panel assumed that fruit sent to packing houses is distributed between regions in proportion to the number of packing houses per region, based on regional and central government records for 2008. Data available on the proportion of fruit going to waste indicate a waste fraction of 3.3% as a central estimate (Gustavsson et al., 2011; WRAP, 2011 and 2012). This waste is brought to the open-air facilities located in the citrus growing area for solar drying and is later used for livestock feeding or bioethanol production (section 3.2.2.4).

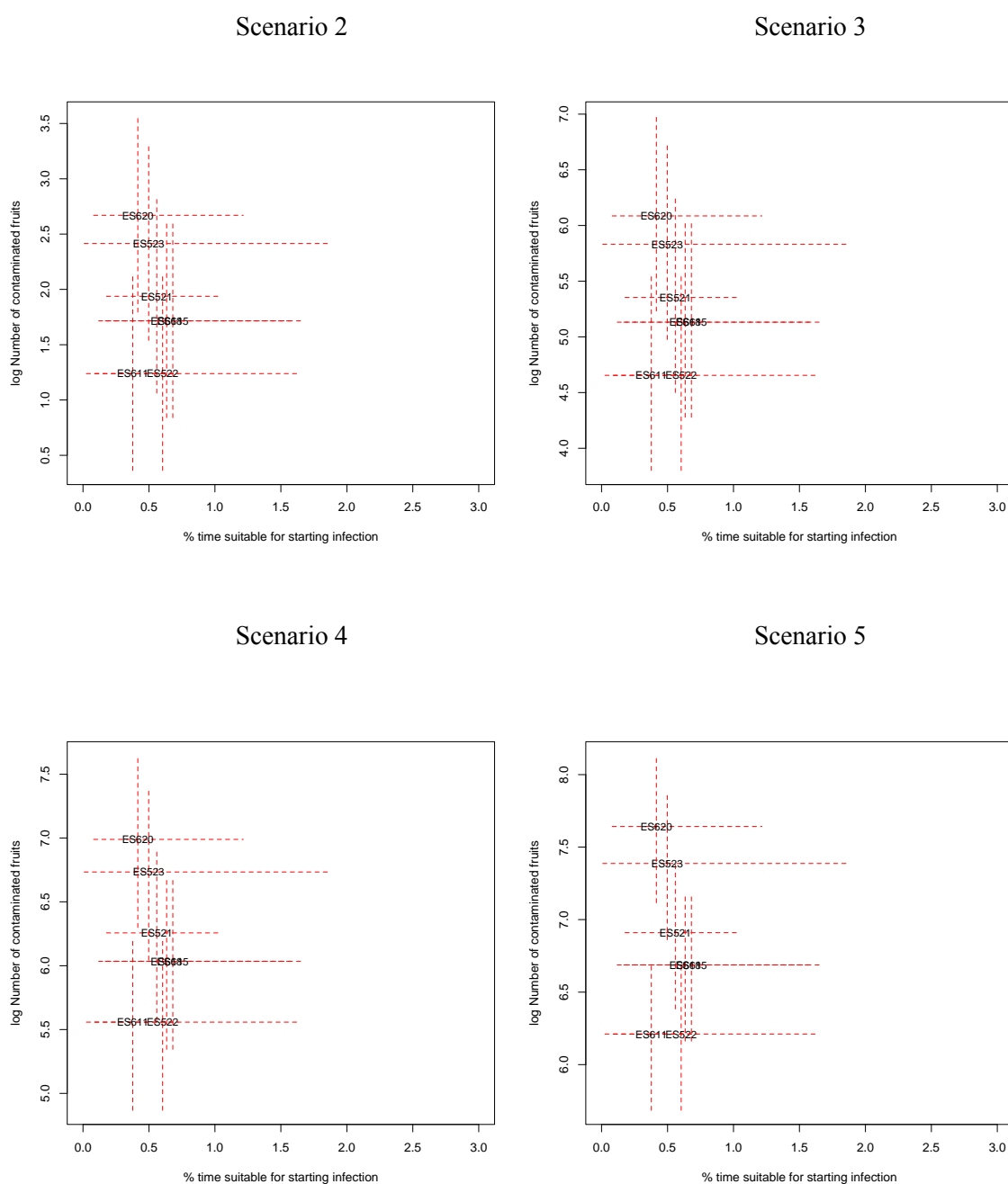
Retailer pathway. Fruit sent direct to retail (not via packing houses) is estimated to be distributed between regions in proportion to their populations, based on official population statistics for Spain. Based on data available (Gustavsson et al., 2011; WRAP, 2011 and 2012) a waste fraction of 2.25 % is considered. The wasted fruit is either sent to landfill or used for composting and the Panel assumed that 5 % of the disposed fruit is then exposed to air.

Consumer pathway. Consumers buying oranges from retail will dispose of peel and fruit that has gone bad before consumption, producing organic waste. Although much of the pulp is consumed, only a very small proportion of peel will be consumed (e.g. in marmalade or after grating) and consumers are likely to avoid using visibly affected peel. Therefore, focusing the model on the relevant part of the fruit (the peel), it is assumed that 100 % of the units purchased will eventually be discarded as waste. The wasted fruit is also either sent to landfill or used for composting and the Panel assumed that 5 % of the disposed fruit is then exposed to open air.

Fruit industry pathway. It is assumed that fruit used by the food industry is distributed in proportion to the number of citrus-processing plants in each region. After pressing the fruit for production of juice, all of the remainder is brought to the open-air facilities located in the citrus growing area for solar drying and is later used for livestock feeding or bioethanol production.



**Figure 17:** Log number of citrus fruits potentially infected by *Phyllosticta citricarpa* in the waste of packing houses located in Spanish provinces for four scenarios. Each Spanish province is identified by its NUTS3 code. Vertical bars indicate the range (min–max) of log number of infected fruits simulated by the pathway model using the most extreme combinations of parameter values. Horizontal bars indicate the range of time (%) suitable for splash dispersal and infection by pycnidiospores predicted for the considered provinces.



**Figure 18:** Log number of citrus fruits potentially infected by *Phyllosticta citricarpa* in the waste of fruit industries located in Spanish provinces for four scenarios. Each Spanish province is identified by its NUTS3 code. Vertical bars indicate the range (min–max) of log number of infected fruits simulated by the pathway model using the most extreme combinations of parameter values. Horizontal bars indicate the range of time (%) suitable for splash dispersal and infection by pycnidiospores for the considered provinces

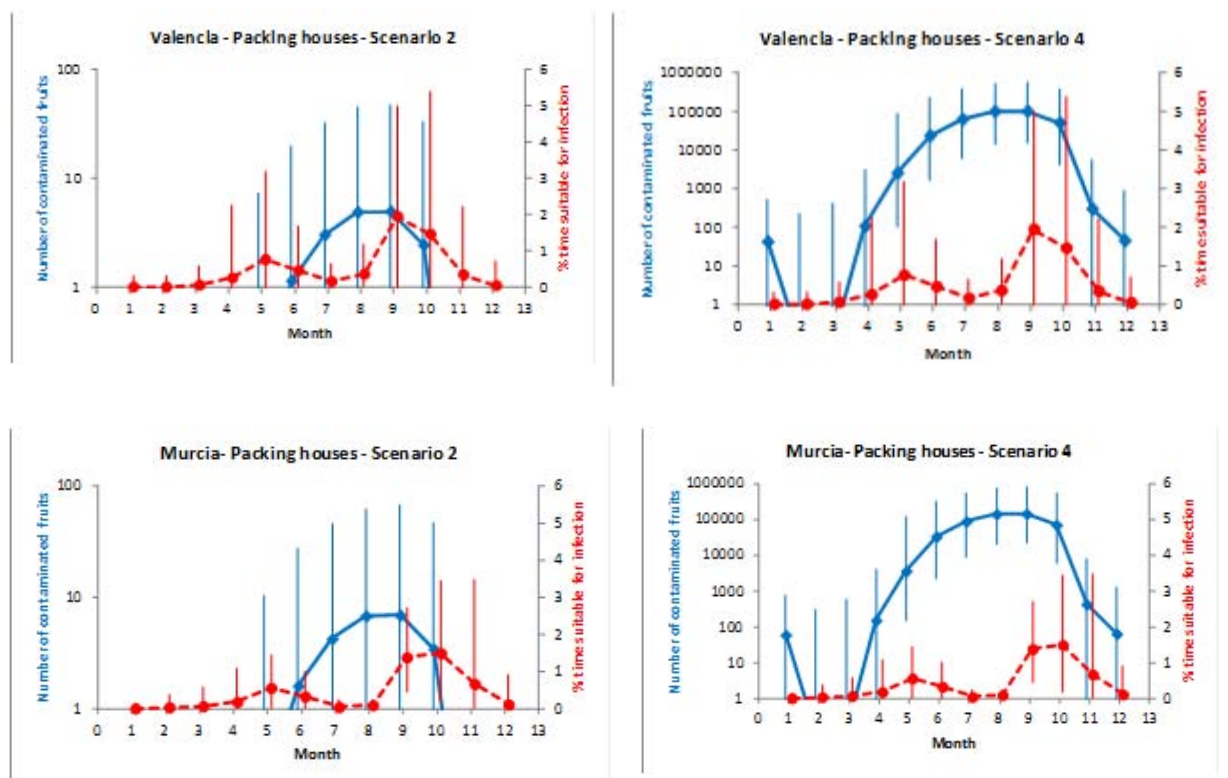
Thus, citrus waste from retailers, consumers and packing houses and fruit processing end up in the same locations at the same proximity to citrus orchards, although with different ways of disposal. Therefore, it was decided to consider them separately also because there are some mitigation options that apply only to the citrus-processing industry (section 4.1.1.7).



The risk of transfer of *P. citricarpa* from imported citrus fruit to a suitable host in the PRA area is a function of the volume of fruit (or citrus by-products) potentially infected by *P. citricarpa* coming into proximity with citrus production areas and the suitability of the local conditions for splash dispersal and subsequent infection by pycnidiospores (section 3.3.2.5). These two factors are then considered and depicted for different Spanish provinces under the different scenarios for regulation for the packing house fruit waste (Figure 17) and fruit industry (Figure 18) pathways.

When considering data from the packing house, while under scenario 2 (current regulation) the total number of waste fruit is very low (0–10 fruit), under scenario 3 (no regulation), the model simulates a considerable increase in the number of fruit, reaching values close 100.000 (log 5) for some provinces (Figure 17). Similarly when data from the fruit industry pathway are considered, the model also simulates a very important increase in the number of contaminated fruit when moving from scenario 2 to scenario 3 (Figure 18).

Since both environmental suitability for the transfer of *P. citricarpa* and the volume of CBS-affected fruit reaching pathway end points vary seasonally, monthly estimates of the percentage suitable time were also calculated for two representative NUTS3 regions in Spain, Valencia (ES 523) and Murcia (ES 620) (Figure 19). In both provinces, the period of time when infected fruit and citrus by-products could be exposed to open air coincides with the times of the season when the percentage of time suitable for splash dispersal and infection by *P. citricarpa* pycnidiospores is at its highest. This is further supported by the fact that most interceptions of *P. citricarpa* at EU borders are concentrated during the months most suitable for dispersal and infection by pycnidiospores (section 3.2.2.1, Figure 13).



**Figure 19:** Monthly values of number of citrus fruit potentially infected by *P. citricarpa* and weather conditions suitable for splash dispersal and infection by pycnidiospores in two Spanish provinces (Valencia and Murcia) for the packing house citrus waste under scenario 2 and 4. The dotted lines indicate the range of variations for each of the variables graphed

Results from the pathway modelling analysis were also compared with the cultivated citrus surface area existing in the Spanish provinces. In general, the highest amount of waste disposed fruit among all Spanish provinces is located in those provinces with the largest citrus cultivation area. For instance, in Valencia province, with a total of 106 841 ha of commercial citrus orchards (MAGRAMA 2013a), the pathway modelling analysis simulates that there will be a total of 289 infected fruits under scenario 2, and a total of 752 492 infected fruits under scenario 3, exposed to open air in close proximity to citrus plants. When considering only lemon, the most susceptible citrus species to CBS (Kotzé, 1981), Murcia is the province of Spain with largest area devoted to this production (23 768 ha, MAGRAMA 2013a). According to the simulation of the pathway modelling analysis, in Murcia the yearly total number of waste infected fruit exposed to open air is 487 fruit under scenario 2 and 1 271 082 fruit under scenario 3.

The pathway model indicates that, under current regulations (scenarios 1 and 2), the number of fruit potentially infected with *P. citricarpa* entering the citrus-growing regions of Spain from CBS-affected countries is likely to be small, in the order of zero to several dozen fruit per region. Thus, under current regulations, entry via the citrus fruit trade pathways is very unlikely, with low uncertainty, owing to the minor amounts of potential inoculum that may reach the trees from the small number of infected fruit moving along the pathway. This finding is of key relevance to the analysis of RROs.

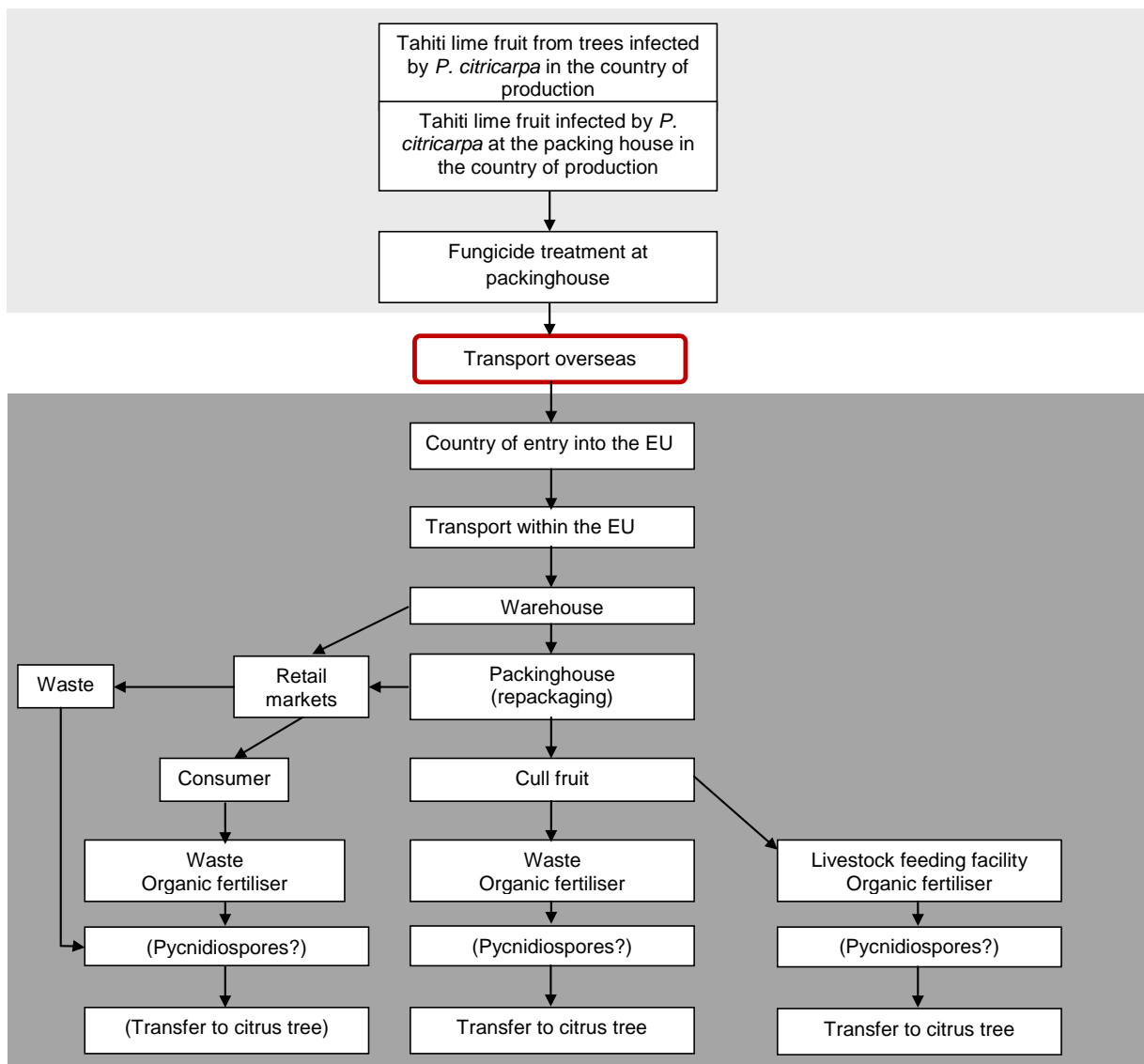
Since regulations currently targeted at *P. citricarpa* are not taken into account in the entry section of the pest risk assessment, only the results from scenarios 3–5 can be considered when assessing the likelihood of entry. Compared with scenarios 1 and 2, scenarios 3–5 all show major increases, by a factor of 10 000 or more (four or more orders of magnitude), in the potential for entry. The uncertainties quantified by the Panel within the model are substantial, but change these estimates by a factor of only 1–2 orders of magnitude and could be either positive or negative (see Appendix E).

The Panel concludes that, without current regulations, the number of citrus fruit potentially infected by *P. citricarpa* entering EU citrus-growing regions from CBS-affected countries and arriving close to citrus orchards in the pest risk assessment area is high. There is a good temporal overlap between the timing of entry of fruit and waste disposal in open air facilities located in citrus-growing areas and the prevalence of weather conditions suitable for rain splash and pycnidiospore infection. Of the four main pathways, the levels of exposure resulting from these pathways is considered to be highest for the citrus processing industry, followed by packing houses, consumers and the retail chain. In relating this conclusion to the qualitative rating according to the EFSA harmonised framework for pest risk assessment (EFSA PLH Panel, 2010), the rating descriptors for entry in Appendix A should be taken into account. For *P. citricarpa*, the likelihood of transfer is the key issue and, even though the volume moving along the pathway is high, this needs to be compared with other pests and other pathways and the Panel considers that the pest “*has some limitations for transfer to a suitable host in the risk assessment area*” and therefore concluded that the pathway should be assessed as moderately likely. Although the uncertainties taken into account in this quantitative model can be considered to be low, when the additional uncertainties outlined in Table 36 in Appendix E are taken into account, the Panel considers that the uncertainty score should be medium.

#### **3.2.4. Entry pathway II: Tahiti lime fruit (*Citrus latifolia*) commercial trade (without leaves and peduncles)**

A graphical pathway model illustrating the entry pathway of *P. citricarpa* with the commercial trade of Tahiti lime (*Citrus latifolia*) fruit, without leaves and peduncles, is shown in Figure 20.

Same reasoning as presented here for Tahiti lime fruit may apply to the trade of sour orange (*C. aurantium*) fruit, Sour orange fruit is used for marmalade and, although statistical data is not available on the import into the EU of this species, this trade is expected to be little.

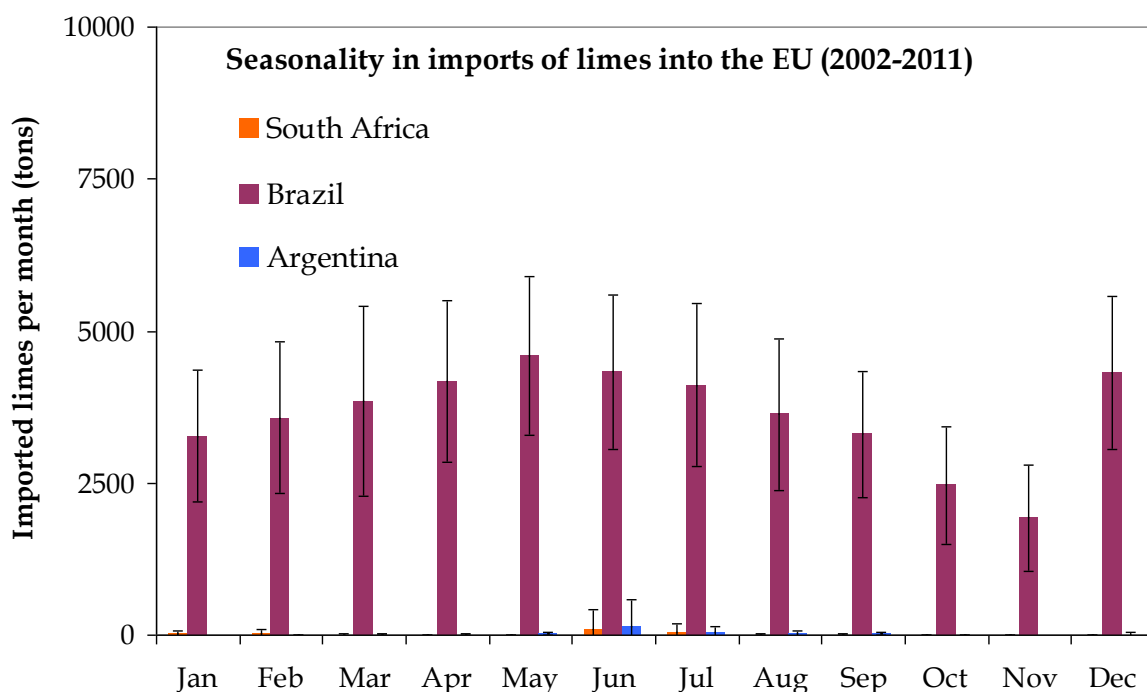


**Figure 20:** A graphical pathway model (pathway II) illustrating steps in the entry pathway of *Phyllosticta citricarpa* for the commercial trade in Tahiti lime (*Citrus latifolia*) fruit, without leaves and peduncles. The pathway starts in infested orchards in a country of origin outside the EU and ends with the transfer of spores of the pathogen to a host within the EU. The scheme is illustrative and departures from the depicted sequence may apply in some countries of origin and certain MSs of the EU depending upon the local characteristics of citrus production, trade and processing. For instance, currently there is import inspection but in the scenarios considered in this opinion, there is no inspection specifically for CBS

#### 3.2.4.1. Probability of association with the pathway at origin

Although confirmatory long term and area-wide field surveys are not available, Tahiti lime (*Citrus latifolia*) fruit are reported not to develop CBS symptoms under field conditions in Brazil, even in areas with high inoculum pressure by *P. citricarpa* (Baldassari et al., 2008). However, in this study conducted in Conchal (Sao Paulo), 2 out of the 11 *Phyllosticta* isolates obtained from peel of fruit of Tahiti lime were identified as *P. citricarpa* and induced CBS symptoms when inoculated in sweet orange fruit. The study did not include inoculations in Tahiti lime fruit.

The major exporter of lime fruit to the EU is Brazil, with limited seasonality in the trade (Figure 21). A total of ~ 435 000, ~ 2 600 and ~ 1 600 tonnes of fruits of Tahiti lime from Brazil, Argentina and South Africa, respectively, were imported into the EU territory between 2002 and 2011. Although *P. citricarpa* is present in these countries, no interceptions on Tahiti acid lime have been recorded in EU border inspections, confirming that no symptoms of CBS were detected on imported Tahiti lime fruit. However, since *P. citricarpa*, although without symptoms (see above), is able to colonise Tahiti lime fruit under natural conditions, the probability of association of the pathogen with the pathway at origin is rated as **likely** with **high** uncertainty owing to the limited amount of evidence available.



**Figure 21:** Seasonality in imports of limes into the EU from the three major exporting third countries (Brazil, South Africa and Argentina, 2002–2011). Error bars are standard deviations

#### 3.2.4.2. Probability of survival during transport or storage

Studies evaluating the survival of *P. citricarpa* in Tahiti lime fruit are not available. However, the same considerations made about the likely survival during transport or storage of *P. citricarpa* as latent mycelia present in asymptomatic fruits of other citrus species may also be applicable to Tahiti lime.

Therefore, the Panel considers that the probability of survival of the pathogen during transport or storage of Tahiti lime fruit is rated as **very likely**, with **high** uncertainty owing to the lack of evidence.

### 3.2.4.3. Probability of survival to existing pest management procedures

Field observations in Brazil indicated that *P. citricarpa* does not induce symptoms in Tahiti lime (Baldassari et al., 2009; Wickert et al., 2009, 2012). Consequently, studies evaluating the efficacy of fungicide sprays, cultural measures or post-harvest treatments for the control of CBS on this citrus species are not available. However, because *P. citricarpa* can survive as latent mycelia in asymptomatic fruits of other citrus species under existing CBS management procedures, it is also likely to survive in Tahiti lime fruit. Therefore, the pathogen is **very likely** to survive existing pest management procedures in Tahiti lime fruit. The level of uncertainty is **high**, owing to the limited information available.

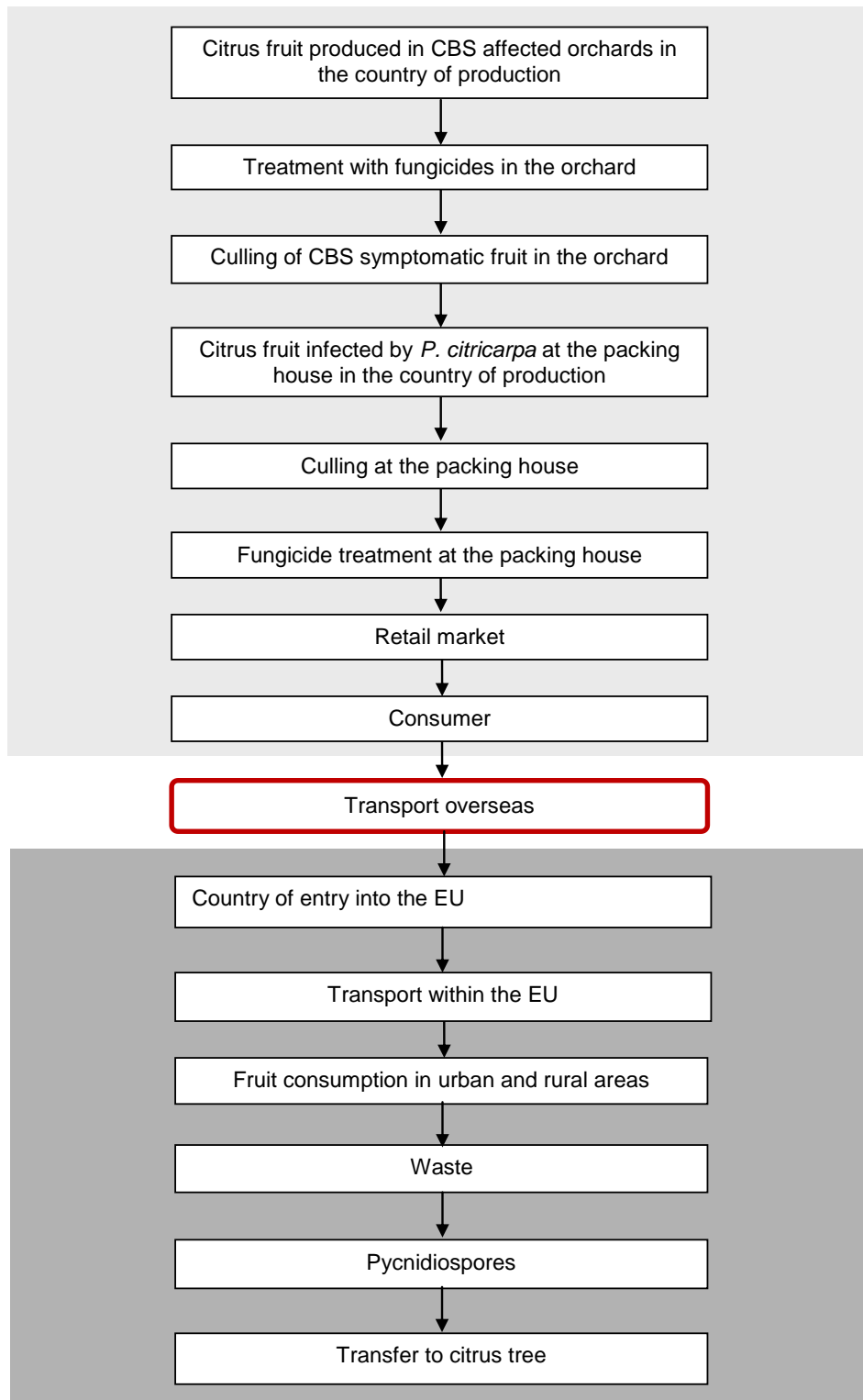
### 3.2.4.4. Probability of transfer to a suitable host

The pathogen can colonise Tahiti lime fruit under field conditions in Brazil (Baldassari et al., 2009), but there are no reports of symptom development or any reproduction of the pathogen on fruits of this citrus species. Nevertheless, it is not known whether CBS symptoms could develop or *P. citricarpa* could reproduce in harvested fruit of Tahiti lime after long storage periods or under waste disposal conditions outdoors. The pathogen could transfer to a suitable host only if it were able to sporulate on fruits or peel of Tahiti lime discarded in the vicinity of citrus trees in the pest risk assessment area, provided that environmental conditions are favourable for spore production, release, dissemination and subsequent infection (see section 3.3.2).

The Panel considers that the probability of transfer is rated as **very unlikely**, with **high** uncertainty owing to the lack of studies on this issue.

### 3.2.5. Entry pathway III: citrus fruit import by passenger traffic

This is a pathway of lesser importance than the commercial fruit pathway, but could still result in pathogen entry. There is generally a lack of information on the volumes of citrus fruit imported by passengers, the probability of survival during transport and the likelihood of interception at points of entry if border inspection was in place. A graphical representation of this pathway is given in Figure 22.



**Figure 22:** A graphical pathway model illustrating steps in the entry pathway of *Phyllosticta citricarpa* with citrus fruit imported by passengers. The pathway starts in CBS-affected orchards in a country of origin outside the EU and ends with the transfer of spores of the pathogen to a host within the EU. The scheme is illustrative, and departures from the depicted sequence may apply in some countries of origin or certain EU MSs owing to local characteristics of citrus production, trade and processing. For instance, in current practice, there is import inspection, but, in the scenarios considered in this opinion, there is no inspection specifically for CBS

### 3.2.5.1. Probability of association with the pathway at origin

Citrus fruit brought into the EU by passengers can be infected by *P. citricarpa* if passengers arrive from countries where the disease is present. In these countries, citrus fruit produced for the local market or picked from backyard trees are likely to have a higher incidence of CBS than fruit produced for export markets. Therefore, citrus fruit bought by travellers into the EU is more likely to be infected with *P. citricarpa* than commercially imported fruit. The presence and severity of CBS in these countries is variable, and this variability will affect the probability of association with the pathway at the origin.

Based on the above considerations, the Panel concludes that the probability of association with the pathway at the origin is rated as **likely**, with **medium** uncertainty owing to the lack of information on the volume and frequency of the movement along the pathway.

### 3.2.5.2. Probability of survival during transport or storage.

Experimental studies on *P. citricarpa* survival during transport by passengers appear to be lacking, but it can be assumed that, if the pathogen can survive commercial transport and storage, it is very likely that it will survive the conditions of transport of individual passengers.

Based on this, the Panel considers that the probability of survival during transport or storage is rated as **very likely**, with **low** uncertainty, despite the lack of information, by analogy with the commercial fruit pathway.

### 3.2.5.3. Probability of survival to existing pest management procedures

Inspections to see whether passengers carry citrus fruit with them when arriving at EU airports from countries where CBS is present are not systematic. There does not seem to be information available about how frequently passengers carry citrus fruit when arriving into the EU from CBS-infected third countries and how likely it is that such passengers will be identified, so that pest management procedures could potentially be applied.

Data on citrus fruit interceptions on individual international passengers are available from two regions of Australia (Central East Region: 8557 citrus fruit seized, Jan 2010–Mar 2011; South Eastern Region: 4892 citrus fruit seized, Jan 2010–Apr 2011; Australian Government, 2011). Considering that most international passengers arriving in Australia fly to these Central/South Eastern Regions, and since there are about 2 million international passengers per month (Australian statistics; this would roughly imply 1 million incoming passengers), a conservative estimate is that about one passenger out of 1 000 carries one citrus fruit.

The figure can be considered as a low estimate if substantial numbers of international passengers fly to Australian airports from outside the Central/South Eastern regions and also taking account of the fact that some citrus fruit may not be noticed. However, only some of this citrus fruit would be affected by CBS because not all passengers carrying fruit arrive from countries where *P. citricarpa* is present.

Based on this information, the Panel considers that the probability of surviving pest management procedures is rated as **very likely**, with **low** uncertainty, despite the lack of information on this pathway, by analogy with the commercial fruit pathway.

### 3.2.5.4. Probability of transfer to a suitable host

The probability that CBS-affected citrus fruit imported by passengers may then transfer the pathogen to a suitable host is influenced by the proportion of passengers that:

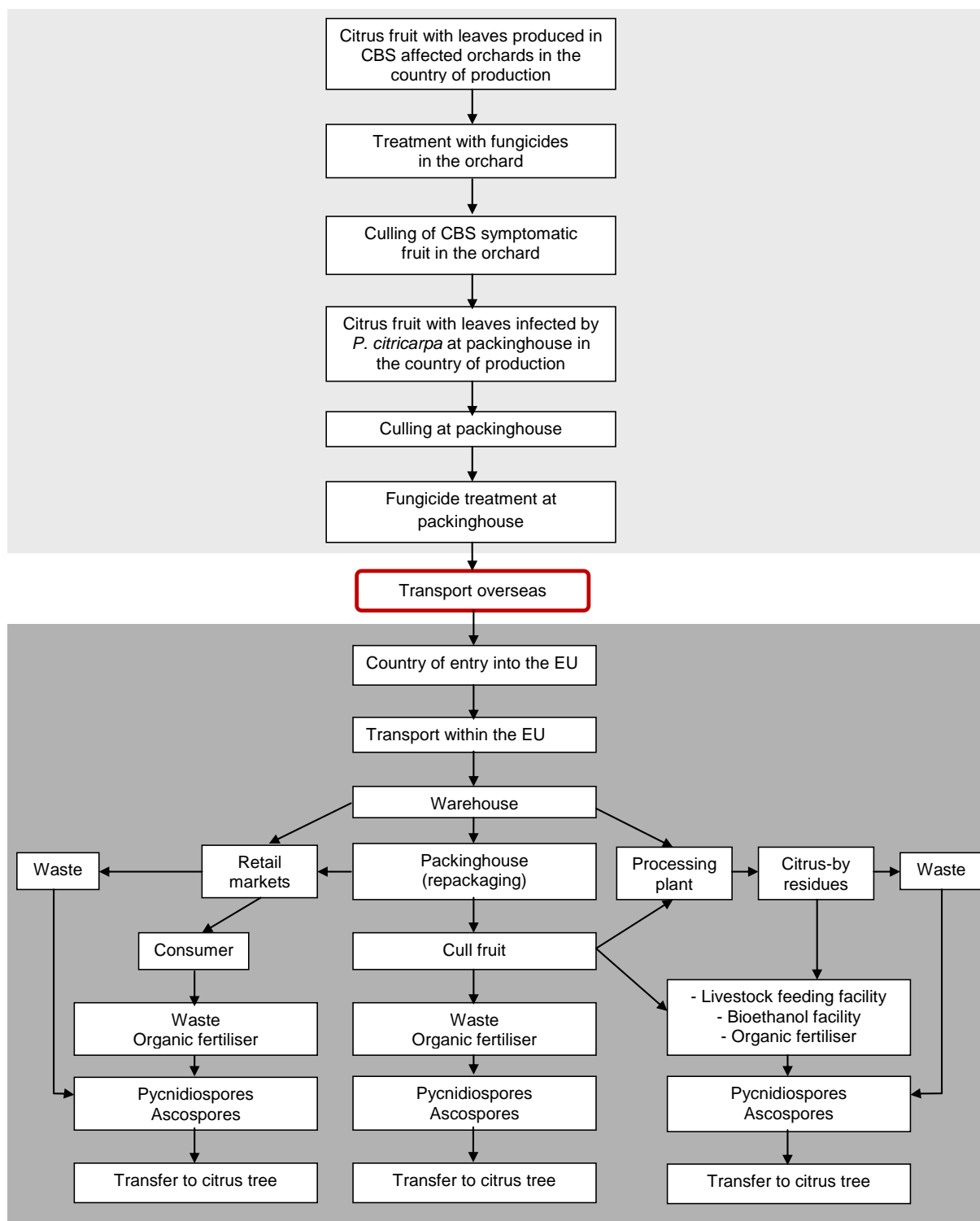
- travel from an area where CBS is present to a citrus-producing EU country

- carry CBS-affected citrus fruit bearing pycnidiospores, which can then be splash dispersed onto citrus trees
- discard citrus peel and fruit waste in proximity to citrus trees (also via household waste or landfill)
- arrive during a period with environmental conditions potentially conducive to infection.

The Panel considers that the probability of transfer of *P. citricarpa* to a suitable host in the risk assessment area by passengers discarding citrus fruit near fruit trees is rated as **unlikely**, with **medium** uncertainty owing to the lack of information on the likelihood that the above-mentioned events will take place.



### 3.2.6. Entry pathway IV: citrus fruit with leaves and peduncles in commercial trade



**Figure 23:** A graphical pathway model illustrating the entry pathway of *Phyllosticta citricarpa* with commercial trade in citrus fruit with leaves and peduncles. The pathway starts in CBS-affected orchards in a country of origin outside the EU and ends with the transfer of spores of the pathogen to a host within the EU. The scheme is illustrative, and departures from the depicted sequence may apply in specific countries of origin or specific MSs of the EU, depending upon local characteristics of citrus production, trade and processing. For instance, in current practice, there is import inspection but, in the scenarios considered in this opinion, there is no inspection specifically for CBS

A graphical representation of the pathway of commercial trade of citrus fruit with leaves and peduncles is shown in Figure 23.

Although importation from third countries of citrus fruit with leaves is currently prohibited by EU legislation, EU countries have made a number of interceptions of consignments of citrus fruit with leaves originating from third countries over the last years (Europhyt data). For example, citrus fruit with leaves have been intercepted in consignments from Bangladesh to Denmark (2007); from Cameroon to Switzerland<sup>12</sup> (2012); from the Dominican Republic to the United Kingdom (2004); from Lebanon to Denmark (2000), France (2001) and the United Kingdom (no year given); from Morocco to the Netherlands (2000); from Pakistan to Germany (2009) and the United Kingdom (no year given); from Sri Lanka to Switzerland (2011); from Thailand to Denmark (no year given), Germany (2006 and 2010), the Netherlands (2000), the United Kingdom (2005 and 2006), Sweden (2000) and Switzerland (2011); from Turkey to Austria (2001); and from Vietnam to the Czech Republic (2009 and 2010), Germany (2006) and Switzerland (2011).

This number of interceptions of commercially traded citrus fruit with leaves should be considered as a conservative estimate because in many cases Europhyt interceptions of citrus “for other reasons including leaves” do not provide the specific reason for the interception, whereas the list above includes only interceptions that specifically mentioned citrus leaves. Moreover, 4 out of the 20 (20 %) above-mentioned interceptions were made by Switzerland, a country whose imports of citrus fruit are much smaller than those of many EU MSs.

#### 3.2.6.1. Probability of association with the pathway at origin

The probability of association with the pathway of citrus fruit with leaves (commercial trade) is similar to that for citrus plants for planting and citrus commercial fruit.

Therefore, taking into account the assessment of entry by these pathways, the pest is **likely** to be associated with the pathway at origin with **medium** uncertainty. In addition, there are the following considerations:

- No trade data are available on the volume of citrus fruit with leaves and peduncles imported into the EU from countries where *P. citricarpa* is present. Nonetheless, the Panel considers that, owing to consumer preference for the consumption of citrus fruit still bearing fresh leaves (Li et al., 2013), there would be a non-negligible volume of citrus fruits with leaves, a fraction of which would be imported into the EU citrus-growing regions.
- Uncertainties include (1) the volume of citrus fruit with leaves that would be imported by EU citrus-growing countries (directly or indirectly through redistribution from non-citrus-growing EU countries) in the absence of the current EU legislation forbidding such imports, (2) the number of imported citrus fruit with leaves with CBS infection and (3) the effectiveness of any potential inspections at the EU points of entry to detect CBS-infected citrus fruit with leaves.

#### 3.2.6.2. Probability of survival during transport or storage

Since commercial citrus fruit with leaves is stored and transported under conditions that are not stressful or damaging for leaf tissues (so as to preserve citrus leaves in fresh condition), the probability that *P. citricarpa* will survive transport and storage of citrus fruit with leaves, exported from countries where *P. citricarpa* is present into the EU, is rated as **very likely**, with a **low** level of uncertainty.

#### 3.2.6.3. Probability of survival of existing pest management procedures

As noted for the plants for planting pathway:

<sup>12</sup> Switzerland is not a EU MS but it records its interceptions in the Europhyt database.

- The application of fungicides in citrus orchards can diminish disease incidence and severity, but does not eradicate infections.
- Visual inspections are most likely to miss latently infected (asymptomatic) fruit and leaves.
- CBS symptoms on fruit are variable and they are rarely observed on leaves, with the exception of lemon leaves; in addition, symptoms may be misidentified during visual inspection, as lesions are similar to those produced by other citrus pathogens.

Therefore, the Panel concludes that it is **very likely** that *P. citricarpa* will survive existing management procedures and remain undetected on commercial citrus fruit with leaves. The uncertainty is considered **medium** owing to the lack of data on the volume of citrus fruit with leaves that could be potentially imported into the EU from infested third countries.

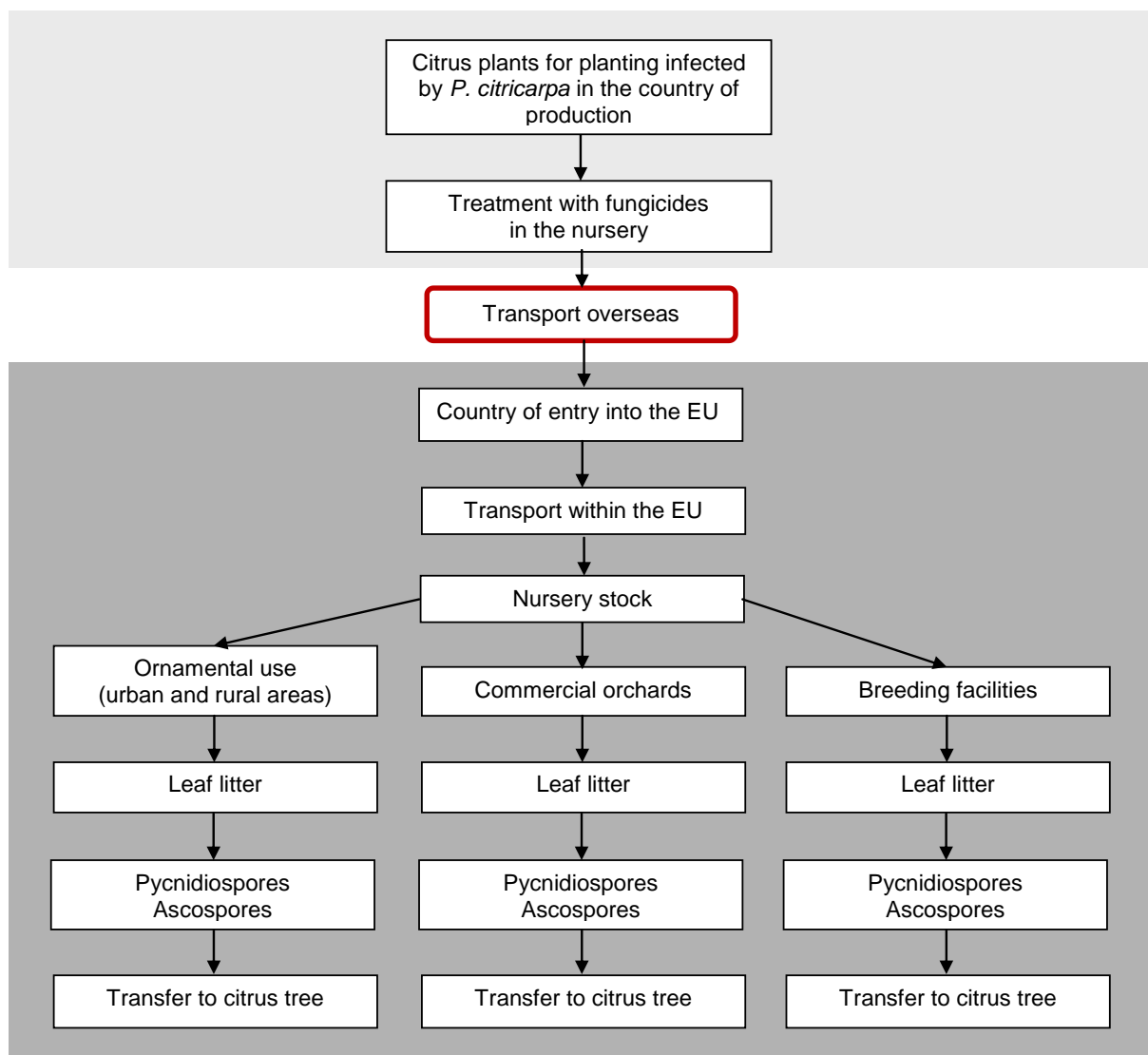
#### 3.2.6.4. Probability of transfer to a suitable host

As noted above for the two main CBS pathways, discarded citrus fruit, peel or other citrus by-products with leaves and peduncles, derived from packing houses, processing plants, fresh fruit markets, households, etc., and their management, would pose a risk of transfer of the pathogen to a suitable host. This is because:

- The long (2–12 months) quiescent period of CBS (McOnie, 1967; Kellerman and Kotzé, 1977; Kotzé, 1981; Aguiar et al., 2012) in many cases would be longer than the time needed for transport of the commodity.
- CBS symptoms on citrus fruit are variable and they are rarely observed on leaves with the exception of lemon leaves; in addition, symptoms can be easily confused with those caused by other pathogens.
- Commercial citrus fruit with leaves and peduncles is likely to be distributed throughout the EU, including citrus-growing regions.
- The latent mycelium present in citrus leaves, if leaves are then improperly discarded, can then develop pycnidia with splash-dispersed pycnidiospores and pseudothecia with wind-disseminated ascospores that can enable the organism to enter new areas.

Thus, in the absence of the current legislation, the pathogen would be **likely** to be able to transfer by various means (wind, water (rain or irrigation), insects) to susceptible host plants, with a **medium** level of uncertainty deriving from the lack of data on the volume of the waste of citrus fruit and leaves that could potentially be disposed in the vicinity of susceptible hosts in the risk assessment area.

### 3.2.7. Entry pathway V: citrus plants for planting



**Figure 24:** A graphical pathway model illustrating the entry of *Phyllosticta citricarpa* with citrus plants intended for planting. The pathway starts with infected plants in a country of origin outside the EU and ends with the transfer of spores of the pathogen to a host within the EU. The scheme is illustrative, and departures from the depicted sequence may apply in specific countries of origin or specific MSs of the EU, depending upon local characteristics of citrus production. For instance, in current practice, there is import inspection, but, in the scenarios considered in this opinion, there is no inspection specifically for CBS.

The trade in citrus plants for planting (Figure 24) is assumed to be a very important potential pathway for the entry of *P. citricarpa* into new areas (Kiely, 1948; Wager, 1949; Whiteside, 1965; Kotzé, 1981; Cortese et al., 2004; MacLeod et al., 2012). This is because citrus plants are normally propagated vegetatively by grafting onto rootstocks. Aerial parts of budwood, scions, rootstocks and nursery plants of citrus species in general may be infected with *P. citricarpa* without or with very few symptoms (see section 3.1.1.2). Nevertheless, there are no authenticated records of CBS introductions into new areas through plants for planting.

#### 3.2.7.1. Probability of association with the pathway at origin

*P. citricarpa* is most likely to be present in citrus propagating material from areas of its current distribution as mycelium in latently infected leaves. Under suitable conditions, *P. citricarpa*

pseudothecia and pycnidia and, in turn, ascospores and pycnidiospores are likely to develop on shed infected citrus leaves, thus making the citrus plant for planting pathway the most effective means of spreading the disease to new areas (Kotzé, 1981).

There are no readily available data on the prevalence of *P. citricarpa* in citrus nurseries in countries where the pathogen is currently distributed. Similarly, there are no detailed data on the location of citrus nurseries in those countries. However, in agreement with MacLeod et al. (2012), the Panel considers that, particularly if citrus nurseries are located near citrus orchards infected by *P. citricarpa*, then it is likely that there will be a high prevalence of the pathogen in citrus planting material for propagation purposes.

Foliar lesions of CBS are rare, especially in young vigorous plants (Kotzé, 1981). Therefore, culling in citrus nurseries in CBS-affected countries is not likely to lead to removal and destruction of seedlings with latent infections, as only symptomatic seedlings are likely to be detected.

The Panel considers it to be highly likely that infected citrus plant propagation material will be asymptomatic. This is because CBS does not generally appear on trees until they are over 10 years old, and it has been known to remain latent for even longer periods (Whiteside, 1965; Kotzé, 1981). In addition, in most varieties, symptoms on leaves are generally absent or very limited, with the exception of lemon (Kotzé, 1981) (see sections 3.1.1.2 and 3.1.1.3 for more details).

As the import of citrus plants into the EU is forbidden, no trade data are available on the volume of citrus plant propagation material imported to the EU from countries where *P. citricarpa* is present. Nonetheless, in agreement with MacLeod et al. (2012), the Panel considers that, owing to the large citrus-growing area in southern EU MSs (Table 6) and with a yearly rate of citrus tree renewal of 7.5 % (Aubert and Vullin, 1997), in the absence of such prohibition high volumes of citrus plant propagation material would be potentially imported into the EU.

Therefore, in agreement with MacLeod et al. (2012), the Panel considers that the pest is **likely** to be associated with the pathway at origin taking into account factors such as cultivation practices and the treatment of consignments, and with **medium** uncertainty, because of the lack of trade data of citrus planting material and on the structure of the trade network for citrus plants for planting in the EU.

#### 3.2.7.2. Probability of survival during transport or storage

Commercial citrus plant propagation material, as is the case with all live plants, is stored and transported under conditions that are not stressful or damaging for plant tissues (and thus also not stressful to the latent mycelium of the pathogen). Therefore, and in agreement with MacLeod et al. (2012), the probability that *P. citricarpa* will survive transport and storage of citrus plant propagation material originating in infested third countries and imported into the EU is assessed as **very likely**, with a **low** level of uncertainty.

#### 3.2.7.3. Probability of survival existing pest management procedures

In agreement with MacLeod et al. (2012), the Panel considers that:

- The application of fungicides in citrus orchards can reduce disease incidence and severity, but it does not eradicate infections. The quiescent period of CBS in affected leaves is likely to be of sufficient duration to extend beyond the time in transit. Visual inspections are most likely to miss asymptomatic citrus plant propagating material infected by *P. citricarpa*.
- If CBS symptoms are present on leaves, they are likely to be relatively similar to those caused by other citrus pathogens (e.g. *Alternaria* spp., *Mycosphaerella citri* Whiteside, *Septoria* spp.) and thus might be misidentified during culling.

- Laboratory testing is needed to reliably detect and identify *P. citricarpa* on citrus plant propagating material (see section 3.1.1.3).

Therefore, the Panel, in agreement with MacLeod et al. (2012), concludes that it is **very likely** that *P. citricarpa* will survive existing management procedures and remain undetected on citrus plant propagating material. Because of the difficulties in identifying CBS symptoms, the uncertainty for this rating is considered **low** despite the lack of published studies on the application of fungicides to control CBS in nurseries where *P. citricarpa* is present.

#### 3.2.7.4. Probability of transfer to a suitable host

With regard to the potential distribution of the imported citrus plants for planting throughout the risk assessment area the Panel considers that:

- Citrus species are extensively grown in EU southern MSs in orchards (see Table 6), in nurseries for production of plant propagation material, as well as in private and public gardens and as ornamentals. In urban areas, citrus trees are also grown along streets and in squares.
- Lemon (*C. limon*), which is considered the citrus species most susceptible to *P. citricarpa* and usually the first to be affected when CBS outbreaks occur in new areas (Kotzé, 1981), is widely grown both in rural and urban regions, covering 63 000 ha—about one-eighth of the total area cultivated with citrus in the EU.
- Citrus plant propagation material potentially imported into the EU would most probably be distributed first to nurseries for planting/grafting and subsequently to orchards, public and private gardens, in both rural and urban areas in the citrus-growing EU MSs.

Therefore, in agreement with MacLeod et al. (2012), the Panel concluded that, if imported, citrus plant propagation material would be distributed **moderately widely** throughout the risk assessment area, with a **low** level of uncertainty.

With regard to the ability of the pathogen to be transferred from the imported plants for planting to susceptible hosts grown in the citrus-producing EU MSs, MacLeod et al. (2012) considered that:

- Although nurseries will tend to grow young citrus trees (after grafting or budding) for 1–3 years before selling and distributing them to customers, CBS has a long quiescent period (2–12 months) and infected leaves (with the exception of lemon leaves) rarely show symptoms during their lifespan (up to about years).
- Despite the latent presence of the pathogen in citrus plant propagating material, nurseries provide favourable environmental conditions (high relative humidity and frequent wetting and drying of leaf litter as a result of irrigation) for the pathogen to produce pycnidiospores and/or ascospores which can be transferred by natural means to susceptible host plants grown nearby.
- If nurseries use infected citrus rootstocks, budwood or scions as propagation material, the pathogen is very likely to be transferred by human assistance to (and infect) susceptible hosts grown at great distances from the nursery.

The Panel also agrees that the intended use of the commodity would **very likely** aid transfer to a suitable host or habitat, with a **low** level of uncertainty because:

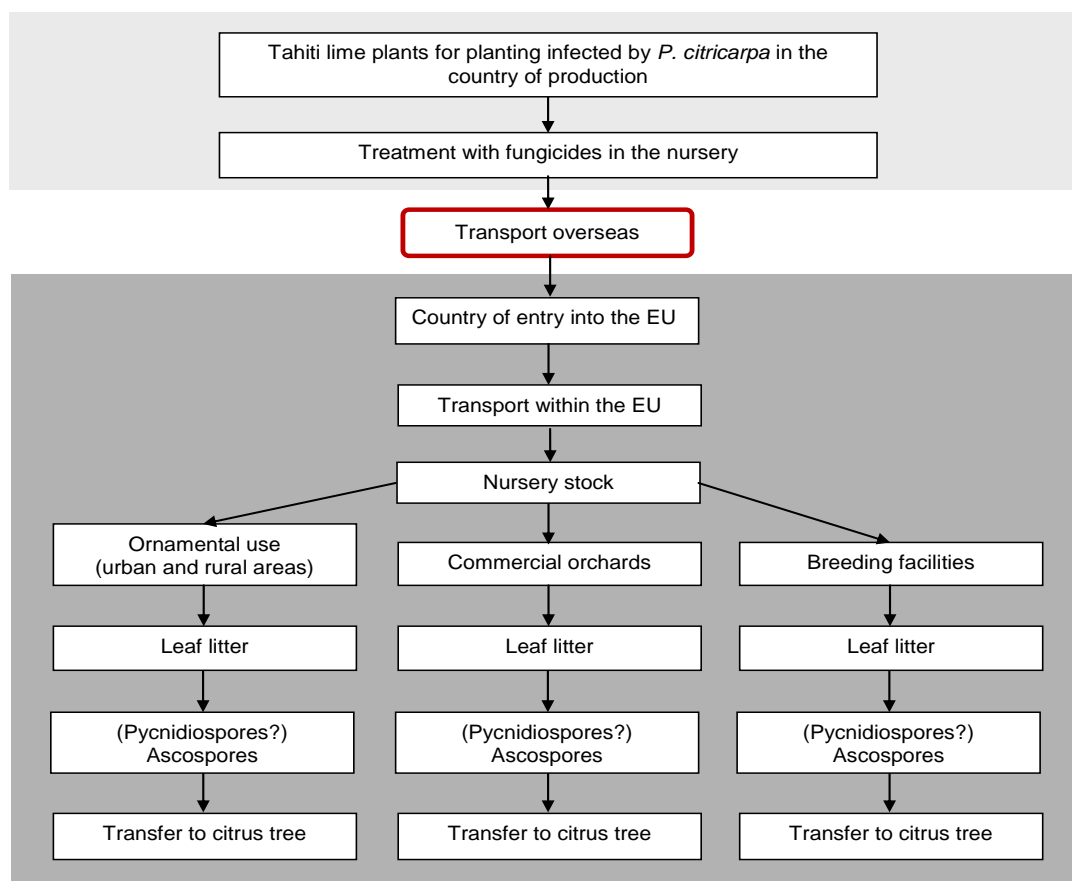
- The intended use of citrus plant propagating material is planting (rootstocks) or grafting (scions, budwood).

- If citrus plant propagating material is infected by *P. citricarpa*, then there will be the opportunity for the pathogen either to infect directly the host plants (if infected budwood/scions are grafted onto citrus trees grown in the risk assessment area) or to be transferred by both natural means and human assistance from infected to susceptible host plants grown in citrus orchards, nurseries and private and public gardens.
- Spread of the pathogen is possible in various ways, naturally through wind and water-splash dispersal, but also with human assistance via infected scions and budwood.
- Improper management of leaf litter in CBS-affected nurseries may also result in transfer of the pathogen to healthy citrus hosts nearby, because the pathogen can produce ascospores and pycnidiospores on leaf litter, which can be spread by wind, rain or irrigation water.

The Panel therefore agrees with the conclusions by MacLeod et al. (2012) that the pest is **very likely** to be able to transfer from the pathway to a suitable host or habitat, with a **low** level of uncertainty.

### 3.2.8. Entry pathway VI: Tahiti lime (*Citrus latifolia*) plants for planting

A representation of the Tahiti lime plants for planting pathway is given in Figure 25.



**Figure 25:** A graphical pathway model illustrating the entry pathway of *Phyllosticta citricarpa* with Tahiti lime (*Citrus latifolia*) plants intended for planting. The pathway starts with infected plants in a country of origin outside the EU and ends with the transfer of spores of the pathogen to a host within the EU. The scheme is illustrative and departures from the depicted sequence may apply in specific countries of origin or specific EU MSs, depending upon local characteristics of citrus production, trade and processing. For instance, in current practice, there is import inspection but, in the scenarios considered in this opinion, there is no inspection for CBS

#### 3.2.8.1. Probability of association with the pathway at origin

In a study conducted in Conchal, Sao Paulo (Brazil), Baldassari et al. (2009) identified two isolates of *P. citricarpa* from a total of seven *Phyllosticta* isolates obtained from Tahiti lime leaves. In addition, ascospores of *P. citricarpa* formed in Tahiti lime leaves were captured using a wind tunnel. In other studies, the population genetics of *Phyllosticta* in Tahiti lime were characterised in two regions in Brazil, Estiva Gerbi/Conchal (Sao Paulo) and Itaborai (Rio de Janeiro) (Wickert et al., 2009, 2012). Leaves were collected from 24 different Tahiti lime trees in each region to obtain one *Phyllosticta* isolate per plant. In addition, 40 leaves per tree were collected from three different trees in each region to obtain 24 *Phyllosticta* isolates from the same plant. A total of 208 *Phyllosticta* isolates were studied. All isolates from Itaborai were identified as *P. capitalensis*, but 8 out of the 18 *Phyllosticta* isolates from Estiva Gerbi inoculated in sweet orange fruit induced CBS symptoms and were identified as *P. citricarpa* based on their morphological and molecular characteristics. Since these studies did not describe how the sampling was conducted, and in particular from which plants and locations the eight *G. citricarpa* isolates were collected, it is not possible to determine precisely the prevalence of *P. citricarpa* in Tahiti acid lime leaves in Brazil. Despite the limited temporal and geographical range of these studies, these results clearly indicate that *P. citricarpa* can colonise and reproduce in Tahiti lime leaves.

Therefore, the pathogen is **likely** to be associated with the pathway at origin, with a **high** level of uncertainty because of the variation in disease prevalence in different regions and the lack of information on this pathway.

#### 3.2.8.2. Probability of survival during transport or storage

Currently, there is no trade in citrus plants for planting imported from third countries into the EU, so the probability of survival of *P. citricarpa* in infected Tahiti lime plants cannot be quantified. However, since the pathogen can colonise Tahiti lime leaves and citrus plants for planting are sold with leaves, there is no reason to consider that the pathogen cannot survive during transport or storage.

This translates into a **very likely** survival during transport or storage, with a low uncertainty.

#### 3.2.8.3. Probability of survival existing pest management procedures

Field trials for the control of *P. citricarpa* on Tahiti lime are not available. However, since *P. citricarpa* can survive under existing management procedures commonly applied to other citrus species, it is also likely to survive in Tahiti lime. Foliar symptoms of CBS are rare in most citrus species, and have been not reported in Tahiti lime. Thus, there is a **very high** probability of the pathogen remaining undetected as latent mycelia in asymptomatic Tahiti lime leaves during potential visual inspection, with **high** uncertainty owing to the lack of studies.

#### 3.2.8.4. Probability of transfer to a suitable host

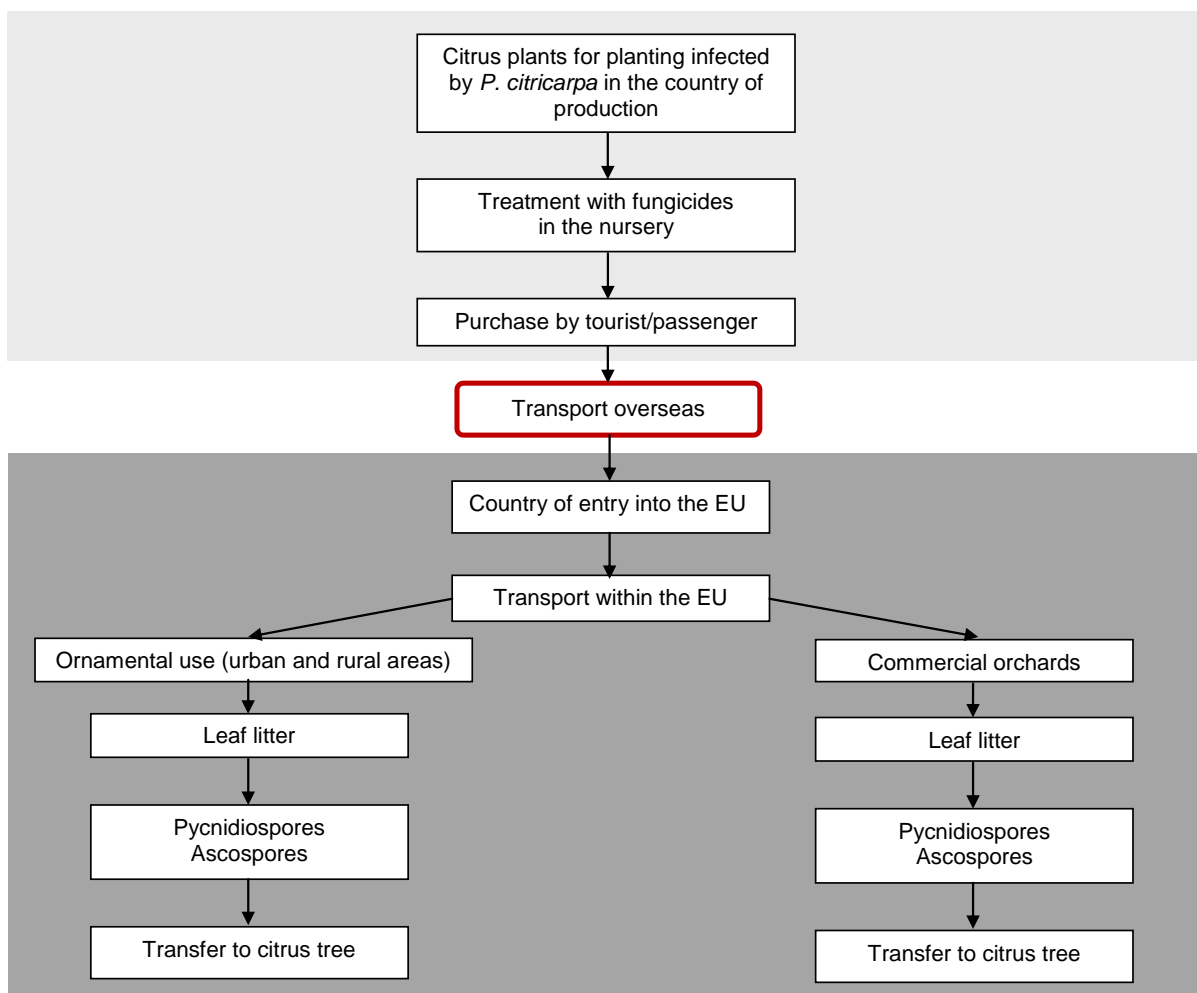
The pathogen can colonise Tahiti lime leaves and reproduce on them forming wind-borne ascospores (Baldassari et al., 2009; Wickert et al., 2009, 2012). If Tahiti lime plants carrying leaves colonised by *P. citricarpa* were planted in the pest risk assessment area, ascospores may be formed on these leaves after falling onto the orchard floor. Once mature, ascospores may be released and disseminated relatively long distances, infecting leaves and fruits of nearby susceptible citrus trees in the area. However, this chain of events would occur only if environmental conditions in the pest risk assessment area were conducive to pseudothecia production, ascospore maturation, release, dissemination and subsequent infection.

Nonetheless, by analogy with the citrus plants for planting pathway, the probability of transfer to a suitable host is assessed by the Panel as **very likely**, with a **high** uncertainty owing to the lack of information on the above-mentioned events.



### 3.2.9. Entry pathway VII: citrus plants for planting import by passenger traffic

As stated above for the pathway citrus plants for planting (commercial trade), infected citrus plants for planting can be a very important potential pathway for entry of *P. citricarpa* into new areas. If passengers imported scions to be used in the risk assessment area as rootstocks or grafting material (scions, budwood), and if such material were infected by *P. citricarpa*, there is the potential for the pathogen to enter the EU. A graphical presentation is given in Figure 26.



**Figure 26:** A graphical pathway model illustrating the entry pathway of *Phyllosticta citricarpa* with citrus plants intended for planting and imported by passengers. The pathway starts with infected plants in a country of origin outside the EU and ends with the transfer of spores of the pathogen to a host within the EU. The scheme is illustrative, and departures from the depicted sequence may apply in specific countries of origin or specific EU MSs, depending upon local characteristics of citrus production. For instance, in current practice, there is import inspection but, in the scenarios considered in this opinion, there is no inspection specifically for CBS

#### 3.2.9.1. Probability of association with the pathway at origin

The probability of association with the pathway at origin is similar to the citrus plants for planting pathway (commercial trade). The pest is thus **likely** to be associated with the pathway at origin, with **high** uncertainty related to the likelihood that passengers will decide to import citrus propagating material on their own without going through the commercial pathway.

### 3.2.9.2. Probability of survival during transport or storage

For the reasons described above in the pathway citrus plants for planting (commercial trade), the probability that *P. citricarpa* will survive transport and storage of citrus plant propagation material, exported from countries of *P. citricarpa* current distribution into the EU by passenger traffic, is **very likely**, with a **medium** level of uncertainty regarding the conditions under which citrus plant propagating material will be transported and stored by passengers.

### 3.2.9.3. Probability of survival existing pest management procedures

Similarly to the commercial pathway citrus plants for planting, it is **very likely** that *P. citricarpa* will survive currently existing management procedures and remain undetected on citrus plant propagating material imported by passengers. The uncertainty is considered **low** despite the lack of information on the application of fungicides to control CBS in orchards and nurseries where *P. citricarpa* is present and from which passengers may decide to take plant propagating material.

### 3.2.9.4. Probability of transfer to a suitable host

Provided that passengers manage to import infected plant propagating material to the pest risk assessment area and that they go on to use this material in private gardens or in commercial orchards in the pest risk assessment area, similarly to the commercial pathway citrus plants for planting, it is **very likely** that the pathogen will be able to transfer from the pathway of citrus plants for planting (passenger traffic) to a suitable host or habitat, with a **low** level of uncertainty, by analogy with the commercial plants for planting pathway.

## 3.2.10. Entry pathway VIII: other citrus plant parts: leaves

Little information is available about the trade of citrus plant parts other than live plants and fruits. Limited quantities of citrus leaves are imported for flavouring food. Lemon (*C. limon*) and kaffir lime (*C. hystrix*) are the main species used for these purposes, although a variety of other exotic citrus species are also employed (Butryee et al., 2009). As stated in section 3.2.1, there is not considered to be a significant trade in leaves and branches for other purposes and so this has not been considered further in this opinion.

### 3.2.10.1. Probability of association with the pathway at origin

The probability of association with the pathway of leaves (commercial trade) of citrus species which are known to be hosts of *P. citricarpa* can be considered to be similar to that for citrus plants for planting and citrus commercial fruit with leaves. However, the status of *C. hystrix* and other exotic citrus species as hosts of *P. citricarpa* is unknown.

Therefore, taking into account the assessment of entry by these pathways, the pest is **likely** to be associated with the pathway at origin with **medium** uncertainty.

Uncertainties include (1) the status of *C. hystrix* and other exotic citrus species as hosts of *P. citricarpa*, (2) the amount of citrus leaves imported by EU MSs, (3) the number of such imported consignments with *P. citricarpa* infection and the effectiveness of surveys operating at the EU points of entry in detecting *P. citricarpa* infection in leaves.

### 3.2.10.2. Probability of survival during transport or storage

As indicated in the case of citrus plants for planting and citrus commercial fruit with leaves, if the commercial transport of citrus leaves is carried out under conditions that are not limiting for *P. citricarpa* survival in these plant tissues (so as to preserve citrus leaves in fresh or dry conditions), then the probability that *P. citricarpa* will survive transport and storage in infected citrus leaves exported from countries where *P. citricarpa* is present into the EU is rated as **likely**, with a **medium** level of uncertainty, given the lack of data on this pathway.

### 3.2.10.3. Probability of survival of existing pest management procedures

As noted above for the plants for planting and citrus commercial fruit with leaves pathways, the application of fungicides in citrus orchards can diminish *P. citricarpa* incidence and severity but does not eradicate *P. citricarpa* infections. In addition, citrus leaves for flavouring or cooking might be produced in untreated or organic orchards to reduce the risk of pesticides residues. Moreover, culling at the country of origin can easily miss asymptomatic citrus leaves infected by *P. citricarpa*: CBS symptoms on leaves are rarely observed and may be misidentified as lesions are similar to those produced by other citrus pathogens.

Therefore, it is **very likely** that *P. citricarpa* will survive the current management procedures and remain undetected on traded citrus leaves. The uncertainty is considered **medium** owing to the lack of data on this pathway.

### 3.2.10.4. Probability of transfer to a suitable host

As noted above for the citrus plants for planting and citrus commercial fruit with leaves pathways, discarded citrus leaves can pose a risk of transfer of the pathogen to a suitable host via airborne ascospores. This is because of (1) the long quiescent period of *P. citricarpa*, (2) the difficulties in detecting CBS symptoms on citrus leaves, (3) the distribution of citrus leaves for flavouring or cooking throughout the EU, including citrus-growing regions and (4) the potential development of pycnidia with pycnidiospores and pseudothecia with ascospores on infected citrus leaves that might be discarded in the vicinity of citrus trees in the pest risk assessment area. However, the transfer from citrus leaves for flavouring or cooking is much less likely to occur because the majority of mycelium and spores will be destroyed by cooking. Moreover, the imported citrus leaves for flavouring or cooking are unlikely to be sorted and packed in packing houses near citrus orchards and any discards may remain in their original packaging.

Thus, the pathogen would be **unlikely** to be able to transfer by various means (wind, water (rain or irrigation), insects) to susceptible host plants, with a **medium** level of uncertainty deriving from the lack of data on this pathway.

### 3.2.11. Conclusion on the probability of entry

The Panel has assessed the overall probability of entry by combining the ratings of the various steps for each pathway, following the rule that within each pathway the overall assessment should not be higher than the lowest probability. The ratings are presented in Table 6 and the justification for the overall ratings is summarised in Table 7.

**Table 6:** Ratings for the probability of entry and uncertainty for relevant entry pathways, under the scenario of absence of EU phytosanitary measures but with application of standard disease management practices in the country of origin, to comply with fruit quality standards

| Pathways  | Probability of association with the pathway at origin |             | Probability of survival during transport or storage |             | Probability of survival to existing pest management procedures |             | Probability of transfer to a suitable host |             | Overall probability of entry along the pathway |             |
|---|---|-------------|---|-------------|--|-------------|--|-------------|--|-------------|
|   | Probability   | Uncertainty | Probability   | Uncertainty | Probability  | Uncertainty | Probability                                | Uncertainty | Probability                                    | Uncertainty |
| I, Citrus fruit trade   | Likely  | Medium      | Very likely   | low         | Very likely  | Low         | Moderately likely                          | Medium      | Moderately likely                              | Medium      |
| II, Tahiti lime ( <i>Citrus latifolia</i> ) fruit trade               | Likely  | High        | Very likely   | high        | Very likely  | High        | Very unlikely                              | High        | Very unlikely                                  | High        |
| III, Citrus fruit import by passengers traffic                        | Likely  | Medium      | Very likely   | low         | Very likely  | Low         | Unlikely                                   | Medium      | Unlikely                                       | Medium      |
| IV, Citrus fruit with leaves trade                                    | Likely  | Medium      | Very likely   | low         | Very likely  | Medium      | Likely                                     | Medium      | Likely   | Medium      |
| V, Citrus plants for planting trade                                   | Likely  | Medium      | Very likely   | low         | Very likely  | Low         | Very likely                                | Low         | Likely   | Low         |
| VI, Tahiti lime ( <i>Citrus latifolia</i> ) plants for planting trade | Likely  | High        | Very likely   | Low         | Very likely  | High        | Very likely                                | High        | Likely   | High        |
| VII, Citrus plants for planting import by passengers traffic          | Likely  | High        | Very likely   | medium      | Very likely  | Low         | Very likely                                | Low         | Likely   | Medium      |
| VIII, Citrus leaves for flavouring or cooking                         | Likely  | Medium      | Likely  | medium      | Very likely  | Low         | Unlikely                                   | Low         | Unlikely                                       | Medium      |

**Table 7:** Justification for ratings of probability of entry

| Rating for entry   | Justification  |
|--|--|
| <p><i>Citrus fruit trade</i></p> <p><b>Moderately likely</b></p>                     | <ul style="list-style-type: none"> <li>• Cultural practices and treatments applied in the current distribution areas of <i>P. citricarpa</i> may reduce the incidence and severity of CBS on citrus fruit imported into the pest risk assessment area, but they will not eliminate the pathogen, as also confirmed by the meta-analysis performed as part of this opinion.</li> <li>• A high volume of citrus fruit is imported every year into the EU from third countries where <i>P. citricarpa</i> is reported. The pathogen has been repeatedly intercepted at the EU borders on commercial citrus fruit imports over the last few years.</li> <li>• There is seasonality in citrus fruit imports, but the traditional period of arrival coincides in part with two periods of host susceptibility (European late spring and early autumn).</li> <li>• <i>P. citricarpa</i> is very likely to survive transport and storage in the form of (i) pycnidiospores within pycnidia in fruit lesions and/or (ii) latent mycelium present in asymptomatic fruit.</li> <li>• <i>P. citricarpa</i> is very likely to survive existing pest management procedures, particularly on latently infected (asymptomatic) fruit and fruit with low disease incidence and severity.</li> <li>• Although citrus fruit consignments are very widely distributed throughout the EU and a considerable part arrive at a time of the year suitable for pest establishment, the intended use of the commodity (processing and human consumption) makes it moderately likely that the pathogen will transfer to a suitable host.</li> </ul> |
| <p><i>Tahiti lime (Citrus latifolia) fruit trade</i></p> <p><b>Very unlikely</b></p> | <ul style="list-style-type: none"> <li>• The probability of association of the pathogen with the pathway at origin is high as latent mycelia in asymptomatic fruits.</li> <li>• The likely survival during transport or storage of <i>P. citricarpa</i> as latent mycelia present in asymptomatic fruits is very high also on Tahiti lime.</li> <li>• Because <i>P. citricarpa</i> can survive as latent mycelia present in asymptomatic fruits of other citrus species under existing CBS management procedures, it is very likely to survive also in Tahiti lime fruit.</li> <li>• The transfer to a suitable host is the limiting factor for this pathway, as pathogen sporulation on whole fruits or peel of Tahiti lime has never been observed.</li> </ul>   |
| <p><i>Citrus fruit import by passengers traffic</i></p> <p><b>Unlikely</b></p>       | <ul style="list-style-type: none"> <li>• In countries where <i>P. citricarpa</i> is present, citrus fruit produced for the local market is likely to have a higher incidence of <i>P. citricarpa</i> infection than fruit produced for export markets.</li> <li>• If the pathogen can survive commercial transport and storage, it is just as possible for it to be transported with citrus fruit carried by passengers.</li> <li>• Data on citrus fruit interceptions from Australia lead to a conservative and rough estimate of about one aeroplane passenger out of 1 000 carrying citrus fruit; given the sheer numbers of passengers flying into the EU, this would make it unlikely for control procedures to be able to stop the pathogen at the borders.</li> <li>• Since passengers are unlikely to discard fruit in the proximity of citrus orchards, and because of the small number of citrus fruit potentially entering the EU on this pathway, the Panel considers that the probability of transfer to a suitable host from this pathway is low.</li> </ul>   |
| <p><i>Citrus fruit with leaves trade</i></p>   | <ul style="list-style-type: none"> <li>• The probability of association with the pathway of citrus fruit with leaves and peduncles (commercial trade) is similar to the citrus plants for planting and citrus</li> </ul>   |

| Rating for entry  | Justification   |
|---|---|
| <p><b>Likely</b></p>  | <p>commercial fruit pathways.</p> <ul style="list-style-type: none"> <li>• Although the importation from third countries of citrus fruit with leaves is prohibited by EU legislation, there have been a number of interceptions over the last few years.</li> <li>• Commercial citrus fruit with leaves is stored and transported under conditions that are not stressful or damaging for leaf tissues and thus to the pathogen.</li> <li>• Pest management procedures (pre- and post-harvest fungicide treatments, culling, physical treatments at packing houses, etc.) do not eliminate the pathogen; CBS symptoms can be misidentified or missed; latent infection is common.</li> <li>• If citrus fruit with leaves are improperly discarded, the latent mycelium present on them can develop pycnidia with pycnidiospores, which can then go on to infect the host under suitable conditions. In addition, in the case of leaves, the pathogen can produce pseudothecia with wind-disseminated ascospores, which may spread the pathogen over long distances</li> </ul> |
| <p><i>Citrus plants for planting trade</i></p> <p><b>Likely</b></p>                         | <ul style="list-style-type: none"> <li>• Particularly if citrus nurseries at the place of origin are located close to infected citrus orchards, it is likely that there will be a high prevalence of the pathogen in citrus plant material for propagation purposes.</li> <li>• Citrus plant propagation material, as with all living plants, is stored and transported under conditions that are not stressful or damaging for plant tissues. The pathogen can survive those conditions. Cultural practices and fungicides applied in citrus nurseries at the place of origin are unlikely to eradicate the pathogen from infected leaves; CBS symptoms on leaves are similar to those of other citrus diseases and latent infections are very common.</li> <li>• The pathogen is very likely to be able to transfer from the pathway to a suitable host in the RA area, because the intended use of plants for planting, including scions and budwood is very likely to aid such transfer.</li> </ul>   |
| <p><i>Tahiti lime (Citrus latifolia) plants for planting trade</i></p> <p><b>Likely</b></p> | <ul style="list-style-type: none"> <li>• The ratings on this pathway were given by analogy with the citrus plants for planting trade pathway.</li> </ul>  |
| <p><i>Citrus plants for planting import by passengers traffic</i></p> <p><b>Likely</b></p>  | <ul style="list-style-type: none"> <li>• The ratings on this pathway were given by analogy with the citrus plants for planting trade pathway.</li> </ul>  |
| <p><i>Citrus leaves for flavouring or cooking</i></p> <p><b>Unlikely</b></p>                | <ul style="list-style-type: none"> <li>• The transfer from citrus leaves for flavouring or cooking is much less likely to occur than from leaves of citrus plants for planting and citrus fruit with leaves because the majority of mycelium and spores will probably be destroyed by cooking.</li> <li>• Moreover, the imported citrus leaves for flavouring or cooking are unlikely to be sorted and packed in packing houses close to citrus orchards and any discarded material is likely to remain in its original packaging</li> </ul>  |

### 3.2.12. Uncertainties on the probability of entry

**Table 8:** Rating and justification for the uncertainty on the probability of entry

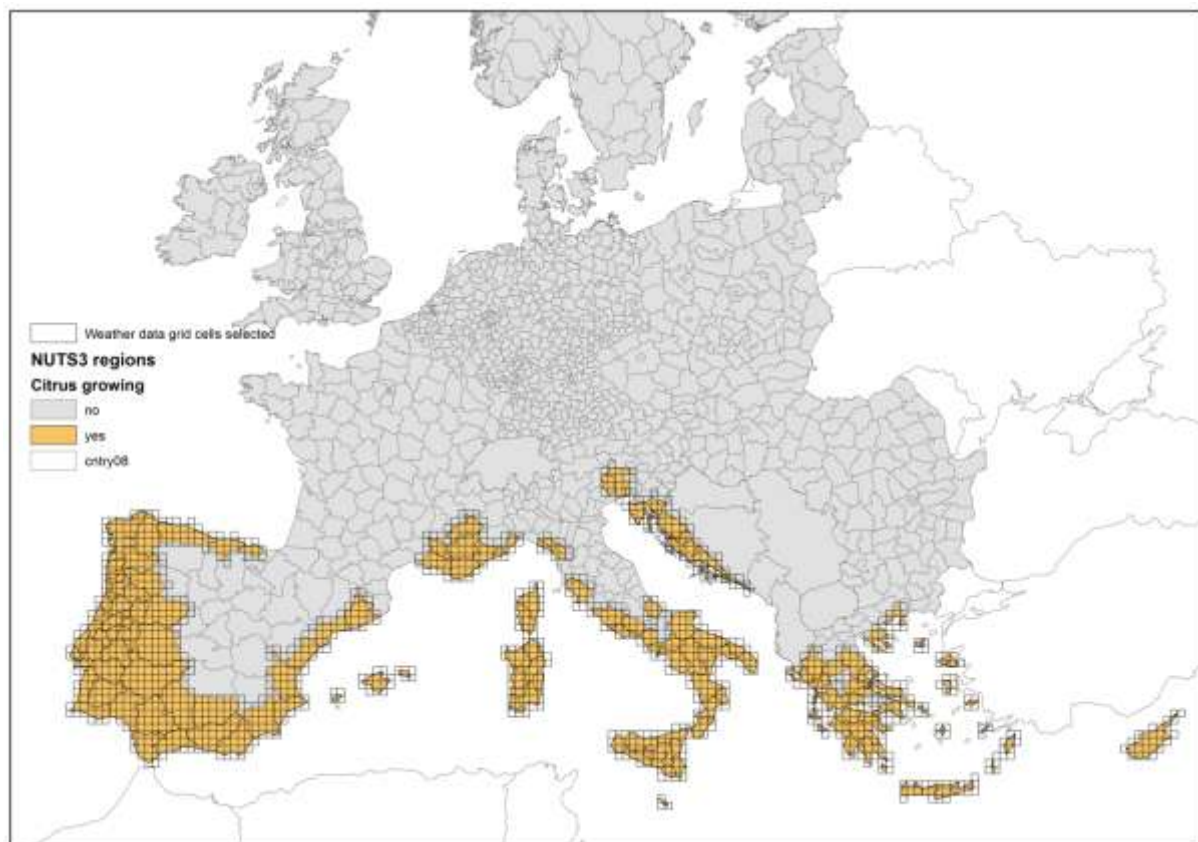
| Rating for uncertainty   | Justification  |
|--|--|
| <p><i>Citrus fruit trade</i></p> <p><b>Medium</b></p>                                      | <p>The main uncertainties concerning this pathway include:</p> <ul style="list-style-type: none"> <li>the prevalence of the pathogen in the various regions of CBS-infested third countries;</li> <li>whether or not pomelo (<i>C. maxima</i>) is susceptible to <i>P. citricarpa</i>;</li> <li>the frequency and quantity of infected fruit/peel or other citrus fruit by-products discarded in close proximity to susceptible hosts in the citrus-growing regions of the pest risk assessment area.</li> </ul> |
| <p><i>Tahiti lime (Citrus latifolia) fruit trade</i></p> <p><b>High</b></p>                | <ul style="list-style-type: none"> <li>There is a high uncertainty about all the stages of this pathway.</li> <li>Most importantly, it is not known if <i>P. citricarpa</i> could develop symptoms and fruiting bodies in harvested fruit of Tahiti lime after long storage periods or under outdoor waste disposal conditions.</li> </ul>   |
| <p><i>Citrus fruit import by passengers traffic</i></p> <p><b>Medium</b></p>               | <ul style="list-style-type: none"> <li>There is a lack of information concerning the volume and frequency of the movement of infected citrus fruit imported by passengers.</li> <li>One key uncertainty is the probability that passengers will dispose citrus peel and whole fruit waste in the proximity of susceptible hosts in the risk assessment area (citrus orchards, private gardens, nurseries, etc.).</li> </ul>  |
| <p><i>Citrus fruit with leaves trade</i></p> <p><b>Medium</b></p>                          | <ul style="list-style-type: none"> <li>There is lack of data on the volume of citrus fruit with leaves that could be potentially imported into the risk assessment area from infested third countries.</li> <li>There is lack of data on the frequency and volume of citrus fruit with leaves that could potentially be discarded in proximity to citrus nurseries and orchards in the risk assessment area.</li> </ul>  |
| <p><i>Citrus plants for planting trade</i></p> <p><b>Low</b></p>                           | <ul style="list-style-type: none"> <li>There is a lack of data on the prevalence of <i>P. citricarpa</i> in citrus nurseries in countries with presence of CBS.</li> <li>Lack of data on compliance with reporting and quarantine rules of plant nurseries, as well as on the likely structure of the trade network of citrus plants for planting.</li> </ul>  |
| <p><i>Tahiti lime (Citrus latifolia) plants for planting trade</i></p> <p><b>High</b></p>  | <ul style="list-style-type: none"> <li>Little is known about the prevalence of CBS on this pathway at origin.</li> <li>Trade in citrus plants for planting imported from third countries into the EU is not allowed, so there is a lack of information on the survival of <i>P. citricarpa</i> in imported Tahiti lime plants.</li> <li>The chain of events that could lead to transfer of the pathogen to the host is also associated with high uncertainty, owing to the general lack of studies.</li> </ul>   |
| <p><i>Citrus plants for planting import by passengers traffic</i></p> <p><b>Medium</b></p> | <ul style="list-style-type: none"> <li>No data exist on the import of such material in the EU by passengers.</li> <li>There is uncertainty concerning the conditions under which citrus plant propagating material will be transported and stored by passengers.</li> </ul>  |
| <p><i>Citrus leaves for flavouring or cooking</i></p> <p><b>Medium</b></p>                 | <ul style="list-style-type: none"> <li>There is a general lack of data on this pathway.</li> </ul>   |

### 3.3. Probability of establishment

#### 3.3.1. Availability of suitable hosts in the risk assessment area

Citrus is grown commercially for fruit production in all the countries of the southern EU with a Mediterranean climate: Croatia, Cyprus, France, Greece, Italy, Malta, Portugal and Spain. From Majorca, eastwards these are mainly in the Köppen–Geiger climate zones CSa and CSb typical of Mediterranean climates with warm or hot dry summers according to maps based on full station data from the Global Historical Climatology Network interpolated to a 0.1° latitude × 0.1° longitude grid published by Peel et al. (2007). However, Cyprus, the Greek islands and most of southern and eastern Spain, including Valencia, are in hot or cold arid zones BSh and BSk with steppe precipitation. Citrus is also grown in northern Portugal and northern Spain, areas that are far from the Mediterranean Sea and have other climates, e.g. Cfb, which is warm temperate, fully humid (i.e. no dry season) and with a warm summer. The maps published by Peel et al. (2007) differ to a certain extent from the map produced by Kottek et al. (2006) based on 1971–2000 global climatic data interpolated to a lower resolution (0.5° latitude × 0.5° longitude), but these also show the hot and cold arid zones BSh and BSk in Spain and the eastern Mediterranean together with the Cfb zone, where citrus is grown in northern Spain.

The cultivated area of orange, lemon and small fruited citrus varieties in the EU by country and NUTS2 region is given in Table 9. A total of 62 854 ha is cultivated with lemon, the citrus species most susceptible to *P. citricarpa* (Kotzé, 1981), covering about 13 % of the citrus-growing area in the EU.



**Figure 27:** EU map of NUTS3 citrus-growing regions based on citrus production data extracted from national statistical databases of Portugal, Spain, France, Italy, Malta, Croatia, Greece and Cyprus (see Appendix F).



The total area cultivated with citrus species in the EU NUTS3 regions has been extracted from the national statistical databases of the EU citrus-growing countries (Portugal, Spain, France, Italy, Croatia, Greece, Malta, Cyprus) (Appendix F). Based on these data, an EU map of NUTS3 citrus-growing regions have been developed to be used for extraction of weather data for the simulations run for this opinion (Figure 27), to allow the comparison of simulation results with the citrus acreage for each NUTS3 region/province.

**Table 9:** The citrus production area (in hectares) in the EU in 2007 (including Croatia, EU MS since 2013). Data extracted from Eurostat (online) on 21/02/2013

| Country/region                 | Orange varieties | Lemon varieties | Small-fruited citrus varieties | All citrus varieties <sup>(a)</sup> |
|--------------------------------|------------------|-----------------|--------------------------------|-------------------------------------|
| EU (28 countries) (*)          | 279 048          | 62 854          | 151 510                        | 493 413                             |
| <b>Croatia</b>                 | 200              | 100             | 1 200                          | 1 500                               |
| <b>Cyprus</b>                  | 1 554            | 665             | 1 766                          | 3 985                               |
| <b>France</b>                  | 28               | 22              | 1 654                          | 1 705                               |
| Provence-Alpes-Côte d'Azur     | 1                | 5               | 1                              | 8                                   |
| Corse                          | 27               | 17              | 1 648                          | 1 692                               |
| France, not allocated          | 0                | 0               | 3                              | 4                                   |
| <b>Greece</b>                  | 32 439           | 5 180           | 6 631                          | 44 252                              |
| Kentriki Ellada, Evvoia        | 6 531            | 1 969           | 0                              | 8 500                               |
| Ipeiros                        | 3 993            | 0               | 0                              | 3 993                               |
| Peloponnisos                   | 17 347           | 1 730           | 3 379                          | 22 458                              |
| Nisia Aigaiou, Kriti           | 883              | 308             | 213                            | 1 405                               |
| Kriti                          | 3 410            | 277             | 356                            | 4 044                               |
| Other Greek regions            | 266              | 885             | 2 598                          | 3 750                               |
| <b>Malta</b> <sup>(b)</sup>    | –                | –               | –                              | 193                                 |
| <b>Italy</b>                   | 73 785           | 16 633          | 21 997                         | 112 417                             |
| Piemonte                       | 0                | 0               | 0                              | 0                                   |
| Liguria                        | 7                | 17              | 3                              | 28                                  |
| Toscana (NUTS 2006)            | 6                | 0               | 0                              | 6                                   |
| Lazio (NUTS 2006)              | 399              | 82              | 178                            | 660                                 |
| Abruzzo                        | 178              | 0               | 0                              | 178                                 |
| Molise                         | 9                | 0               | 9                              | 18                                  |
| Campania                       | 689              | 954             | 634                            | 2 278                               |
| Puglia                         | 3 462            | 146             | 4 059                          | 7 668                               |
| Basilicata                     | 4 640            | 39              | 2 093                          | 6 774                               |
| Calabria                       | 17 273           | 967             | 10 774                         | 29 015                              |
| Sicilia                        | 43 731           | 14 338          | 3 106                          | 61 176                              |
| Sardegna                       | 3 387            | 86              | 1 138                          | 4 612                               |
| <b>Portugal</b>                | 12 416           | 494             | 3 235                          | 16 145                              |
| Norte                          | 734              | 52              | 133                            | 920                                 |
| Centro (PT) (NUTS95)           | 401              | 27              | 54                             | 482                                 |
| Lisboa e Vale do Tejo (NUTS95) | 256              | 196             | 37                             | 490                                 |
| Alentejo (NUTS95)              | 1 585            | 11              | 247                            | 1 844                               |
| Algarve                        | 9 437            | 206             | 2 763                          | 12 407                              |
| <b>Spain</b>                   | 158 824          | 39 859          | 116 225                        | 314 908                             |
| Principado de Asturias         | 0                |                 | 0                              | 1.00                                |
| Extremadura                    | 278              | 0               | 38                             | 317                                 |
| Cataluña                       | 2 080            | 20              | 10 777                         | 12 877                              |

| Country/region       | Orange varieties | Lemon varieties | Small-fruited citrus varieties | All citrus varieties <sup>(a)</sup> |
|----------------------|------------------|-----------------|--------------------------------|-------------------------------------|
| Comunidad Valenciana | 76 593           | 9 127           | 90 878                         | 176 599                             |
| Illes Balears        | 660              | 397             | 98                             | 1 156                               |
| Andalucía            | 64 158           | 5 646           | 9 999                          | 79 804                              |
| Región de Murcia     | 14 514           | 24              | 4.433                          | 43 509                              |
| Canarias (ES)        | 538              | 104             | 0                              | 643                                 |

(a): Calculated.

(b): Data for citrus production area for Malta are provided according to FAOSTAT (online) for the year 2011. The detailed production structure is as follows: tangerines, mandarins, clementines (6 ha); grapefruit including pomelo (1 ha); lemons and limes (38 ha); oranges (95 ha); citrus fruit others (53 ha).

### 3.3.1.1. Periods of susceptibility of citrus leaves and fruits in the risk assessment area

Citrus leaves are susceptible to *P. citricarpa* for 8–10 months (Truter et al., 2004; Truter, 2010) In South Africa, fungicide sprays cease four months after fruit set because fruit is then considered resistant, although this ontogenic resistance has not been confirmed experimentally (McOnie, 1964b, c; Kotzé, 1981). The lack of fungicide sprays during the final stages of fruit development can also be a consequence of low inoculum levels and unfavourable weather conditions at that time. Studies conducted in Brazil and Ghana, under non-limiting inoculum and weather conditions for infection, indicated a susceptibility period of six and seven months after fruit set, respectively (Reis et al., 2003; Baldassari et al., 2006; Aguiar et al., 2012; Brentu et al., 2012), but longer periods were not evaluated. In countries of the EU with commercial citrus fruit production, citrus trees have three main leaf flushes per year and fruit set is concentrated in spring, around the beginning of May (Agustí, 2012; García-Mari et al., 2002). Therefore, in the citrus-growing regions of the EU, susceptible leaves are present all year around and susceptible fruits from May to December. In the case of lemons, one or two additional flowering periods may occur in summer (July–September), and fruit with different growth stages coexist in the tree so susceptible fruits are present all year around (Cutuli et al., 1985; Agustí, 2012).

### 3.3.2. Suitability of environment

Climate is the key environmental factor that determines the potential for *P. citricarpa* establishment in the EU. The Panel has tackled this issue by:

- Summarising the role played by climatic factors in the life cycle of *P. citricarpa*.
- Reviewing the different methods (principally Paul et al. (2005), EFSA (2008), Magarey et al. (2011), MacLeod et al. (2012), Yonow et al. (2013) and Fourie et al. (2013)) that have previously been used to assess, *inter alia*, the potential distribution of *P. citricarpa* in Europe. An evaluation of their advantages and disadvantages has been conducted in order to select the most appropriate method to employ in this pest risk assessment.
- Assessing the climatic suitability of *P. citricarpa* in Europe using the most suitable method identified.

#### 3.3.2.1. Summary of the role played by climatic factors in the life cycle of *P. citricarpa*

The geographical distribution of *P. citricarpa* shows that it generally occurs in humid regions characterised by rainy summers (Kotzé, 1981, 2000), but citrus-growing areas in the Eastern Cape Province in South Africa are also affected by CBS (Paul et al., 2005; Yonow et al., 2013; Carstens et al., 2012) and these lie within the arid steppe Köppen–Geiger zones (Kottek et al., 2006; Peel et al., 2007), which also occurs in Spain and islands in the Eastern Mediterranean. It has been stated that *P. citricarpa* has failed to establish in Mediterranean climates (Paul et al., 2005; Yonow et al., 2013), but the extent to which the pathogen has or has not become established under Mediterranean climatic

conditions depends on the definition of the Mediterranean climate. However, definitions vary between the general, found in dictionaries (e.g. <http://www.oxforddictionaries.com>), which describes the climate in the area surrounding the Mediterranean Sea; (ii) a climate distinguished by warm, wet winters under prevailing westerly winds and calm, hot, dry summers, as is characteristic of the Mediterranean region and parts of California, Chile, South Africa and SW Australia; (iii) the specific Köppen–Geiger climate zones CSa (warm temperate, steppe precipitation and hot summer) and CSb (warm temperate, steppe precipitation and warm summer); and (iv) 11 components related to climate, vegetation, soils and fire regimes adapted from Aschmann (1973). Metzger et al. (2005) provide an even more detailed analysis based on climatic variables, oceanicity, northings, geomorphology, geology and soil. Furthermore, climate zones (e.g. Köppen–Geiger) may not necessarily represent the environmental factors that are critical for the pathogen and its host, especially when considering the influence of microclimate (Vicent and García-Jiménez, 2008).

Several environmental variables are associated with the biology of *P. citricarpa* and the epidemiology of CBS. As described in section 3.1.1.2, *P. citricarpa* has two infection cycles, with a primary cycle driven by ascospores produced by sexual fruiting bodies (pseudothecia) in the leaf litter and a secondary cycle involving pycnidiospores produced by asexual fruiting bodies (pycnidia) on lesions in fruit, twigs and leaf litter. Warm temperatures and high soil moisture have been associated with rapid leaf litter decay, limiting further pseudothecia and ascospore development (Lee and Huang, 1973). The formation of pseudothecia in the leaf litter and the production and release of ascospores is influenced by the temperature and water regime. Pseudothecia develop 23–180 days after leaf drop, depending on the frequency of wetting and drying as well as on the prevailing temperatures and the maturation of ascospores occurs almost simultaneously on infected leaves abscised throughout the year (Kotzé, 1963, 1981; McOnie, 1964c; Lee and Huang, 1973). According to Lee and Huang (1973), the optimum temperature for pseudothecia formation is 21–28 °C and no pseudothecia are produced below 7 °C or above 35 °C. When mature asci within pseudothecia in the leaf litter are moistened with water, ascospores are ejected into the air and are disseminated by air currents (Kiely, 1948, 1949; Wager, 1949; McOnie, 1964b; Huang and Chang, 1972; Kotzé, 1981). In the presence of water, ascospores are released when temperatures are between 5 and 25 °C (Kotzé, 1963).

Pseudothecia formation and subsequent ascospore maturation and release in the Limpopo province of South Africa have been modelled by Fourie et al. (2013) using temperature sums and the moisture conditions in the leaf litter (resulting from rain, dew, or irrigation). Both ascospore germination and infection are driven by temperature and moisture conditions, where infection requires moisture in the specific form of a wet leaf surface for infection to occur (Kotzé, 1981). The requirements for ascospore germination on agar media varied between 15 and 29.5 °C and 15 and 38 hours of wetness (Kotzé, 1963). McOnie (1967) demonstrated that ascospores can infect when there has been at least 15 hours of continuous wetness, but no records of the temperatures were reported in this study. Timossi et al. (2003) evaluated the germination rate of ascospores of *Phyllosticta* spp. at different temperatures and incubation durations. The tested ascospores were produced on artificial media which, according to Baayen et al. (2002), are suitable for ascospore production only in *P. capitalensis* and not in *P. citricarpa*. No conclusive strain identification was provided by Timossi et al. (2003), so these data were no further considered.

Temperature also influences the secondary infection cycle by determining the duration of the incubation period, symptom expression and consequently the formation of pycnidiospores on fruit lesions. Disease incidence and pycnidiospore production in naturally infected sweet orange fruit increased significantly at 27 °C compared with 20 °C. Light also augmented disease incidence and pycnidiospore production on fruit (Brodrick and Rabie, 1970). Field studies conducted in Brazil also showed that temperature was the main environmental factor affecting symptom expression (Ninin et al., 2012). Pycnidiospores produced on infected fruits and twigs in the canopy are mainly disseminated by rain-splash (Whiteside, 1967) and are considered to be epidemiologically important in areas of Brazil (Spósito et al., 2007, 2008, 2011), where high rainfall frequently occurs during infection periods. Garrán (1996) only found pycnidiospore inoculum during the early stages of CBS epidemics

in the northeast of Entre Rios, Argentina. Although pycnidiospores produced in twigs might add to the risk of infection in spring and early summer in some regions and/or years in the pest risk assessment area where ascospore availability is delayed due to low temperatures, the lack of quantitative data on pycnidiospore production in twigs and its relation with environmental parameters limited any further modelling effort. Therefore, conclusions about the suitability of environment in the pest risk assessment area are based only on potential ascospore infections.

### 3.3.2.2. Review of the different methods used to assess the climatic suitability of the EU for *P. citricarpa*

Four methods have been employed, some in combination, when assessing climatic suitability of the EU for *P. citricarpa* establishment. This review gives a brief description of each method, lists the applications, describes the advantages and disadvantages and finally provides a conclusion concerning their applicability for the assessment of *P. citricarpa* climatic suitability in the EU.

#### (i) Qualitative assessment based on the literature and expert judgement with or without model outputs:

- Description of the method:
  - This has been the standard method of pest risk analysis since schemes were first developed in the early 1990s. It can be a general description of risk, e.g. EPPO (2007), or a detailed qualitative PRA scheme that requires a risk rating and an uncertainty score supported by a documented, referenced justification based on all the evidence including model outputs, e.g. EFSA PLH Panel, (2010) and EPPO (1997, 2011). Risk ratings and uncertainty scores can be provided for each factor, e.g. climatic suitability, or just for each section, e.g. establishment.
- Applications:
  - The *P. citricarpa* datasheet in EPPO (1997) has a paragraph on phytosanitary risk to Europe based on a general review of the evidence without risk ratings and uncertainty scores.
  - The Prima phacie project (MacLeod et al., 2012) assessed the risk posed by *P. citricarpa* to the EU based on the literature and the model evaluations and runs provided by the EFSA Panel on Plant Health (PLH) (EFSA, 2008) and answered the question: “How similar are the climatic conditions that would affect pest establishment, in the risk assessment area and in the current area of distribution?” The risk was rated as moderately similar, with an uncertainty score of medium.
- Advantages:
  - It provides a clear written summary of risk and uncertainty that is based on the evidence presented and can be compared with other species.
  - It integrates all the evidence available, not just the results from one model that will itself have uncertainties and often a range of plausible outputs.
  - It is familiar to risk assessors and risk managers in the EU and elsewhere.
  - It follows international guidelines (ISPM 11 by FAO, 2004) that do not stipulate that assessments should be quantitative.
  - It follows the EFSA harmonised framework for pest risk assessment ((EFSA PLH Panel, 2010).
- Disadvantages:
  - Even if based on published data and model outputs, there are likely to be elements of subjectivity, e.g. due to inconsistencies between assessors in selecting appropriate risk ratings and uncertainty scores.
  - There can be a lack of transparency on how the different sources are combined and how risk ratings have been derived from the available information.
- Conclusions
  - This is a well-recognised method for assessing risk that integrates model outputs and uncertainties with evidence from the literature.

- The results may depend on the assessor's subjective views.
- Qualitative scores are often difficult to interpret.

(ii) Climate matching and correlative models

- Description of the method
  - Climate matching methods, e.g. CLIMEX Match Climates, compare climates at one weather station or area with that in another using a variety of algorithms. Correlative models, e.g. MaxEnt (Elith et al., 2011) and BIOCLIM, use a wide variety of statistical methods or machine learning techniques to assess climatic suitability. Classification rules are developed from the climatic variables at the locations where the pest is present and extrapolated to new areas.
- Applications
  - The CLIMEX Match Climates method (Sutherst et al., 2007) has been used for *P. citricarpa* by Paul (2006), a study that was evaluated by the EFSA Panel on Plant Health (PLH) (EFSA, 2008).
  - Climate response surfaces (Huntley et al., 1995).
    - Paul (2006), evaluated by the EFSA Panel on Plant Health (PLH) (EFSA, 2008).
- Advantages
  - Climatic matching methods are relatively simple to use and they provide preliminary indications of climatic similarity that can be used for further analysis.
  - The advantages of correlative methods are summarised by, for example, Eyre et al. (2012). They are generally open access and relatively quick to use and the outputs are more likely to be consistent between different modellers.
- Disadvantages
  - The outputs of the climate matching methods expressed as climatic similarities, match indices, etc., are based on combinations of climatic variables and time periods that are unlikely to reflect the specific climate responses of the pest and the key periods during which they are important in the pest's life cycle.
  - Correlative methods greatly depend on (a) the extent to which location data (for both presence and absence) are representative of the areas where the climate is suitable, (b) the climatic factors selected and (c) the methods for selecting thresholds for establishment (Dupin et al., 2011; Eyre et al., 2012).
  - The use of small presence/absence datasets may lead to inaccurate results (Dupin et al., 2011).
  - In both methods, the outputs are difficult to relate to pest biology and epidemiology.
  - The accuracy of the results of matching methods depends critically on the correctness of the assumption that physiological and ecological traits of organisms are identical between the area of origin and the area for which the potential for establishment is evaluated, and that these traits will remain unchanged over time. While this assumption of fixed traits is a valid null hypothesis to initiate the assessment, there are many examples of adaptation of invasive organisms to novel environments. The area for potential establishment will become larger than initial assessments would indicate if an organism adapts to selective forces in a new environment. Therefore, in principle, matching methods have a fundamental weakness in demonstrating unsuitability of a geographic region for an organism, especially if a region is on the margin of suitability, posing opportunity for adaptation. In the case of *P. citricarpa*, very little information is available regarding diversity in ecophysiological traits and its propensity for adaptation. Broadbent (1995) stated the following, indicating the risks of diversity and adaptation in the pathogen: "*Black spot (caused by Guignardia citricarpa Kiely) causes serious losses in coastal orchards in New South Wales (Kiely 1948), but does not survive or cause symptoms in hot dry inland orchards (Barkley 1988). By contrast, black spot in South Africa was first reported in 1929 only in the cool misty areas of Natal, but in 1945 assumed more serious proportions when it spread to the hot dry subtropical East and North Transvaal (Wager*

1952). *Introduction to Australia of strains with a broader physiologic diversity could threaten export markets and reduce the viability of inland citrus*".

- Conclusions
  - Climate matching methods are useful primarily as a preliminary guide and not for detailed analysis.
  - Given the paucity of representative location data and the complex relationship of the pest with climatic variables, it will be difficult to interpret the results of any correlative models applied to *P. citricarpa*.

(iii) Models combining correlative and deductive elements

- Description of the method
  - The CLIMEX Compare Locations model (Sutherst et al., 2007) can be parameterised by utilising a species' climate response data and by inference from its known distribution. The potential for establishment is based on the ecoclimatic index (EI), which combines a growth index, representing the suitability of the location for growth and development of the organism studied, and a stress index that is estimated according to the degree to which the climate is too wet, dry, hot or cold. Once the parameters have been manipulated so that CLIMEX has satisfactorily emulated a pest's current distribution, EIs can be calculated from climatic data in the risk assessment area and mapped.
- Applications
  - CLIMEX Compare Locations
    - Paul et al. (2005), evaluated by EFSA (2008)
    - Yonow et al. (2013) enhancing Paul et al. (2005) and responding to EFSA (2008)
- Advantages
  - CLIMEX can integrate detailed climatic response data, e.g. temperature and soil moisture thresholds, with the climate in the area where the pest is present to mirror the current distribution that can then be projected onto the climate in the pest risk assessment area.
  - Yonow et al. (2013) state that CLIMEX: "*is well suited to predicting the potential distribution of G. citricarpa because of the important influence of climate in the epidemiology of CBS*", but this argument is valid for any model taking into account climatic variables, not only for CLIMEX. This point is discussed further below.
  - Yonow et al. (2013) also state that: "*CLIMEX has been successfully used to predict the potential distribution of other pathogens (Brasier and Scott, 1994; Venette and Cohen, 2006; Watt et al., 2011a,b; Yonow et al., 2004)*." However, as discussed below, the success of these predictions has not been systematically evaluated.
- Disadvantages
  - EFSA (2008) noted that: "*it is difficult to reflect the relationship between pathogen infection and host phenology. All pest risk maps have to take into account the spatial presence of suitable hosts but, for many pathogens, temporal availability is also critical since infection may only occur if climatic conditions are suitable at specific host phenology stages. CLIMEX takes the whole year's climatic data into account so cannot readily be constrained to analyse just the period of suitable host phenology*". EFSA (2008) also noted "*the importance of complex variables, such as leaf wetness, that are not taken into account by CLIMEX and may act at a much shorter time scale (hours) than that utilised by CLIMEX (weeks for the moisture index)*". Yonow et al. (2013) responded by stating that: "*EFSA (2008) argues that the climate during the period of host susceptibility alone should be considered, rather than the climate over the entire year. Whilst it is true that climatic conditions must be suitable at the appropriate time of host susceptibility for the presence of G. citricarpa spores to result in an infection, conversely, it is not true that a window of opportunity for host infection will necessarily lead to the permanent establishment of a population of G. citricarpa. An infection incident will not result in the establishment of a pathogen population unless the climate is suitable for the persistence of that population until the next infection incident can occur and a full life cycle can be*

completed. Suitably timed and repeated recurrence of such circumstances is required for there to be an opportunity for permanent establishment”. Yonow et al. (2013) also state that “it is true that CLIMEX does not consider the effects of a whole range of complex variables (which may or may not be driven by climate), such as leaf wetness, and it is true that the time scale at which a factor such as leaf wetness occurs is very short by comparison to the time scale at which CLIMEX operates. However, such issues are related to the first factor, where EFSA (2008) argues that only short periods of climate should be considered, and the counter-argument remains the same: short periods of suitability that may result in an infection incident will not necessarily result in the establishment and persistence of the pathogen”. The Panel agrees with Yonow et al. (2013) that modelling infection alone is insufficient. However, not only has the climate (primarily temperature) to be suitable for development and spore production, but the timing of spore release also has to coincide with key stages in host phenology. For successful establishment, suitable hourly temperature and leaf wetness conditions required for infection to take place need to coincide with the availability of inoculum (i.e. spore presence) and host phenology (i.e. citrus hosts in a susceptible phenological stage). This complex combination of climatic factors and host phenologies requires models such as those proposed by Fourie et al. (2013) and Magarey et al. (2005) that, unlike CLIMEX, can operate at a high temporal resolution related to the timing of key epidemiological events, utilise parameters such as leaf wetness and can be constrained to interact with host phenology.

- EFSA (2008) noted that there are “discrepancies between the pathogen and host’s climatic responses. The pathogen’s climatic responses may be much greater than the range suitable for the host” Yonow et al. (2013) state that “a pathogen and its host may indeed have differing climatic responses. In the case of *G. citricarpa* and citrus, there is evidence in both South Africa and Australia that despite the extended absence of restrictions on the movement of citrus propagation material from CBS-infected areas into CBS-free areas, the disease has never established in these areas. These areas are thus evidently climatically suitable for citrus, but unsuitable for *G. citricarpa*. The current CLIMEX model predicts correctly that several citrus regions are unsuitable for the long-term persistence of *G. citricarpa* and it also predicts potential climatic suitability for *G. citricarpa* in some parts of the world that are not suitable for citrus production. Our model therefore appropriately provides for differentiation between potential distribution of the host and pathogen”. The ecoclimatic index calculated by Yonow et al. (2013) for *P. citricarpa* is highest in areas of Europe, e.g. southern Romania, where the winters are too cold for commercial outdoor citrus production. The Panel accepts that species distribution models may predict potential establishment based on climate in areas that are not climatically suitable for their host. Such discrepancies highlight the importance of taking host distribution into account when assessing the area of potential establishment.
- The successful use of CLIMEX in predicting the potential distribution of pathogens is subjective and has never been properly analysed. The “success” of a model in projecting the distribution of any organism, whether or not it is a pathogen, depends on many factors, e.g. the complexity of the life cycle, the extent to which distribution is dependent on climate and whether the key climatic factors are represented in CLIMEX and are available at an appropriate spatial and temporal resolution. In addition, the volume, quality and spatial distribution of locations where the pest is known to be present (Eyre et al., 2012) and the extent to which the pests is known to have high/low incidence at these locations are also important. Moreover, the extent to which CLIMEX has been successful in predicting the potential distribution of the pathogens may be difficult to evaluate because evidence is limited. The paper by Brasier and Scott (1994) is particularly difficult to assess because they modelled a root pathogen (*Phytophthora cinnamomi* Rands) that lives in an edaphic microclimate that is very different from that measured by weather stations and did not provide the model parameters and justification for their selection. Model parameterisation and outputs are strongly influenced not only by the availability of

reliable climatic response data and representative presence data, but also by the likelihood of continuing spread and disjunct distributions. The distribution of citrus and therefore CBS in South Africa and Australia is highly disjunct and is also affected by major geographical features (principally the sea) and irrigation. This makes it difficult to determine with confidence the factors that are critical in setting the limits to the distribution of *P. citricarpa*.

- Although Yonow et al. (2013) state that: “*Climatic suitability can be broadly categorised as follows: EI = 0 (unsuitable), 1 ≤ EI ≤ 4 (marginal), 5 ≤ EI ≤ 9 (suitable), 10 ≤ EI ≤ 29 (highly suitable), and 30 ≤ EI (optimal)*”, classifying outputs into marginal, suitable and optimal is difficult and species specific. Stephens et al. (2007) stated that: “*The assignment of classifications to EI values is usually an arbitrary process, as the resulting patterns are species-specific*”. Sutherst et al. (2004) provide some suggested guidelines: “*an EI = 0–0.49 indicates that the climate is unsuitable; the species cannot persist in an area under average environmental conditions, an EI of 0.50–9.99 indicates marginal conditions, an EI of 10–19.99 indicates suitable conditions and an EI of 20+ indicated optimal conditions. An EI of 100 indicates that conditions are perfect all year round, and there are few environments that are stable enough to provide perfect habitat year round*”. Baker et al. (2011) stated that the ecoclimatic index “*can be classified by looking at where the pest is: (a) present but with very low populations, (b) present but not abundant and (c) generally abundant and if (a), (b) and (c) are clearly primarily influenced by climate and not other factors they can be used to classify the EIs. EI values close to zero can be considered marginal, and we would generally expect that a species distribution in climatically marginal habitats would be patchy, and restricted to more climatically favourable sites. In this zone, we would also expect that a species presence would be patchy in time, and metapopulation dynamics might play a strong role in maintaining its presence on a regional basis. If the EI, which is scaled from 0–100, is greater than 30, the climate can generally be considered to be very favourable for establishment (Sutherst et al., 2007; Pinkard et al., 2010). However, the maximum climate suitability that a species can experience under field conditions depends upon the interplay between the seasonality of temperature and moisture variables and the individual species’ climatic niche. In climatic terms, it is possible to have too much of a good thing. As noted by Brown (1996), biotic factors tend to define a species range where resources are abundant. These factors underline why the climate suitability classification needs to be considered on a species-specific basis*”.
- Fitting the distribution simulated by CLIMEX to the actual distribution of the organism by the iterative adjustment of parameters can be difficult and can lead to difficulties of interpretation if the values selected are significantly different from those in the literature. As noted above, a key advantage of CLIMEX, compared with other species distribution models, is that it can be parameterised with climatic response data that have been published on the species of interest. For example, the minimum temperature threshold for development is available for many species (Jarosik et al., 2012), including some data on certain life cycle stages of *P. citricarpa* (Kotzé, 1963; 1981). All parameters, both those that have been obtained from the literature and those, such as the stress indices, that are inferred from the species distribution, can be modified by a process of iteration to match the distribution simulated by CLIMEX with the known distribution. Where there are no published data, the modification of parameters has few constraints. Departing from published climate response thresholds is justified when there is considerable uncertainty, experimental data vary or there is evidence that data obtained from laboratory experiments do not accurately represent field conditions. Since the literature on the minimum temperature threshold for development of *P. citricarpa* as summarised by Yonow et al. (2013) does not provide one clear value, there is considerable scope for parameter variation. Nevertheless, the published literature all points to a threshold at or below 15 °C (though one unpublished South African report states that subsequent infection has not been observed at these temperatures). However, Yonow et al. (2013) have selected a



threshold of 20 °C, justifying the much higher temperature solely on the basis that this was the only way they could find of excluding the simulated distribution of *P. citricarpa* from the Western Cape Province of South Africa where the disease is absent. The decision to select a minimum temperature threshold for development that is considerably outside the published range makes their model results very difficult to interpret.

- Conclusions
  - CLIMEX Compare Locations can provide misleading results for this species because of the lack of data from sites where the pest is marginal, the difficulty of addressing key events in the life cycle of the pathogen and their relation to host phenology together with the short time scale over which some key events in the life cycle operate.

(iv) Deductive models (generic infection, leaf wetness and temperature models)

- Description of the method
  - These models focus on the key processes in the life cycle that determine whether the life cycle can be completed and perpetuated. Phenology models, based on degree-days, are often used to determine whether there is sufficient temperature above the minimum threshold to complete development. In the case of foliar fungal pathogens, typically moisture, in addition to temperature, is modelled to determine whether conditions are suitable for spore development, release and germination.
- Applications
  - Generic infection (temperature and leaf wetness) models
    - Magarey and Borchert (2003) using the generic infection model
    - EFSA (2008) using the generic infection model (Magarey et al., 2005)
    - Magarey et al. (2011) using the generic infection model (Magarey et al., 2005).
  - Inoculum production and release models (combined temperature and moisture models: degree-day models with or without moisture restriction to predict the release of ascospores)
    - Fourie et al. (2013) contradicting EFSA (2008).
- Advantages
  - The models directly simulate key processes in the pathogen life cycle on which establishment depends
- Disadvantages
  - The models need very high temporal resolution climatic data. Leaf wetness (required for the generic infection model by Magarey et al., 2005) is not commonly measured at meteorological stations.
  - The models are difficult to parameterise because they need experimental data to estimate the minimum, optimum and maximum temperatures for successful infection, the minimum and maximum wetness durations for successful infection and the tolerance to short dry periods. Only limited experimental data are available to estimate the parameters of the Magarey et al. (2005) model for *P. citricarpa*.
  - The timing of life cycle events must be closely related to host phenology to help predict the likelihood of establishment.
- Conclusions

It was concluded that a combination of model based assessments could give a better insight into the risk of establishment of *P. citricarpa* in the EU territory. Three models have been used (1) a model by Fourie et al. (2013) describing the timing of pseudotheticia maturation in *P. citricarpa*; (2) a model by Fourie et al. (2013) describing the seasonal time course of ascospore release; and (3) the model by Magarey et al. (2005) describing when environmental conditions (temperature, humidity) are suitable for infection. The results of these three models have been combined with records and expert knowledge on the phenology of susceptible host tissues. The overall conclusions have been based on a qualitative assessment of the establishment potential following the EFSA guidance document (EFSA PLH Panel, 2010).

### 3.3.2.3. Analyses of climate suitability done by the Panel

The suitability of the environment was analysed by the Panel mainly using two different types of model simulations:

- simulations of pseudothecium maturation and ascospore release with the models of Fourie et al. (2013) (section 3.3.2.4);
- infection simulations with the generic infection model of Magarey et al. (2005) (section 3.3.2.5).

Environment suitability was evaluated from these simulations and from the periods of susceptibility of citrus leaves and fruits derived from the scientific literature and from technical documents (see section 3.3.1.1).

In addition, the Panel undertook a limited investigation of the CLIMEX model parameterisation for *P. citricarpa* done by Yonow et al. (2013) (section 3.3.2.6).

### 3.3.2.4. Simulations of pseudothecium maturation and ascospore release

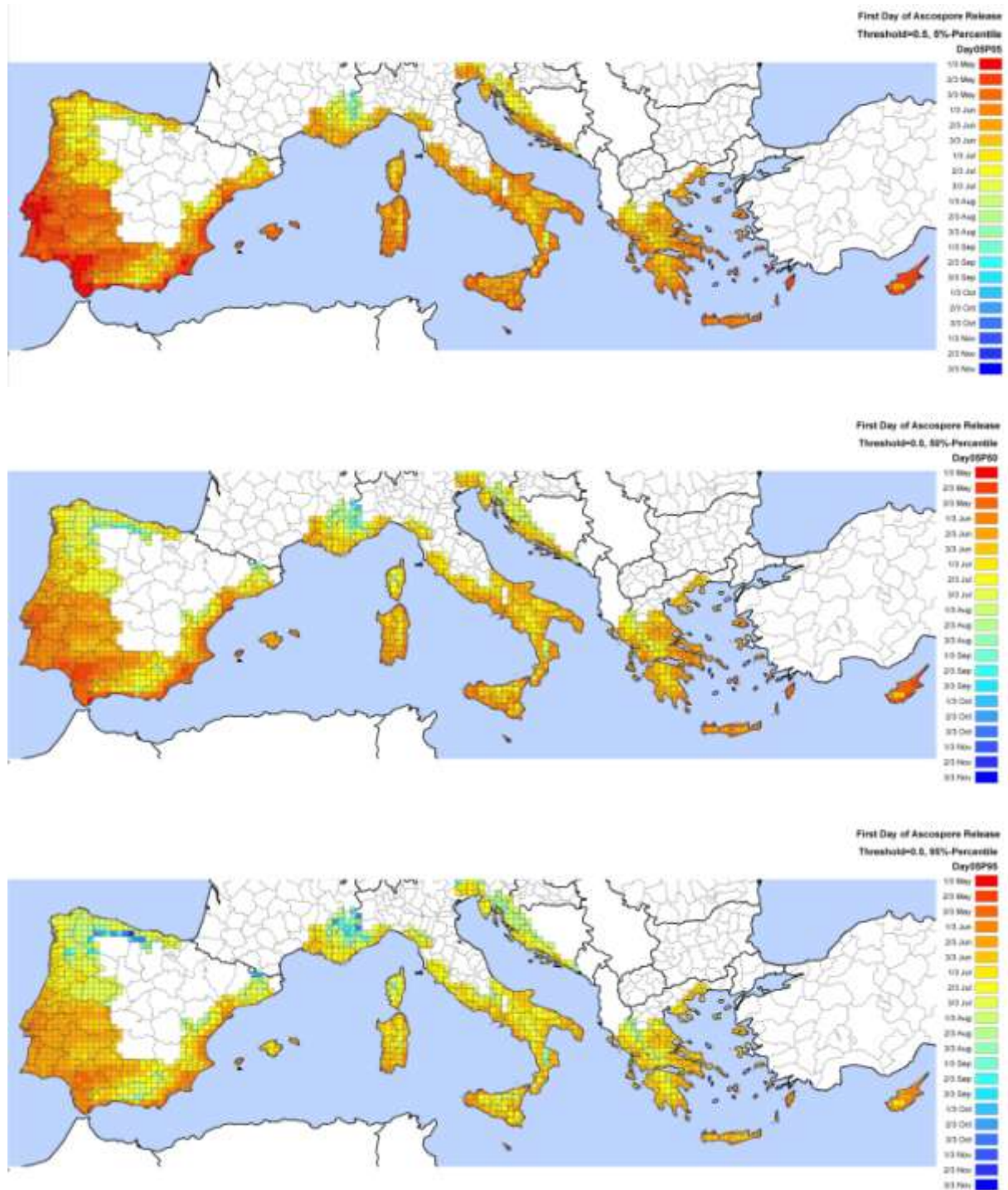
Fourie et al. (2013) parameterised models to predict pseudothecium maturation and the onset and seasonal course of ascospore discharge of *Phyllosticta* spp. (*P. citricarpa* and *P. capitalensis*). These models were previously developed for the pear scab pathogen, *Venturia pyrina* Aderh., by Rossi et al. (2009). The models of Fourie et al. (2013) were fitted to ascospore trap data collected in the Limpopo province of South Africa. The authors compared several variants of their models and finally recommended two models:

- A model based on a Gompertz equation predicting the onset of ascospore release as a function of degree-day accumulation from daily weather data using mid-winter (i.e. 1 January in the northern hemisphere and 1 July in the southern hemisphere) as the biofix and 10 °C as the base temperature (further referred to as **Model 1**). Time of onset is defined in this model as the moment at which the probability of spore discharge on days that are suitable for such discharge (three-day cumulative rainfall > 0.2 mm or vapour pressure deficit < 5 hPa) pass a predefined threshold. Fourie et al. (2013) recommend probability thresholds of 0.5 and 0.7. The capture of spores on days that are suitable for spore release is thus used as evidence that the pseudothecia are mature.
- A model based on a Gompertz equation predicting the cumulative proportion of ascospores trapped per season as a function of degree-day accumulation only on days with measurable rainfall (>0.1 mm) or vapour pressure deficit < 5 hPa) (further referred to as **Model 2**). This model does not define a relationship between the predicted proportion of ascospore release and CBS incidence/severity.
- Model 1 was run by Fourie et al. (2013) using average monthly climatic data for CBS-free locations, including Valencia (Spain), Messina (Italy) and Pontecagnano (Italy) in Europe in addition to CBS-affected sites in Brazil, South Africa and the USA. However, model outputs were not compared (evaluated/validated) with ascospore trapping data at any of these CBS-affected locations. When the original model developed for *V. pyrina* by Rossi et al. (2009) in Italy was compared with ascospore trapping data from dissimilar climatic regions (e.g. Norway, Belgium and Australia), differences between the predicted and observed date of ascospore release were up to 24 days too early and 15 days too late (Eikemo et al., 2011). Model 1 is a parameterisation for *Phyllosticta* spp. of the model by Rossi et al. (2009). Thus, in the absence of area-wide evaluation/validation, extrapolations of this model to climatic regions different from where it was developed are likely to be affected by the same problems of over- or under-estimation. According to Figure 1 in Fourie et al. (2013), the onset of

ascospore release would occur between May and June in Valencia and Messina and between June and July in Pontecagnano, based on the probability thresholds of 0.5 or 0.7. However, the between-year variability in the onset of ascospore release was not investigated and the uncertainty of the model prediction was not analysed by the authors. Fourie et al. (2013) concluded that the bulk of ascospores in Mediterranean-type climates would most likely be released during the dry summer months, but did not run Model 2 to predict the dynamics of ascospore release for any European location. Fourie et al. (2013) indicated a threshold for ascospore release of 18 °C derived from ascospore catches in CBS-affected orchards in Limpopo Province, South Africa. This value represents the average temperature in this region when sufficient degree-days were accumulated for pseudothecia maturation. Kotzé (1963) demonstrated that ascospores can be readily released from mature pseudothecia over a temperature range from 5 °C to 25 °C. Thus, prevailing temperatures during ascospore release may vary depending on the dynamics of degree-day accumulation in different climatic regions.

Model 1 was run by the Panel with daily weather data interpolated to a 25-km grid for the EU citrus-growing areas to predict the potential onset of ascospore release at these locations. The 0.5 and 0.7 thresholds were evaluated using a weather dataset consisting of daily data from 29 consecutive years (1983–2011). The results of the simulations using the 0.5 probability threshold are shown in Figure 28; other outputs are included in Appendix F. The model predicted the onset of ascospore release from the beginning of May to the end of June, depending on locations and years. In general, onset of ascospore release occurred earlier in coastal citrus-growing regions than in inland areas. The areas predicted to have May as the dominant period (50th percentile) for the onset of ascospore release are Cyprus, Malta, some of the islands in Greece and some areas in southern Spain. Model 1 was also run for eight agrometeorological stations located in citrus-growing regions in Italy (Caronia Buzza, Lentini, Mineo, Misilmeri, Paterno, Ribera, Riposto and Siracusa) to obtain the biofix to run Model 2 and predict the subsequent dynamics of ascospore release (Figure 36 and Figures 59 to 66 in Appendix C).

When running the ascospore maturation and release model (Fourie 2) it was observed that a minor proportion of the spores would not mature within one growing season, and would not be released until the following season. This might be a consequence of extrapolating these models to a region with a climate markedly different from that in the area where they were developed and evaluated. Although semi-arid conditions are not particularly detrimental for leaf litter survival, the data available on citrus leaf litter decomposition indicate that it is unlikely that fallen leaves will maintain their integrity as a substrate for inoculum production for such a long period (Lee and Huang, 1973; Mondal and Timmer, 2002; Mondal et al., 2003; Upadhyaya et al., 2012; Bassimba et al., 2014). Therefore, only predictions for the first year have been considered (Figure 36 and Figures 59 to 66 in Appendix C).



**Figure 28:** Onset of ascospore release predicted by Model 1 (Fourie et al., 2013) for a 25 km-grid interpolated climatic data for the EU citrus-growing areas from 1989 to 2009 (Probability threshold set to 0.5 and the upper, middle and lower map shows respectively the 5th, 50th and 95th percentiles of the results for the 21 years)

For all studies in which weather or climate data are used to assess the suitability of environment for a pest in a new area, it is a prerequisite that the weather data should be recorded under standard meteorological conditions in order to be comparable. Standard meteorological conditions mean that measurement equipment is placed in an open field with a standard cover of a grass lawn kept cut short

on a regular basis. The sensors measuring air conditions such as air temperature, wind speed and relative humidity should be placed at the standard height of two metres above ground. When these data are used as inputs to model based simulations, in which the key parameter values originate from laboratory experiments where the measurements underlying the estimates of environmental conditions are not recorded under standard meteorological conditions, this can cause error and introduce additional uncertainty into the model results. Taking air temperature as an example, the air temperature close to citrus leaf litter lying on the ground, or close to the surface of a living citrus leaf, can differ by several degrees from the air temperature recorded at the same time at a nearby meteorological station where the air temperature is recorded two metres above ground in an open field. Ribeiro et al. (2005) evaluated daily and seasonal changes in leaf temperature in relation to the variation of meteorological elements (global radiation and air temperature) and air vapour pressure deficit in field-grown citrus plants and recorded differences between leaf and air temperatures up to 8 °C. For instance, during daytime hours, under clear skies, leaf litter is warmer than air temperature (Jiménez-Bello et al., 2011). The increment of temperature in the leaf litter might favour ascospores release particularly in those areas where low temperature can be a limiting factor for ascospores release.

### 3.3.2.5. Infection simulations with the generic infection model of Magarey et al. (2005)

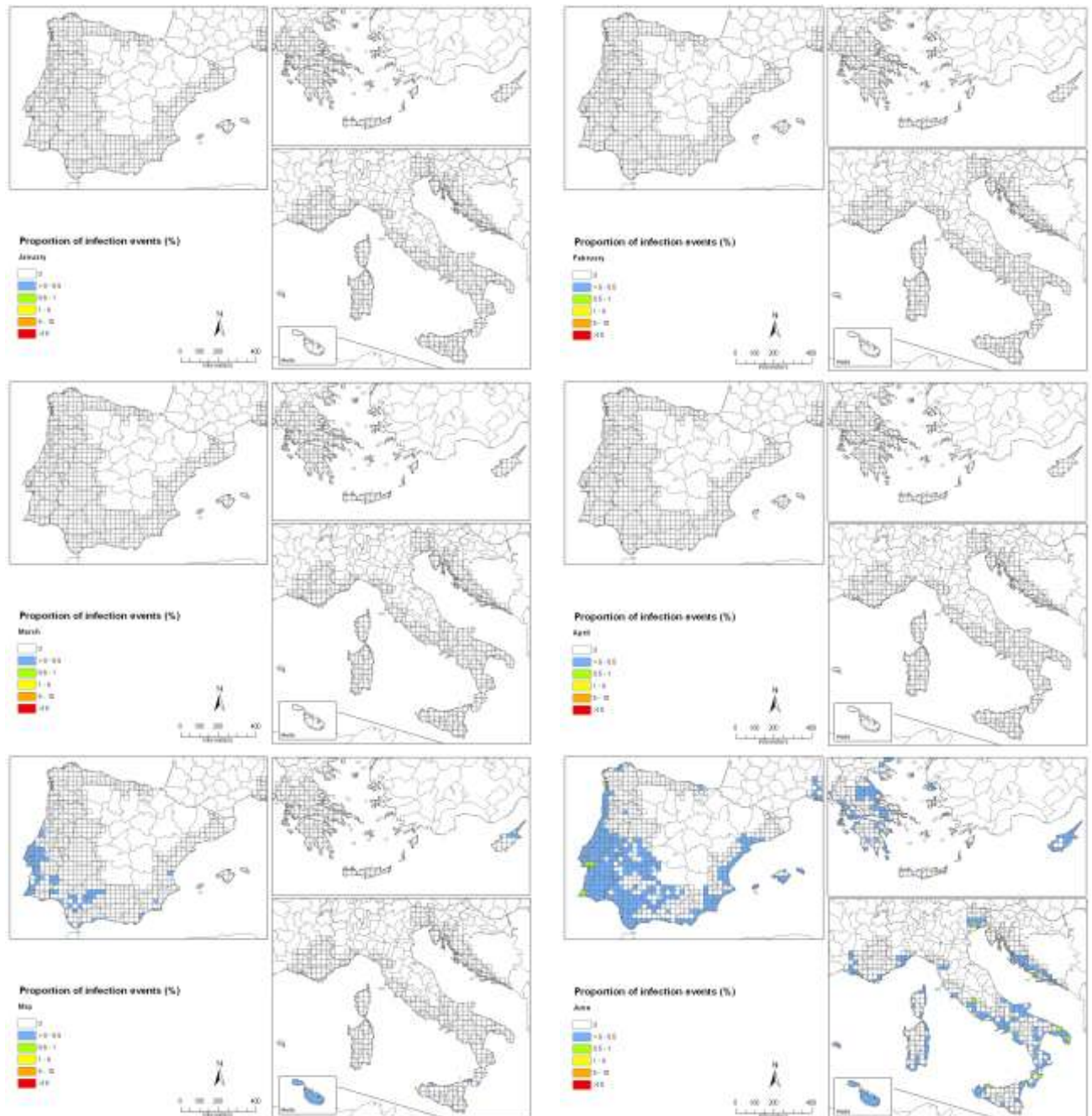
This model was first run for *P. citricarpa* for EU citrus-growing areas by EFSA (2008) with meteorological data from the MARS Crop Yield Forecasting System (MCYFS; JRC Monitoring Agricultural Resources Unit) interpolated to a 50-km grid for the EU citrus-growing areas with simulated wetness data (Bregaglio et al., 2010, 2011) and with agro-meteorological station data (14 Spanish stations and 10 Italian stations) equipped with on-site wetness sensors. The model was also applied by EFSA (2008) to climatic datasets from locations where CBS is present as well as extra-EU locations where it is not known to occur. In this scientific opinion, the model simulation results for climatic suitability for *P. citricarpa* infection in EU citrus-growing areas were updated using a four times higher spatial resolution (25 km) grid of interpolated weather data from the JRC-MARS database (JRC, 2012; JRC-MARS, online). The data for the years 1989 to 2009 were extracted for 1 518 grid cells of a 25 × 25 km grid covering all European NUTS regions of level 3 with citrus production (see Figure 27). This approach allowed climate suitability to be analysed at the continental scale and at higher spatial and temporal resolution, but without considering some specific microclimatic features of citrus orchards, which might provide suitable conditions for infection even under unfavourable macroclimate (Vicent and García-Jiménez, 2008). Therefore, available data from on-site agroclimatic weather stations in EU-citrus growing locations were also included for simulation.

The model by Magarey et al. (2005) requires estimates of the three cardinal temperatures ( $T_{max}$ ,  $T_{min}$ ,  $T_{opt}$ ), of two wetness duration thresholds ( $W_{max}$ ,  $W_{min}$ ) and a parameter describing tolerance to dry interruptions ( $D_{50}$ ). When based on infection data, the model computes the leaf surface wetness duration requirement to produce 20% disease incidence or 5% disease severity on inoculated plant parts at a given temperature. However, this threshold does not apply when infection efficiency data are not available. In any case, the model does not define a relationship between the predicted number of infection events (hourly or daily) and the incidence/severity of the disease. The criteria for potential disease development based on the frequency and distribution of infection events is set by the modeller and may differ depending on the pathogen and its host (Magarey et al., 2007). The parameter values for *P. citricarpa* were estimated by EFSA (2008; Table 3, page 36) based on published experiments on germination or infection by pycnidiospores and ascospores separately. Studies on the infection efficiency of *P. citricarpa* spores under different combinations of temperature and wetness durations are not available. Thus, parameters values were mainly obtained from published data on spore germination and mycelial growth. A sensitivity analysis carried out by EFSA (2008) indicated that model uncertainty was mainly due to the parameters  $D_{50}$  and  $T_{min}$ . EFSA (2008) set the  $T_{min}$  for ascospores to 15 °C, based on the studies by Kotzé (1963), who reported germination of *P. citricarpa* ascospores at this temperature. However, lower temperatures were not tested in this experiment and therefore the possibility of infection below 15 °C cannot be excluded. For pycnidiospores  $T_{min}$  was set at 10 °C based on Noronha (2002). The value of  $T_{max}$  for both pycnidiospores and ascospores was set

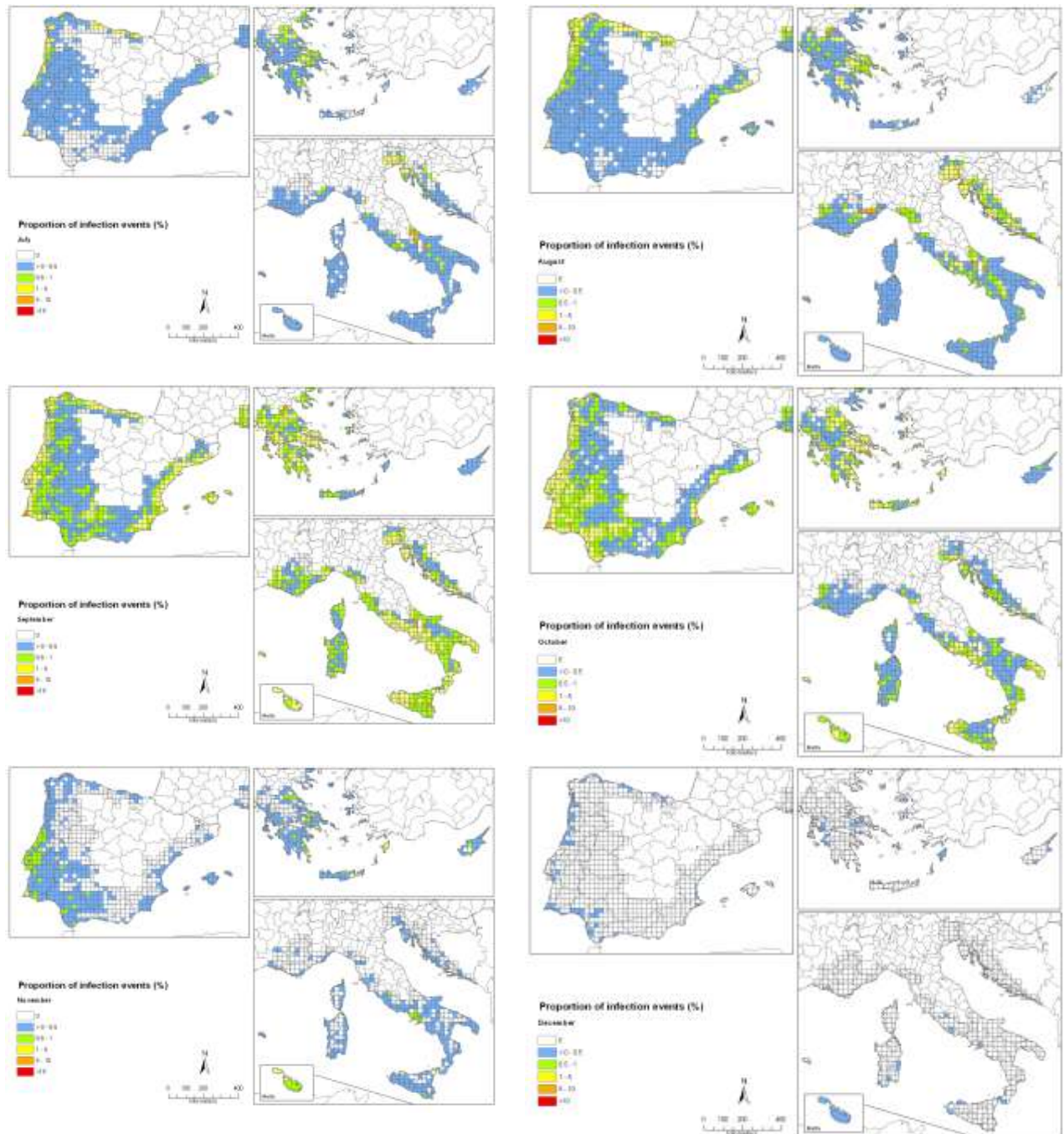
at 35 °C, as indicated by Magarey et al. (2005), when there is no information on the upper temperature limit for infection, as is the case for *P. citricarpa*. With regard to  $T_{opt}$ , Kotzé (1963) obtained the highest germination rate at 29.5 °C, which was also the highest temperature tested. The optimal temperature for the growth of *P. citricarpa* on liquid basal synthetic medium is 27 °C (Kotzé, 1981) and the optimal temperature for hyphal growth is 25–28 °C (Chiu, 1955). Noronha (2002) obtained peaks of appressoria formation for pycnidiospores at 25 °C for most incubation periods. Therefore, EFSA (2008) used a  $T_{opt}$  of 27 °C for ascospores and 25 °C for pycnidiospores. McOnie (1967) demonstrated that ascospores can infect with at least 15 hours of continuous wetness. This value is supported by Kotzé (1963), who obtained 15.7 % germination of ascospores after 15 hours of incubation at 29.5 °C, showing consistency between germination and infection data. An appressoria formation rate of approximately 30 % was observed for pycnidiospores after 12 hours of incubation (Noronha, 2002). Thus, EFSA (2008) set the value of  $W_{min}$  to 15 hours for ascospores and 12 hours for pycnidiospores. A  $W_{max}$  value of 38 hours for ascospores was selected by EFSA (2008) according to the results of Kotzé (1963) and 35 hours for pycnidiospores based on Noronha (2002). No information was found in the literature on the sensitivity of *P. citricarpa* to dry interruptions during infection, so  $D_{50}$  was set to three hours as a value which is often found in the literature as being a generally acceptable period of leaf wetness interruption (Xu and Butt, 1993; Rossi et al., 2007). In the case of pycnidiospores, to account for the splash dispersal requirement, only those infection events preceded by rains were considered. The parameters of EFSA (2008) were later used by Magarey et al. (2011).

The model used by EFSA (2008) predicted numerous pycnidiospore and ascospore infection events over a 10-year period (1998–2007) at agro-meteorological stations and 50-km grids. With the gridded data, few infection events were predicted in summer (June–August), but some events occurred at many locations in the spring and significant numbers were predicted in late summer and early autumn. In general, data from agro-meteorological stations followed the same pattern, although with a somewhat longer infection period, reflecting the effect of microclimate variability. The results obtained from the new simulations conducted for this scientific opinion, with a higher spatial and temporal resolution using the 25-km grid and the 21-year period (1989–2009), are in line with EFSA (2008). Infection events by pycnidiospores and ascospores were predicted from May to November in most citrus-growing areas in the EU (Figures 29, 30, 31 and 32 and Appendix F). In general, the highest values of weather suitability for infection by pycnidiospores and ascospores were obtained in September and October. The same trend was observed when the model was applied to data from on-site agro-meteorological stations in Italy (Figure 36 and Figures 59 to 66 in Appendix C and Appendix F).

In addition, an analysis of the duration of periods with weather conditions unsuitable for infection by *P. citricarpa* ascospores during the 21-year period (1989–2009) was conducted for the 25-km grid cells representing citrus production (Figure 33). When considering the longest duration of unsuitable conditions across the 21-year period (1989–2009), coastal areas showed durations lower than one or two years and, in inland areas, values increased to more than two or three years. Taking into account the fact that leaves can survive on the tree for at least two years (Spiegel-Roy and Goldschmidt, 2008) and that commercial citrus production in the EU is strongly concentrated in coastal areas, these results indicate that suitable weather conditions for ascospore infection occurs in every growing season over the 21-year period studied in key citrus-growing regions in the risk assessment area.

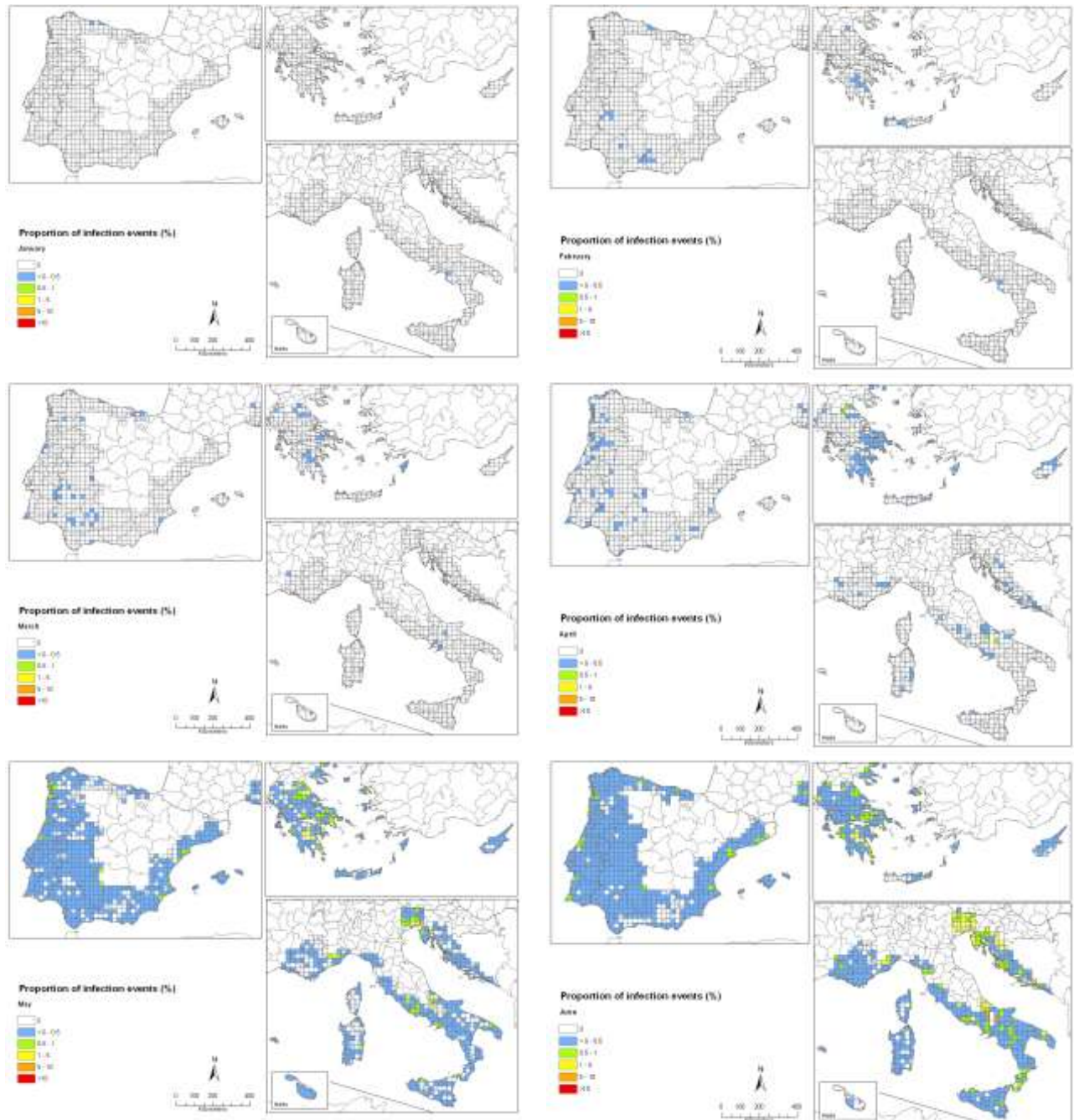


**Figure 29:** Percentage of hours with weather conditions suitable for successful infection events by *Phyllosticta citricarpa* pycnidiospores (generic infection model for foliar fungal pathogens by Magarey et al. (2005) with  $D_{50} = 3$  hours and  $T_{min} = 10$  °C) with additional requirement of a rain event per day of infection using interpolated weather data (January to June) from 1989-2009 period (JRC 2012; JRC-MARS, online) in citrus-growing grid cells (based on NUTS3 citrus production data) updating previous simulations by EFSA (2008) for the 1998-2007 period

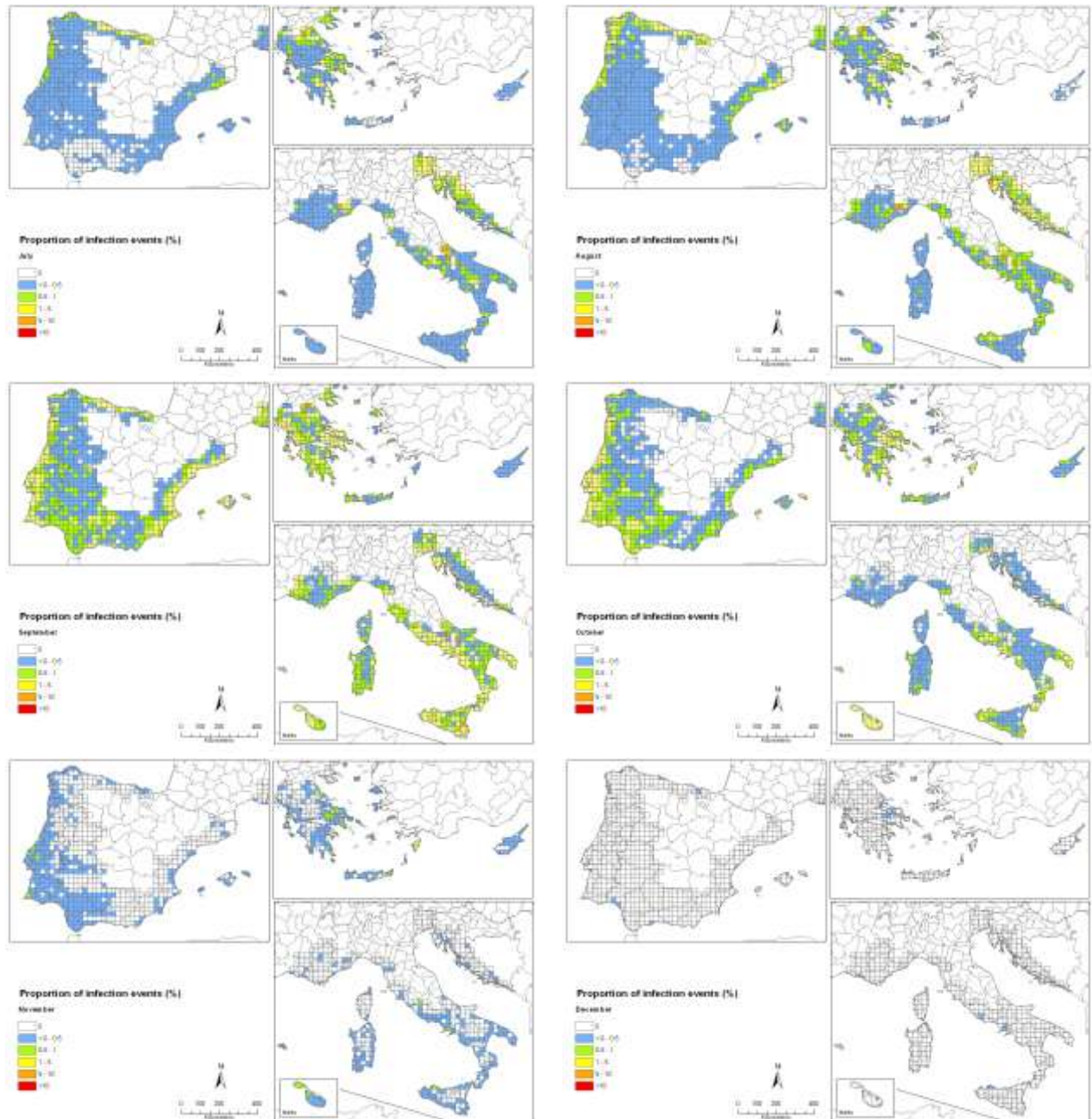


**Figure 30:** Percentage of hours with weather conditions suitable for successful infection events by *Phyllosticta citricarpa* pycnidiospores (generic infection model for foliar fungal pathogens by Magarey et al. (2005) with  $D_{50} = 3$  hours and  $T_{min} = 10$  °C) with additional requirement of a rain event per day of infection using interpolated weather data (July to December) from 1989-2009 period (JRC, 2012; JRC-MARS, online) in citrus-growing grid cells (based on NUTS3 citrus production data) updating previous simulations by EFSA (2008) for the 1998-2007 period

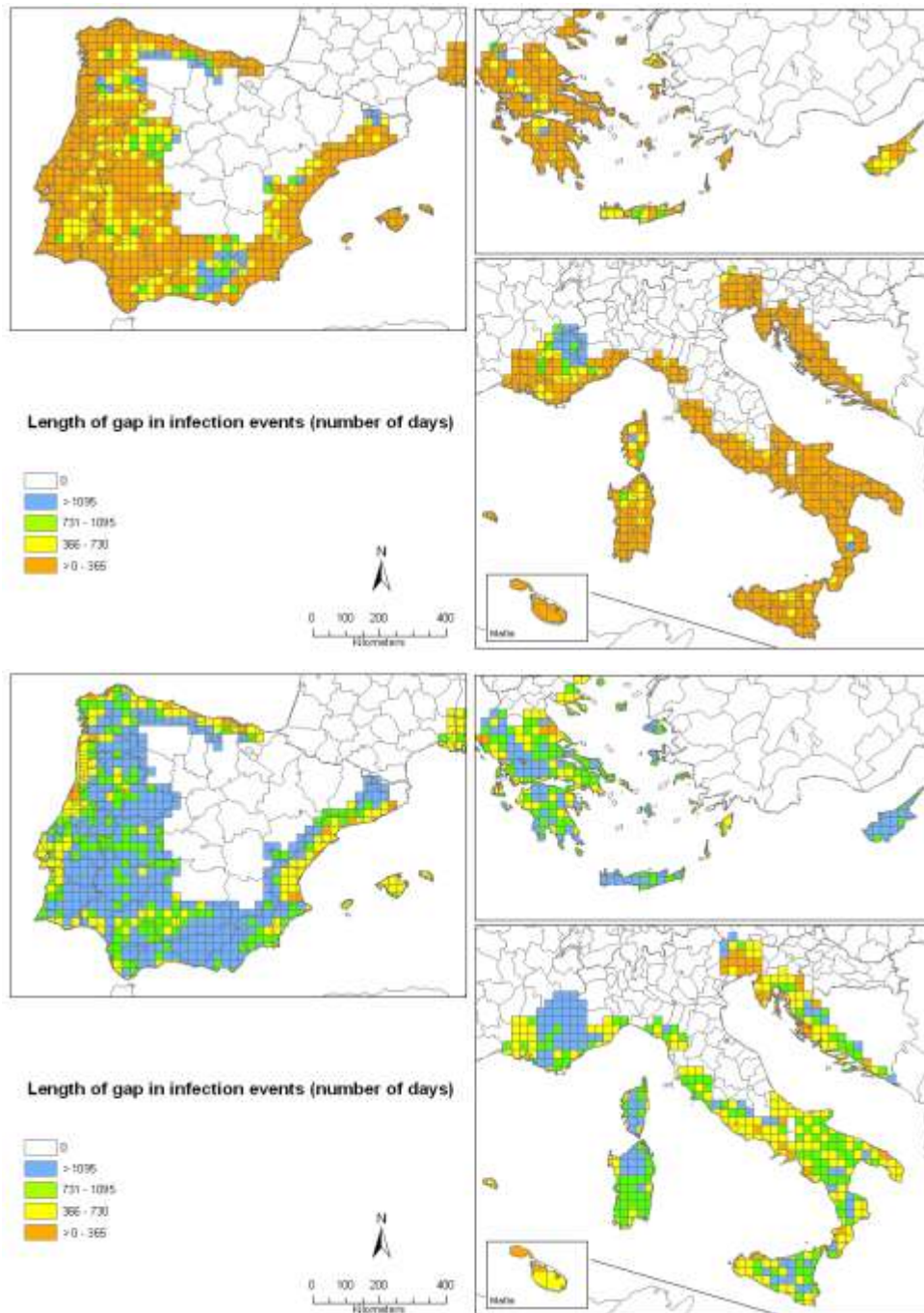




**Figure 31:** Percentage of hours with weather conditions suitable for successful infection events by *Phyllosticta citricarpa* ascospores (generic infection model for foliar fungal pathogens by Magarey et al. (2005) with  $D_{50} = 3$  hours and  $T_{min} = 15$  °C) using interpolated weather data (January to June) from the 1989–2009 period (JRC, 2012; JRC-MARS, online) in citrus-growing grid cells (based on NUTS3 citrus production data) updating previous simulations by EFSA (2008) for the 1998–2007 period



**Figure 32:** Percentage of hours with weather conditions suitable for successful infection events by *Phyllosticta citricarpa* ascospores (generic infection model for foliar fungal pathogens by Magarey et al. (2005) with  $D_{50} = 3$  hours and  $T_{min} = 15$  °C) using interpolated weather data (July to December) from the 1989–2009 period (JRC, 2012; JRC-MARS, online) in citrus-growing grid cells (based on NUTS3 citrus production data) updating previous simulations by EFSA (2008) for the 1998–2007 period



**Figure 33:** Average (top) and maximum (bottom) duration of periods with weather conditions unsuitable for successful infection events by *Phyllosticta citricarpa* ascospores (generic infection model for foliar fungal pathogens by Magarey et al. (2005) with  $D_{50} = 3$  hours and  $T_{min} = 15$  °C) using interpolated weather data from the 1989–2009 period (JRC, 2012; JRC-MARS, online) in citrus-growing grid cells (based on NUTS3 citrus production data)

### 3.3.2.6. Conclusions from model simulations on inoculum availability and infection

The duration of leaf litter integrity on the orchard floor is an important factor for pseudothecia and ascospore production. Warm temperatures together with high soil moistures have been shown to enhance citrus leaf litter decay in South Africa and Taiwan (Kotzé, 1963; Lee and Huang, 1973), limiting further pseudothecia and ascospore development. No data have been found from the semi-arid areas in South Africa where CBS is present. Few experiments on citrus leaf litter decomposition are available, but general studies on leaf litter decomposition indicate that the decomposition rate increases with both mean annual temperature and precipitation, mainly due to the enhanced activity of the decomposer organisms (Zhang et al., 2008). In Spain, young immature mandarin leaves affected by *Alternaria* brown spot that have fallen on the orchard floor survived for up to 76 days until complete decay. The survival of mature leaves was not studied, but the authors indicated that they will survive for longer in semi-arid conditions than in humid climates (Bassimba et al., 2014). In the humid and tropical conditions of India, complete leaf decomposition of mature citrus leaves takes up to five months (Upadhyaya et al. 2012). Taking into account that under Mediterranean climate conditions high rainfall amounts seldom coincide with warm temperatures, the survival of mature citrus leaves infected by *P. citricarpa* on the orchard floor is not considered to be a limiting factor for the production of *P. citricarpa* ascospores in the EU citrus-growing areas.

Pseudothecia and ascospores are produced in the leaf litter after periods of alternate wetting and drying (Kiely, 1948; Lee and Huang, 1973; McOnie, 1964b). In addition to the seasonal rains, the high frequency of dews through the year in the Mediterranean citrus-growing areas might enhance the formation and maturation of *P. citricarpa* pseudothecia in the leaf litter (Vicent and García-Jiménez, 2008). The extensive use of surface, sprinkle and micro sprinkle irrigation in the EU citrus-growing areas (section 3.3.3.1) might add to the suitability of the environment since it has the potential to lengthen the periods of leaf wetness aiding infection. Both sprinkle and micro sprinkle directly wet the tree canopy. In the case of surface irrigation, Scherm and van Bruggen (1995) found that surface irrigation in lettuce crops significantly increased wetness duration and air humidity close to the soil surface when compared with sub-surface drip irrigation, where soil surface is not wetted. An increase in leaf wetness duration due to irrigation would be expected particularly for citrus growing areas where sprinkler or micro-sprinkler irrigation is more common (section 3.3.3.2).

For a polyetic disease like CBS, epidemics take place when annual disease cycles merge into a multi-year continuous sequence (Zadoks and Schein, 1979). For this type of epidemic, a regular overlap between susceptible host, inoculum availability and weather conditions conducive to infection is generally required. As indicated above (section 3.3.1.1), susceptible leaves are present all year around in the citrus-growing regions of the EU and susceptible fruits are present from May to December. In the case of lemons, susceptible fruits are present all year around. With regard to inoculum availability, it is important to note that citrus is an evergreen tree and leaves remain active in the canopy for more than two years before they fall (Spiegel-Roy and Goldschmidt, 2008). Once fallen on the orchard floor, it takes about 23 to 180 additional days for pseudothecia maturation and subsequent ascospore formation on the leaf litter, depending on the prevailing temperature and humidity regimes (Lee and Huang 1973; Kotzé 1981). Consequently, infected leaves can fall off and may produce inoculum at a time relatively long after they originally were infected by *P. citricarpa*. In addition, *P. citricarpa* can colonise and sporulate on dead twigs in the canopy, and this is considered to be an important source of inoculum in field epidemics (Whiteside, 1967; Spósito et al., 2011). Pycnidiospores produced in affected twigs may infect nearby leaves, which will eventually fall on the orchard floor, forming leaf litter in which the pathogen may reproduce and spread through ascospores. The duration of the infectious period of *P. citricarpa* in affected twigs is not known, but it is likely to be conditioned by the survival of the plant substrate itself. Due to the lack of quantitative data, infections by pycnidiospores produced in twigs were not quantified and are discussed here only in the context of overwintering (see also section 3.6.1.2). Taking into account that, in addition to the leaf litter, the pathogen can overwinter between seasons in infected leaves and twigs in the canopy, the absence of infection for a few years, will not necessarily lead to a break in the multi-year disease cycle. Figure 33 shows that during 1989–2009 the EU areas where the bulk of commercial citrus production is located

had weather conditions suitable for *P. citricarpa* ascospore germination and infection on average every growing season, with a maximum duration of unsuitable periods of less than two years. These results, together with the low inter-year variability of potential inoculum availability obtained by the ascospore maturation models, indicate that environmental conditions conducive to CBS development are continuously met in parts of the risk assessment area allowing its potential establishment and spread.

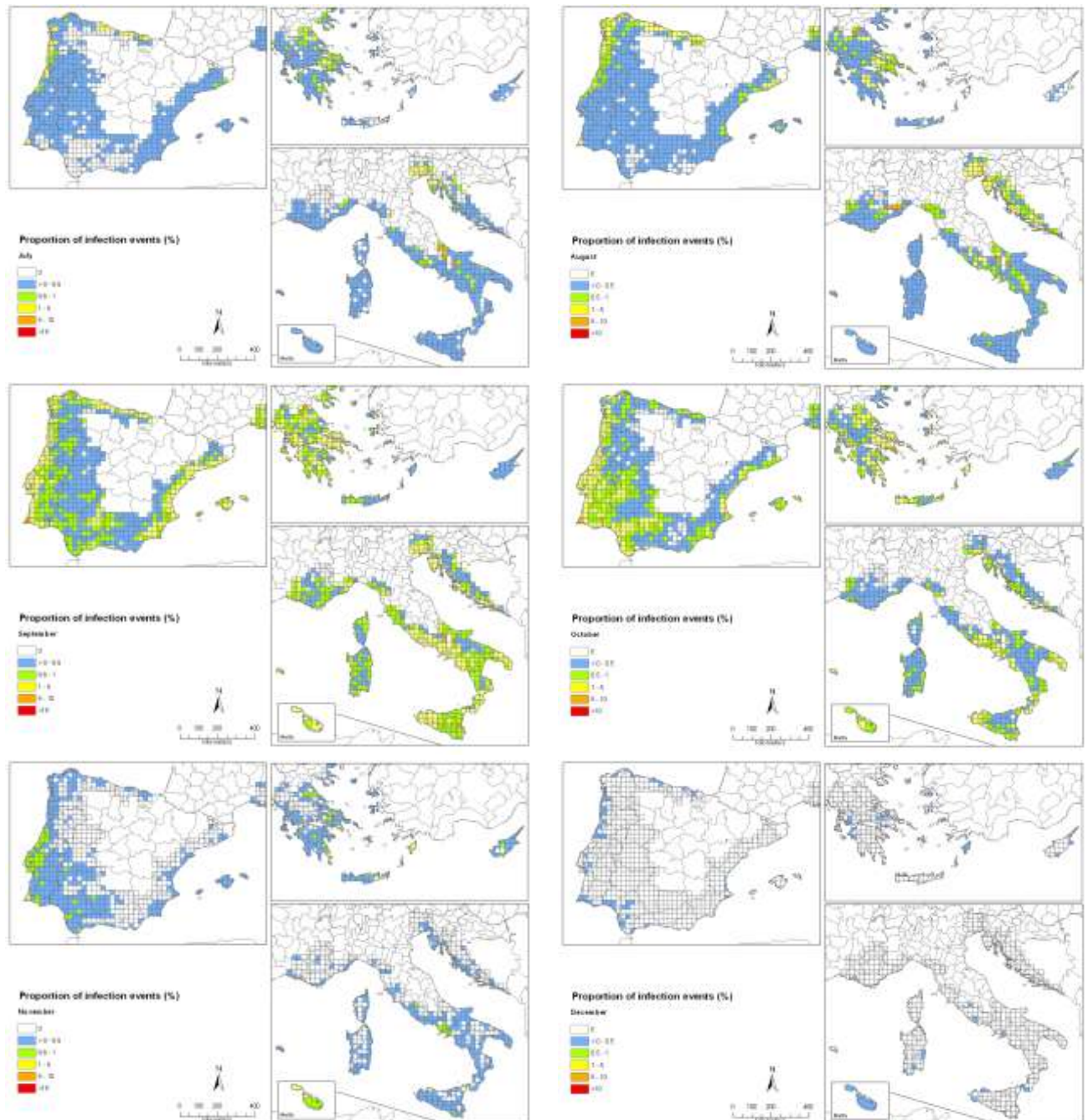
Figures 34 and 35 show maps that superimpose the model outputs on ascospore inoculum availability (Fourie et al., 2013) and infection (Magarey et al., 2005) on the principal European citrus-growing areas. These maps show that no significant infection periods by ascospores of *P. citricarpa* are estimated before May. The number of grid cells (Figures 34 and 35) and the proportion of years (Figures 56 and 57 in Appendix F) show that environmental conditions suitable for inoculum availability and infection progressively increase, reaching a maximum in September, when in some grids weather conditions suitable for infection was present 5 % of the time. The potential for infection was lower in November and December. These results indicate that late summer and early autumn are the most likely period of infection for *P. citricarpa* ascospores in the main EU citrus-growing areas.

Figure 36 and the Figures 59–66 in Appendix C show the monthly dynamics of ascospore release predicted for six years at eight Italian agro-meteorological stations with the two Fourie et al. (2013) models, together with the average proportion of hours with environmental conditions favourable for ascospore infection predicted by the Magarey et al. (2005) model as described in EFSA (2008) with  $D_{50} = 3$  hours. Data on potential ascospore release were obtained on a daily basis whereas hourly estimates of the weather conditions for infection were produced. Monthly summaries of the outputs from both models have been presented for clarity.

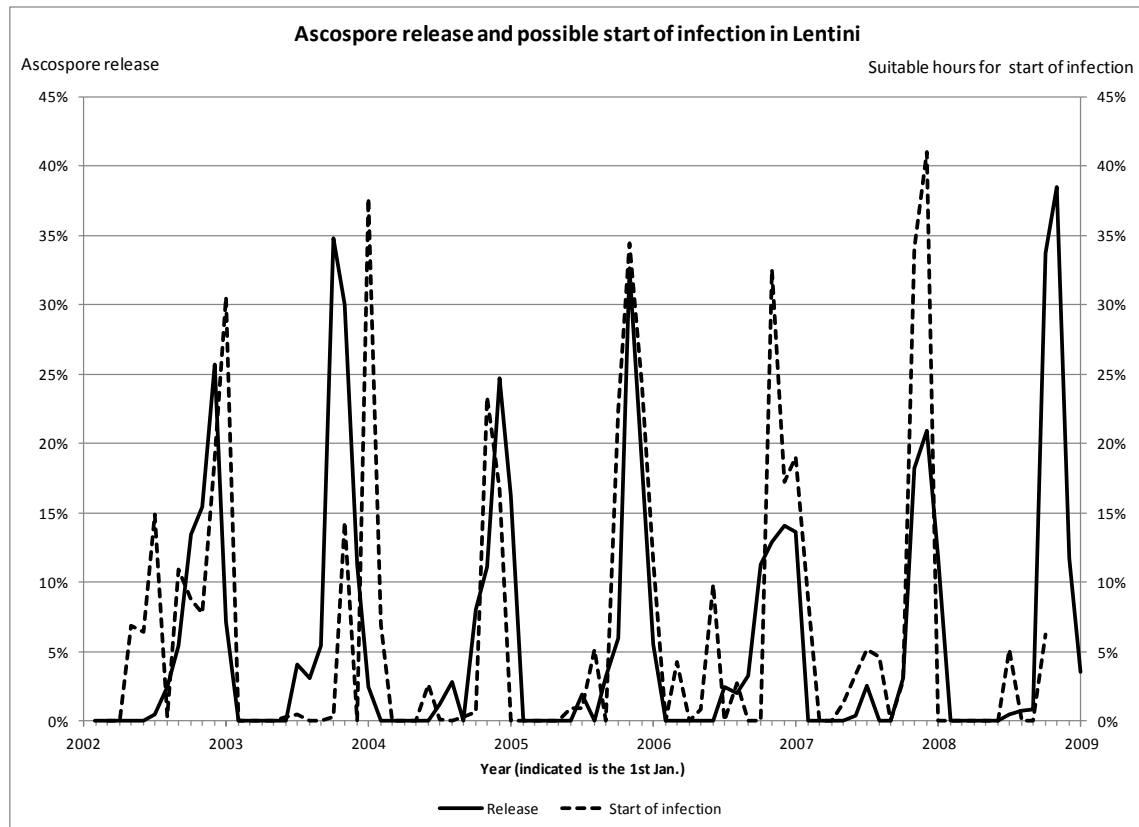
The results from the on-site agro-meteorological stations confirm the outputs obtained using the gridded climatology (Figures 34 and 35) that there is generally an overlap between potential ascospore release and the weather conducive to infection, with peaks in late summer and early autumn when susceptible leaves and fruits are widely available in the risk assessment area (section 3.3.1.1). From these results, it can be concluded that the climate in the risk assessment area would sustain the reproduction, dissemination and infection of *P. citricarpa* ascospores, at least at some European locations.



**Figure 34:** Percentage of hours with weather conditions suitable for *Phyllosticta citricarpa* ascospore availability and infection (Fourie model 1: threshold = 0.7; Fourie model 2: average cumulative ascospore release  $\geq 1\%$ ) and ascospore infection (generic infection model for foliar fungal pathogens by Magarey et al. (2005) with  $D_{50}=3\text{h}$  and  $T_{min}=15^{\circ}\text{C}$ ) in citrus-growing grid cells (based on NUTS3 citrus production data) with environmental conditions suitable for *P. citricarpa* ascospore production and release using interpolated weather data (January to July) from 1989-2009 period (JRC, 2012; JRC, 2013; JRC-MARS, online).



**Figure 35:** Percentage of hours with weather conditions suitable for *Phyllosticta citricarpa* ascospore availability and infection (Fourie model 1: threshold = 0.7; Fourie model 2: average cumulative ascospore release  $\geq 1\%$ ) and ascospore infection (generic infection model for foliar fungal pathogens by Magarey et al. (2005) with  $D_{50}=3\text{h}$  and  $T_{min}=15^{\circ}\text{C}$ ) in citrus-growing grid cells (based on NUTS3 citrus production data) with environmental conditions suitable for *P. citricarpa* ascospore production and release using interpolated weather data (July to August) from 1989-2009 period (JRC, 2012; JRC, 2013; JRC-MARS, online).



**Figure 36:** Comparison of the dynamics of ascospore release (2002–2007) and average percentage of hours suitable for start of a successful infection by *P. citricarpa* ascospores (2002–2008) predicted for the station of Lentini in Italy

**Uncertainty:** The results of the Fourie et al. (2013) models should be interpreted with caution for several reasons and the Panel considers that there is a high uncertainty related to these predictions because:

- The models were developed and evaluated in the Limpopo province of South Africa, a region characterised by a summer rainfall pattern. The capability of the models to predict ascospore release in other areas has not been investigated and it is likely to be negatively affected by climatic differences (Eikemo et al., 2011).
- Although the standard deviations of the parameter estimations are reported in Table 5 by Fourie et al. (2013), the consequences of the uncertainty concerning the parameter values on model predictions were not analysed by the authors.
- The results presented in Figure 1 of Fourie et al. (2013) are based on average monthly climatic data. The authors do not report on the between-year variability of ascospore release.
- Model 2 was not used by Fourie et al. (2013) to predict the proportion of ascospore release in Europe.
- Rossi et al. (2009) used a base temperature of 0 °C to calculate degree-days, but Fourie et al. (2013) chose 10 °C. With this higher base temperature, negative values are obtained for some days in many Mediterranean locations. The Panel adopted the general practice of considering negative values as zero values in the degree-days calculation (De Wit and Goudriaan, 1978).



- The models were originally fitted to ascospore trap data consisting of a mixture of two species, *P. citricarpa* and *P. capitalensis*, in unknown proportions.

Experiments carried out to determine the temperature and wetness duration requirements of *P. citricarpa* were reviewed by EFSA (2008) and showed that, owing to the scarcity of experimental data available, there is a high uncertainty concerning the values of the parameters describing the climatic requirements for infection. The minimum and optimum temperatures for infection and the degree of tolerance to dry periods were considered as highly uncertain by the PLH Panel (see section 2.3.2 of EFSA, 2008). The uncertainty analysis performed by EFSA (2008) for the Magarey et al. (2005) generic infection model using agro-meteorological station data showed that the simulations of infection were highly uncertain (see section 2.3.5.3 of EFSA, 2008). As no new experimental study has been made available to estimate the parameters of the infection model since 2008, the Panel considers that the level of uncertainty concerning these aspects is unchanged.

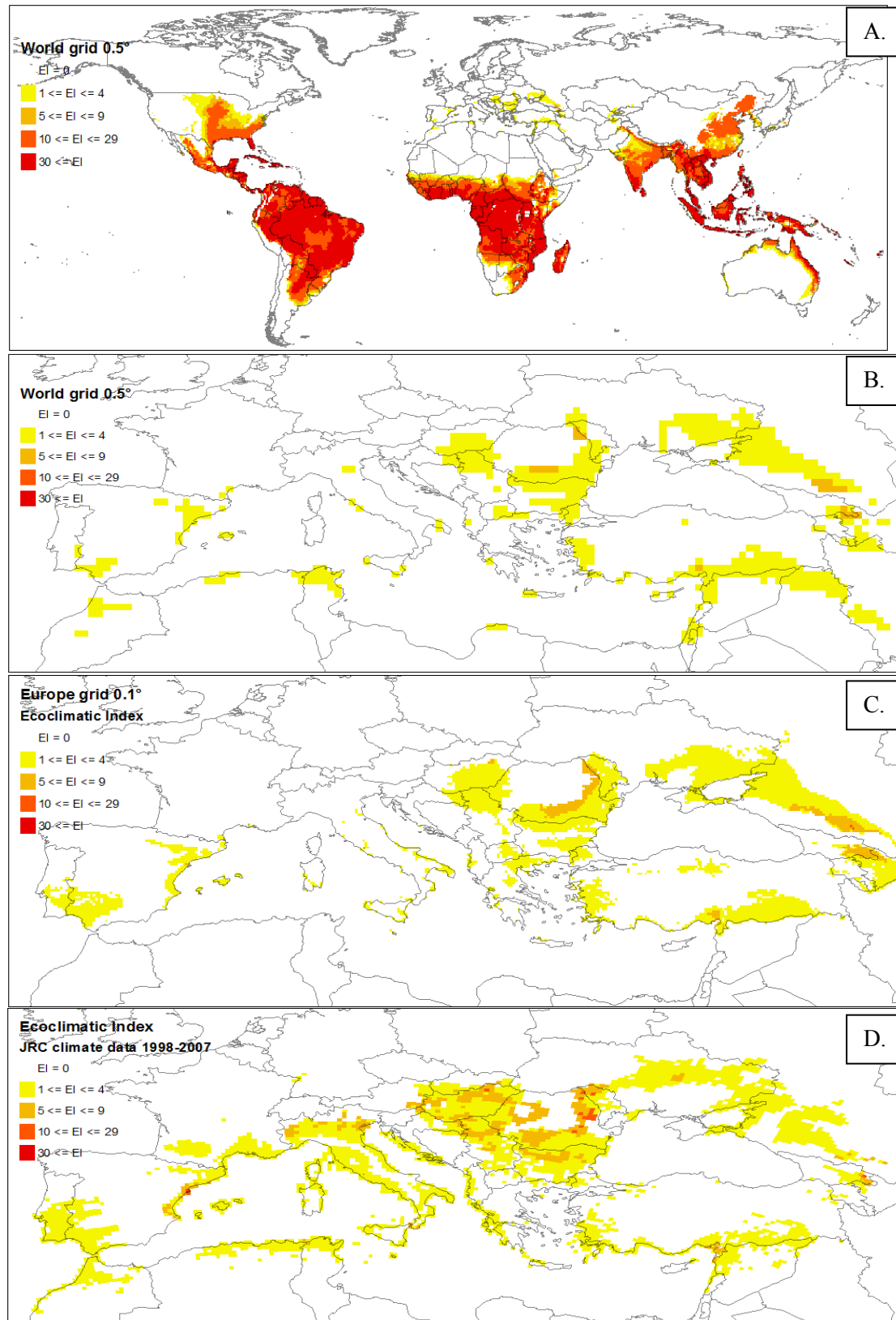
The Panel concluded that the only way to further reduce the uncertainty concerning the climatic requirements of *P. citricarpa* would be to conduct new experiments in order to determine more precisely the temperature and wetness duration requirements of this fungus. Based on the sensitivity analysis presented in EFSA (2008), the parameters that have the strongest influence on the wetness requirements calculated by the wetness model of Magarey are the minimum temperature requirement for infection and the degree of tolerance of the dry period (see Table 6 in section 2.3.5.3 of EFSA, 2008) are. Thus, it would be useful to carry out experiments to estimate these parameters more accurately.

### 3.3.2.7. CLIMEX model parameterised to model the potential global distribution of the citrus black spot disease by Yonow et al. (2013)

In 2008, the EFSA Panel on Plant Health evaluated a pest risk assessment for *Guignardia citricarpa* conducted by South Africa (Hattingh et al., 2000) and additional supporting material (Paul et al., 2005; Paul, 2006). In its evaluation, the EFSA (2008) scientific opinion expressed concerns about the appropriateness of applying the CLIMEX modelling approach underpinning the pest risk assessment for *Guignardia citricarpa* conducted by South Africa. These concerns expressed by EFSA (2008) were recently challenged by Yonow et al. (2013), who published a new set of CLIMEX parameters for *P. citricarpa* in order to model the potential global distribution of the pathogen with a particular focus on the risk posed to Europe.

In the preparation of this scientific opinion the Panel explored the basis for the arguments raised by Yonow et al. (2013) (see section 3.3.2.2). In addition, the Panel analysed the sensitivity of the CLIMEX model outputs for Europe to climate data inputs at different spatial resolutions and time periods (see Figure 37).

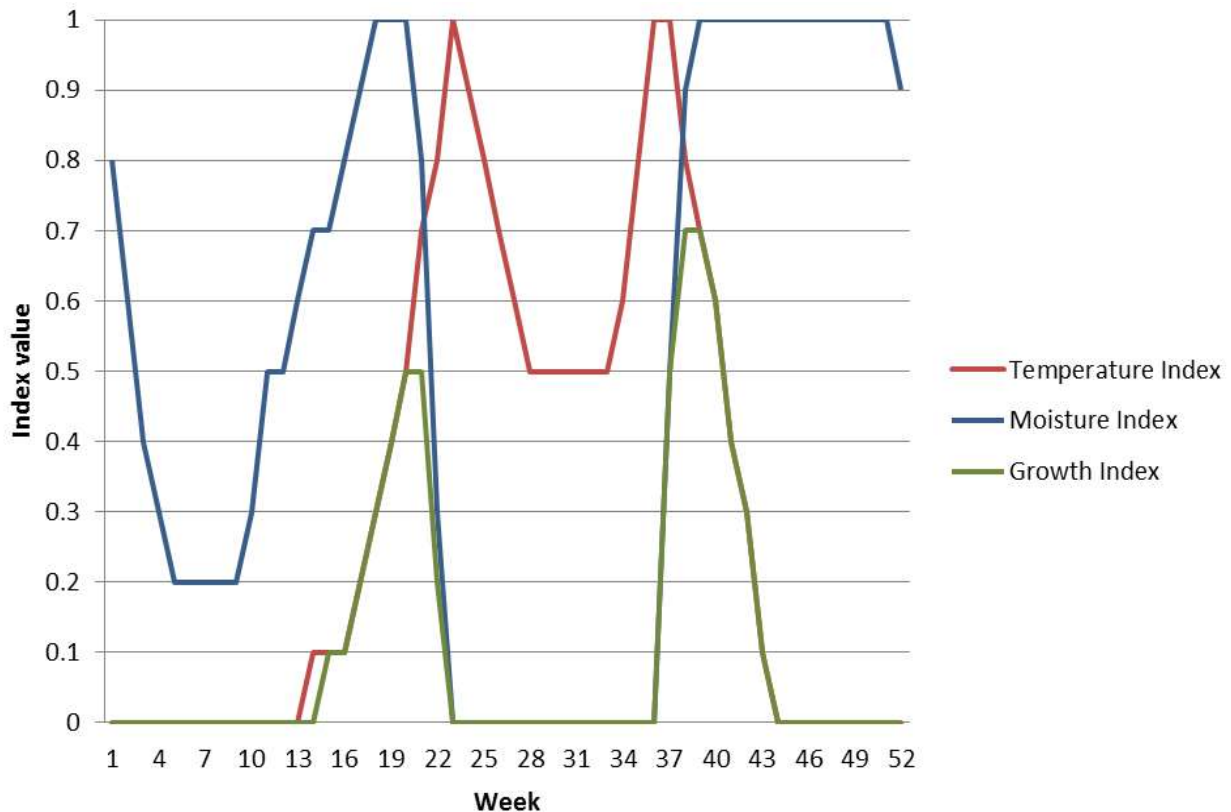
To display the results of their CLIMEX model, Yonow et al. (2013) used a 0.5° latitude × 0.5° longitude grid with interpolated monthly 1961–1990 climatic data. When this is replaced by a higher spatial resolution (0.1° latitude × 0.1° longitude) 1961–1990 climatology (New et al., 2002) (see Figure 37C), a larger area of citrus production in Europe is suitable for *P. citricarpa* based on the classification utilised by Yonow et al. (2013). When the Yonow et al. (2013) CLIMEX model is run with more recent climate data for 1998–2007 (JRC, 2012) at a different spatial resolution (25 km × 25 km), a larger area is predicted as being suitable (Figure 37D) and some areas have a higher EI than that predicted for the period 1961–90. According to the classification of the EI by Yonow et al. (2013), one area is even predicted to be highly suitable. This corresponds to the area of the Ebro delta in eastern Spain, where the northernmost commercial citrus production in the country takes place. Overall, it can be concluded from these analyses that the suitability of climate for *P. citricarpa* as predicted by the CLIMEX model parameterised by Yonow et al. (2013) is very sensitive to the spatial resolution and time period of the climate data inputs.



**Figure 37:** Prediction of the potential global distribution of *P. citricarpa* according to the CLIMEX model developed by Yonow et al. (2013) based on (A) 0.5° latitude × 0.5° longitude global spatial resolution 1961–90 average climate data, (B) the latter zoomed to Europe, (C) 0.1° latitude × 0.1° longitude spatial resolution 1961–90 climate data and (D) 1998–2007 JRC climatic data at 25 km resolution.

A detailed examination of the output from the Yonow et al. (2013) CLIMEX model in EU citrus-growing areas shows that positive EIs occur where sufficiently high temperatures and moisture for pathogen development coincide. With the exception of a small accumulation of cold–wet stress, the outputs for all the other stress indices included in the model do not exceed zero, indicating that no stresses accumulated during the season that is unfavourable for development.

In Figure 38, the results for the two key factors promoting growth in CLIMEX, namely the Temperature Index and the Moisture Index, and the product of these two, the Growth index, have been plotted at the weekly resolution on which CLIMEX operates at one location in the Ebro delta.



**Figure 38:** Weekly CLIMEX Temperature, Moisture and Growth Index values from the Ebro delta region in eastern Spain for 1961–90 at 0.1° latitude × longitude spatial resolution

The Panel concludes from these results that CLIMEX parameterisation by Yonow et al. (2013) appears to emulate the temperature and moisture requirements of the fungus in a similar way to the modelling approach for spore maturation and infection adopted by the Panel, but with a lower level of detail. The accumulation of the Growth Index for *P. citricarpa* occurred during weeks 15–22 in spring and weeks 37–43 in autumn (Figure 29). More detailed analyses can be found in Appendix D.

Yonow et al. 2013 (drew) the conclusion from their CLIMEX modelling of the potential global distribution for citrus black spot disease that “*Within European citrus producing regions, suitable areas are highly constrained, never more than marginally suitable, and all have lower levels of suitability than any area in South Africa and Australia where G. citricarpa is known to occur*”. However, the results presented here from a limited analysis of this new CLIMEX model support the Panel’s concerns about the extent to which the CLIMEX Compare Locations procedure can provide reliable results for this species. This is because the CLIMEX model for *P. citricarpa* parameterised by Yonow et al. (2013):

- Is shown to be highly sensitive to the spatial resolution and time period of the climate data inputs with regard to whether the EU citrus-growing areas are suitable for establishment of *P. citricarpa*. Thus, for some of the EU citrus-growing areas, the climatic suitability classification varies from “marginally suitable”, through “suitable” and even to “highly suitable” based on the classification of the EI by Yonow et al. (2013) when changing either the spatial resolution or the temporal period of the climate data inputs.
- For the EU citrus-growing areas, the outputs from the Yonow et al. (2013) CLIMEX model mainly show where high temperatures and moisture coincide, as very little stress is accumulated in these areas
- The summary of high temperature and moisture coincidences provided by CLIMEX cannot be used to draw reliable conclusions about the extent to which EU citrus-growing areas have a suitable climate for *P. citricarpa*.

### 3.3.3. Cultural practices and control measures

#### 3.3.3.1. Irrigation

Practically all the commercial citrus orchards existing in the EU are irrigated nowadays (Carr, 2012). However, the type of irrigation system employed is not uniform throughout the EU citrus orchards. This is important because the irrigation system employed and its management can potentially influence the development of *P. citricarpa* primarily by affecting the decomposition rate of leaf litter and ascospore production and release due to changes in its moisture content. Orchard microclimate can be also potentially affected by irrigation practices, like surface flood irrigation, which may induce higher humidity levels and longer wetness durations close to the soil surface (Schermer and van Bruggen, 1995). On the other hand, irrigation is recommended in some CBS-affected areas as a part of the integrated disease control programs. In South Africa, the incidence of CBS is higher on wilted trees due to water stress induced by improper irrigation. Wilting during the months just prior to harvest appeared to encourage disease development more than wilting periods during flowering (Kotzé, 1963; 1971). In Brazil, irrigation is recommended to concentrate flowering and avoid the coexistence of fruit with different growth stages in order to minimise infections of young fruits by pycnidiospores produced in mature fruits (Spósito et al., 2005).

The irrigation systems used in the EU citrus orchards are surface irrigation, sprinkler irrigation and micro-irrigation (see Stewart and Nielsen, 1990 for more details). Whereas 40 years ago most citrus plantations were surface irrigated, the general trend is to replace surface irrigation methods with pressurised systems (sprinkler and micro-irrigation), reducing soil evaporation, increasing the overall orchard irrigation efficiency and minimising the volume of water applied.

#### Surface irrigation

In these irrigation systems, the irrigation water is applied at one edge of an orchard and flows across the soil surface by gravity. As water moves over the soil, water infiltrates into the rootzone. Irrigation applications generally take place every 13–25 days, watering the soil to a depth of 40–80 mm at each irrigation event. Two main types of surface irrigation methods are applied in Europe: (1) flooding, in which the entire orchard floor is irrigated, and (2) the graded furrow, in which, before the first irrigation application, furrows to convey the water across the field are ploughed between the crop rows. In the furrow irrigation method, the proportion of the soil that is wetted might vary from 30 % to 70 % of the entire citrus orchard floor. In this case, most of the ground shaded by the tree, where most of the fallen citrus leaves will remain, will be wetted. Even with the furrow method most of the leaf litter on the ground will be wetted.

#### Sprinkler irrigation

In these systems water is supplied in a pressurised network and emitted from sprinkler heads mounted on either fixed or moving supports. In European citrus orchards, only set sprinkler irrigation systems are found. Set systems are those in which the sprinklers are placed in a fixed grid or spacing. The entire orchard floor is wetted and the water applications are applied over the tree canopy, so the irrigation water completely wets the tree canopy in the same way as rainfall. Sprinkler irrigation is generally applied every 7–20 days, giving an amount of water equivalent to 20–60 mm of rainfall. In addition to irrigation, set sprinkler systems can also be used for frost protection. Sometimes citrus orchards use other types of irrigation system employing sprinkler irrigation only for frost protection.

### Micro-irrigation

Micro-irrigation includes methods that are more commonly known as drip irrigation and other low-pressure systems. Water is generally distributed in plastic conduits and emitted by trough drippers, tricklers, foggers, micro-sprinklers or sprayers. In European citrus orchards, two main types of micro-irrigation systems are found.

- *Drip irrigation*, in which water is allowed to drip slowly to the soil through an emitter with a low discharge rate (0.8–8 litres per hour). The main features are that (1) only a small proportion of the entire orchard floor is wetted by the irrigation system (15–35 % of the soil beneath each tree) and (2) applications of water are frequent (generally 1–4 mm per irrigation event, with a daily application during the summer months). Another subtype of drip irrigation is sub-surface drip irrigation, in which the pipelines transporting water and the emitters are located in the sub-soil at a depth of 30 cm, and where the water does not reach the soil surface. However, sub-surface irrigation systems are rarely used in citrus orchards in Europe because their installation is expensive and they are complex to maintain.
- *Micro-sprinkler* is another type of micro-irrigation system in which water is applied by sprayers located underneath the tree canopy, 45–70 cm above the soil. This wets 30–70 % of the entire orchard floor and some of the lower part of the tree is also directly wetted by the irrigation system.

#### 3.3.3.2. Regional differences in citrus irrigation

##### Spain

The Spanish citrus orchards are mostly irrigated by either flood or drip irrigation using low-pressure operating emitters located at the soil surface. In the Valencia region, according to Pons (2008), 67 % of the citrus orchards are irrigated using drip systems, while 32 % is under surface flooding irrigation. Micro-sprinkler irrigation is used only in the remaining 1 % of the Valencia citrus orchard plantations, where it is employed to provide some frost protection. However, this sprinkler system is not overhead and wets only the lower part of the tree canopy.

In the southern citrus-growing areas of Spain (Andalucía and Murcia), where citrus orchards plantations are generally younger (particularly in Andalucía), drip irrigation systems are more common, with 81 % of the citrus orchards using drip systems and the remaining 19 % using surface flooding irrigation (MAGRAMA, 2013b).

##### Italy

In Sicily, the dominant irrigation system is a type of micro-sprinkler irrigation which uses low-pressure sprayers that often wet most of the orchard floor (Liberati, 2008). Irrigation is applied every 8–25 days and applications range from 20 to 60 mm per session. Drip irrigation is applied in the remaining 10 % of the citrus irrigated area. Overhead sprinkler systems are used in some areas of Sicily and particularly in the regions of Calabria and Campania, but the percentage of the citrus area irrigated with overhead sprinkler systems in these two regions is only 6 % (Consoli, 2010). In the Apulian region, citrus orchards cover an area of 15 897 ha. Drip irrigation is employed in 59 % of the

irrigated area, while the percentage of orchards irrigated by micro-sprinkler, overhead irrigation and surface irrigation is 30 %, 8 % and 3 % respectively (ISTAT, 2010).

### Portugal

In Portugal, most of the commercial irrigated citrus orchards are located in the Algarve region. According to Norberto (2011), in this region, 88 % of citrus orchards are irrigated by drip irrigation, 8 % by micro-sprinklers applied below the tree canopy, at about 100 cm above the soil surface, and 4 % by surface flooding irrigation.

### Greece

According to a review by Shirgure (2012), micro-irrigation and surface flooding irrigation are the two main systems used in Greek citrus-growing areas. In Argolis county, in the South-Eastern Peloponnese (Prefecture of Argolida, Subject: Data on irrigation systems of citrus in the prefecture of Argolida, 28/11/2012), of a total citrus area of 12 500 ha, 1 000 ha is irrigated by surface flooding irrigation (8 %), 300 ha by drip irrigation (2.4 %) and 11 200 ha by low-pressure micro-sprinkler sprayers (89.6 %). In the low-pressure system, the sprayers are located at a height of 40 cm above the orchard floor with one sprayer per tree at a distance of 40–80 cm from the trunk. This means that the water drops are ejected up to a height of 60 cm, wetting most of the lower parts of the tree canopies. During the winter months, sprayers are used to protect citrus trees from frost in an area of 2 000–3 000 ha.

### Cyprus

In Cyprus, traditionally farmers have used the flooding method to irrigate citrus orchards. However, with modernisation, 26 % of the orchards are now drip irrigated. In the remaining 74 % of the irrigated citrus orchards, surface flooding irrigation that wets the entire orchard floor is applied (Mehmet and Ali Biçak, 2002).

### Malta

In Malta, the most reliable information comes from the study by Attard and Azzopardi (2005). They reviewed the irrigation systems used and water use efficiency in irrigated Maltese agriculture. Drip irrigation use has steadily increased in recent years, and 46 % of citrus is drip irrigated (National Statistics Office, Malta 2010). However, 52 % of the irrigated citrus orchards are still flood irrigated. The remaining 2 % of the orchards are irrigated by other systems other than flood and drip irrigation.

### France

In the French citrus orchards, mainly located in Corsica, 43 % of the plantations are under sprinkler overhead irrigation, while drip and micro-sprinkler irrigation are used in 28 % and 29 % of the citrus orchards, respectively (Dr Jean Bouffin, INRA, personal communication, November 2013).

### Summary of the irrigation practices in European citrus orchards and their potential role in *P. citricarpa* development

In summary, it is clear that the trend is to move away from the irrigation systems, e.g. flood and sprinkler irrigation that use large amounts of water, wet the soil surface of the whole orchard and are likely to have an influence on the microclimate within the orchard than the micro irrigation systems. However, drip irrigation and micro-sprinkler are used at a much higher frequency (days) than surface or sprinkler irrigation (weeks), so in absence of rains they may compensate for the periods of alternate wetting and drying necessary for pseudothecia and ascospore development in the leaf litter. While most of the micro irrigation systems use much less water and are likely to have a minor effect on orchard microclimate, micro-sprinkler irrigation uses spray jets located under the tree canopy that also wet the lower hanging leaves and fruit in the tree canopy increasing leaf wetness duration, that may enhance infection by *P. citricarpa* ascospores and pycnidiospores. In addition, the potential role of micro-sprinkler irrigation aiding the splash dispersal of pycnidiospores from discarded fruit to nearby citrus trees cannot be overlooked when assessing the likelihood of entry of *P. citricarpa* in the EU. As

indicated above, micro-sprinkler irrigation is particularly common in citrus-growing regions in Sicily and Greece. Even though some of the information on irrigation practices is not from recent publications, micro sprinkler irrigation together with flood and sprinkler irrigation systems are thought to be still widely used in several citrus producing regions in the EU. Although precise calculations cannot be made due to the lack of specific studies, irrigation practices used in the EU citrus-growing areas could influence in some way the likelihood of *P. citricarpa* entry and establishment by providing greater opportunities for completing the life cycle than predicted by the models based on climatic variables alone (section 3.3.2).

#### 3.3.3.3. Citrus-growing habits and other cultural practices in the EU

The range of variation in tree growth habits exhibited by the citrus trees as a whole is very wide: from the straggly, shrub-like citron to the large, highly symmetrical trees of most of the sweet oranges and grapefruits and some of the mandarins. Citrus trees are generally pruned to a central leader or a modified central leader shape, and pruning operations are conducted annually, reducing the apical dominance of the natural tree branches. A full canopy of leaves is normally maintained in order to protect the bark of the trunk and branches from direct sun and potential sunburn. Trees often have branches close to the ground (a full skirt) in order to maximise photosynthesis and therefore tree productivity (Agustí, 2012). In addition, since practically all the commercial citrus orchards in the EU are manually harvested and the labour costs are high, it is important to maintain trees that can be easily harvested by hand operators. Because of this, in general, the European citrus orchards tend to be restricted to small trees with a height that is often less than three metres (Vacante and Calabrese, 2009). In addition, modern plantations now use citrus scions grafted onto semi-dwarfing rootstocks, which limits tree height to less than 2.5 metres (Legua et al., 2011). Under these situations (Figure 16d), the weight of developing fruit generally pulls some branches down very close or even in direct contact with the orchard floor (Fake, 2012).

#### 3.3.3.4. Citrus disease management in the EU

It is considered that current fungicide spray schedules in EU citrus-growing areas generally will not prevent the establishment of *P. citricarpa*. Some late-maturing sweet orange cultivars are sprayed in autumn with fungicides such as fosetyl-Al for the control of brown rot caused by *Phytophthora* spp. These fungicides are specific for oomycetes and are ineffective against fungi such as *P. citricarpa* (Tuset, 1987). Some late-maturing mandarin hybrids, such as 'Fortune', 'Nova' and 'Murcott', are routinely sprayed in spring and autumn with copper or mancozeb for the control of Alternaria brown spot (Vicent et al., 2007, 2009). Although these chemicals are not among the most effective for the control of *P. citricarpa* (see section 3.6.1.1), they could to some extent prevent possible infections of *P. citricarpa*. However, the areas grown with cultivars susceptible to Alternaria brown spot represent a very minor proportion of the EU citrus-growing area, whereas in the most of the EU citrus-growing areas no fungicide sprays are usually applied.

### 3.3.4. Other characteristics of the pest affecting the probability of establishment

Very little is known of the rate of inoculum build-up from small initial populations of *P. citricarpa*. *P. citricarpa* may be present as latent mycelia in asymptomatic citrus fruit and leaves with a long lag phase between the first establishment and subsequent epidemic development. In South Africa, symptoms were present for over three decades before control measures became necessary (Kotzé, 1981; for more details see section 3.4.3).

### 3.3.5. Conclusions on the probability of establishment

A summary regarding the assessment of the components of the probability of establishment is presented in Table 10 below.

**Table 10:** Assessment of the components of the probability of establishment

| Rating for establishment   | Justification  |
|--|--|
| <p><i>Availability of suitable host(s)</i></p> <p><b>Widely available</b></p>  | <ul style="list-style-type: none"> <li>• Citrus is grown in southern areas of the EU with a sufficiently warm climate that is only rarely exposed to frosts.</li> <li>• Within the citrus-growing regions, the host plants are grown in commercial citrus orchards and nurseries, as well as in streets and private and public gardens, both in urban and rural areas</li> <li>• Citrus leaves are susceptible to <i>P. citricarpa</i> for 8–10 months and citrus trees have three main flushes: in spring, summer and autumn. Sweet orange fruits are susceptible for at least 6–7 months after fruit set in spring.</li> </ul>   |
| <p><i>Suitability of environment</i></p> <p>– <i>Similarity of climatic conditions in the current area of distribution</i></p> <p><b>Slightly similar</b></p>  | <ul style="list-style-type: none"> <li>• This rating is limited to the citrus-growing areas of the EU. <i>P. citricarpa</i> mainly occurs in subtropical citrus-growing regions characterised by a summer rainfall pattern and high annual precipitation. However, the disease is also present in semi-arid areas, such as the Eastern Cape province in South Africa, with an annual rainfall of approximately 400 mm, which is comparable to the rainfall in some EU citrus-growing areas. Based on simulation results, conditions in the EU citrus-growing areas during September and October are generally suitable for ascospore development and infection whereas late spring to early summer infections are limited to specific locations, e.g. in southern Spain, southern Italy, Portugal, Malta, Cyprus and the Greek islands.</li> </ul> |
| <p><i>Cultural practices and control measures</i></p> <p>– <i>To what extent is the managed environment in the risk assessment area favourable for establishment?</i></p> <p><b>Highly favourable</b></p> <p>– <i>How likely is it that existing pest management practice will fail to prevent establishment of the pest?</i></p> <p><b>Likely</b></p> | <ul style="list-style-type: none"> <li>• For several reasons, EU citrus orchards are designed and maintained as small trees and their height is often lower than three metres. The weight of developing fruit generally pulls some branches down very close or even in direct contact with the orchard floor and this will aid splash dispersal of pycnidiospores.</li> <li>• Irrigation techniques wetting the leaves and fruit are still in use in parts of the EU citrus-growing areas, thus creating a micro-environment favourable to the establishment of the disease</li> </ul>   |
| <p><i>Other characteristics of the pest affecting the probability of establishment</i></p> <p><b>Likely</b></p>  | <ul style="list-style-type: none"> <li>• Small populations are likely to become established as there is evidence that shows that it may take decades from initial introduction until epidemics reach damaging levels of impact.</li> </ul>   |



| Rating for establishment  | Justification  |
|---|--|
| <p><i>Overall probability for establishment</i></p> <p><b>Moderately likely</b></p> | <ul style="list-style-type: none"> <li>Based on the modelling of ascospore maturation and release, the Panel found additional evidence that part of the risk assessment area has climate conditions favourable for inoculum production for <i>P. citricarpa</i>.</li> <li>The results from these simulations based on both the gridded and station weather data show that there are locations where the period of host susceptibility, inoculum availability and suitable environmental conditions for infection overlap.</li> <li>The likelihood of establishment is assessed as <b>moderately likely</b>: owing to the simultaneous occurrence of host susceptibility and weather conditions suitable for ascospore production and infection (primary infection cycle).</li> </ul> |

### 3.3.6. Uncertainties on the probability of establishment

Overall, uncertainty on the probability of establishment is rated as high, mainly because of a lack of knowledge on how *P. citricarpa* will respond under the EU climatic conditions. Although the environmental factors that are important in the various stages of the life cycle are known, there is insufficient scientific evidence to determine the thresholds of the values the organism requires, e.g. for the temperature and wetness levels and durations (Table 11). Further validation of the models applied by the Panel, especially for marginal areas within the current distribution of the citrus black spot disease, would be needed to reduce the uncertainty on the probability of establishment of *P. citricarpa* in the EU.

**Table 11:** Uncertainties concerning the probability of establishment

| Level of uncertainty  | Justification   |
|---|---|
| <p><i>Availability of suitable host(s)</i></p> <p><b>Low</b></p>  | <ul style="list-style-type: none"> <li>The citrus varieties grown in the EU are known to be susceptible to <i>P. citricarpa</i></li> </ul>  |
| <p><i>Suitability of environment</i></p> <p>– <i>Similarity of climatic conditions in the risk assessment area and in the current area of distribution</i></p> <p><b>High</b></p> | <ul style="list-style-type: none"> <li>Only limited data are available to precisely define some of the climatic requirements of the pathogen (temperature and wetness duration thresholds). The previous EFSA opinion showed that the outputs of the epidemiological model of Magarey et al. (2005) were highly sensitive to some parameters (<math>D_{50}</math> and <math>T_{min}</math>).</li> <li>As the relationship between the proportion of ascospore release and infection efficiency has not been studied, there is a high level of uncertainty concerning the threshold of percentage of ascospore release that must be set for the Fourie 2 model to determine whether ascospore release occurs or not (it has been set to 1 % in this opinion).</li> <li>There is limited information on pathogen presence/absence, as well as disease development data, from semi-arid areas within the current area of distribution, e.g. the Eastern Cape Province of South Africa. If marginal areas within the current distribution could be defined, detailed weather data from such marginal areas could reduce uncertainty about suitability of climate in areas outside the current distribution .</li> </ul> |

| Level of uncertainty  | Justification   |
|---|---|
| <p><i>Cultural practices and control measures</i></p> <ul style="list-style-type: none"> <li>- <i>Managed environment favour establishment</i></li> </ul> <p><b>Low</b></p> <ul style="list-style-type: none"> <li>- <i>Existing management practices will fail to prevent establishment</i></li> </ul> <p><b>Low</b></p> | <ul style="list-style-type: none"> <li>• The cultural practices in use are relatively well known and some quantitative data on the cultivation practices in use in the EU citrus production area are available.</li> <li>• The meta-analysis of fungicide trials shows that most fungicide treatments have limited efficacy in eliminating the pathogen. It could therefore be assumed that there is low uncertainty about the likely failure of existing non-targeted management practices to prevent pathogen establishment.</li> </ul> |
| <p><i>Other characteristics of the pest affecting the probability of establishment</i></p> <p><b>High</b></p>   | <ul style="list-style-type: none"> <li>• Very little is known on the rate of inoculum build-up from small initial populations of <i>P. citricarpa</i>.</li> </ul>   |
| <p><i>Overall probability for establishment</i></p> <p><b>High</b></p>  | <ul style="list-style-type: none"> <li>• The uncertainty is high, mainly because of (i) the uncertainty on key biological parameters of the pathogen, (ii) the need for more experimental data covering a wider range of climatic and citrus growing conditions to model the establishment potential, (iii) the lack of knowledge on the relationship between ascospore proportion and infection efficiency and (iv) the lack of knowledge about the rate of inoculum build-up for this pathogen.</li> </ul>                              |

### 3.4. Probability of spread after establishment

#### 3.4.1. Spread by natural means

Natural spread of *P. citricarpa* occurs by ascospores and pycnidiospores, the former by wind dispersal and the latter primarily by splash dispersal. The pycnidiospores are formed into asexual fruiting bodies (pycnidia) on lesions in fruit, twigs and leaf litter. Pycnidiospores are splash dispersed or washed off by rain for relatively short distances, infecting susceptible leaves and fruit. On fruit and twigs only pycnidiospores are formed, while on citrus leaf litter both ascospores and pycnidiospores are formed. Data on ascospore dispersal distances are not available. Pazoti et al. (2005), referring to a paper by Goes (2002), stated that “*ascospores are spread not only on short, but also on long distances: the wind can spread it, infecting orchards at kilometres of distance*”. However, no data on ascospore dispersal were provided by Goes (2002). In still air, pycnidiospores in infected fruit were rain splashed vertically at a height of over 60 cm and horizontally at a distance of at least 70 cm. With the combined effect of rain and wind, pycnidiospores disseminated up to eight metres downwind, reaching heights up to 75 cm and even higher as a result of fine droplets becoming aerosolised (Perryman and West, 2014).

Spatial analysis conducted in Sao Paulo, Brazil, using several statistical indices indicated an aggregated pattern of CBS-symptomatic trees in the orchards, but no data on ascospores/pycnidiospore dispersal were provided. The mean distances between symptomatic trees were close to the spacing between neighbouring trees (four to six metres), indicating dispersion of the disease over short distances. Disease incidence varied from 0.6 % to 84.9 % of symptomatic trees and the maximum radius of symptom aggregation was 24.7 metres, but observed only in one year and in one orchard (Spósito et al., 2007). These studies were conducted in a high-rainfall zone in Brazil and spatio-

temporal analyses of disease spread in other climatic regions where CBS is present, such as particular semi-arid areas in South Africa, are not currently available.

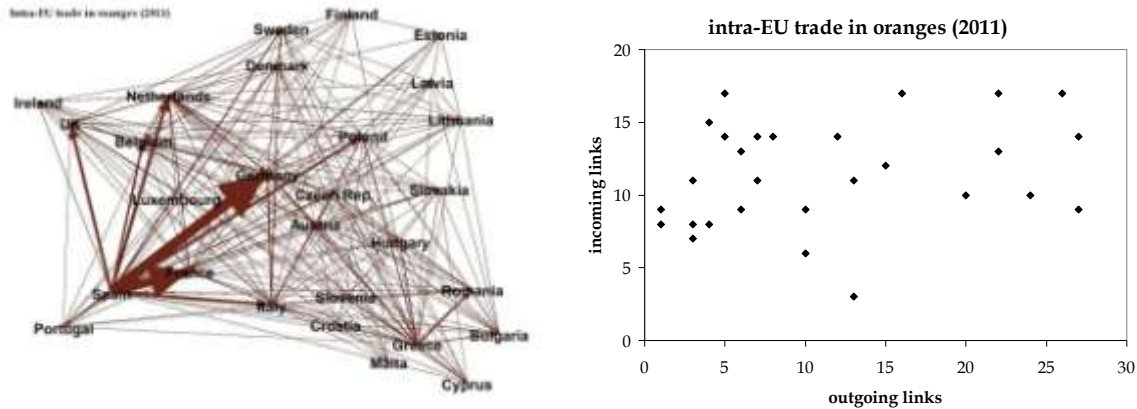
Kiely (1948) observed an equal distribution of CBS through the vertical plane on the northern side of the trees, suggesting that wind-borne ascospores were more important in disease spread than water-borne pycnidiospores. In contrast, Kotzé (1963) reported that in South Africa CBS incidence was significantly higher in the upper halves of the tree (52.83 %) than in the lower halves (28.44 %). This phenomenon was presumed to be induced by differences in environmental conditions between the upper and lower portions of the canopy during the period of disease development, but not to more infections taking place in the tree tops (Kotzé, 1963). The spatial pattern of CBS incidence within citrus trees in Sao Paulo, Brazil, was aggregated. The low frequency of infection on the upper third and the high aggregation of diseased fruits on the upper and middle thirds of the trees suggested that splash-dispersed pycnidiospores have an important role in increasing the disease in citrus trees in Brazil. The high severity at low incidence observed was also indicative of relatively short-distance spread of *P. citricarpa* pycnidiospores (Spósito et al., 2008). Further experiments conducted in the absence of leaf litter demonstrated that CBS-affected fruit and dead twigs on the tree were able to spread the disease in the canopy. The recorded distance of disease spread was less than 80 cm from these inoculum sources (Spósito et al., 2011). Nevertheless, none of the studies indicated above include ascospore or pycnidiospore assessments and results are also likely to be influenced by environmental and host factors.

#### 3.4.2. Spread by human assistance: fruit trade

The citrus fruit trade networks shown in this section were created using Gephi, an open-source and free software for network visualisation and analysis (<https://gephi.org/>). Networks are sets of ‘nodes’ (in this case, EU MS) connected by links (in this case, consignments of citrus fruit during 2011). For the trade of citrus fruit, the networks are directed, because export of a certain amount of citrus fruit from country A to country B in year Y does not imply that the same amount (and type) of citrus fruit is exported from country B to country A in the same year Y, so that it is important to keep track of the direction of trade flows.

The network based on the intra-EU trade data for oranges in 2011 (Eurostat, online) is shown in Figure 39. Croatia is included because this country joined the EU in July 2013. The network has 28 nodes and 320 links (320 incoming and 320 outgoing), and thus a connectance ( $C = L/N^2$ ) of 0.41. This means that 41 % of the potential links are realised. The total volume of sweet oranges traded in 2011 was about 2 million tons.

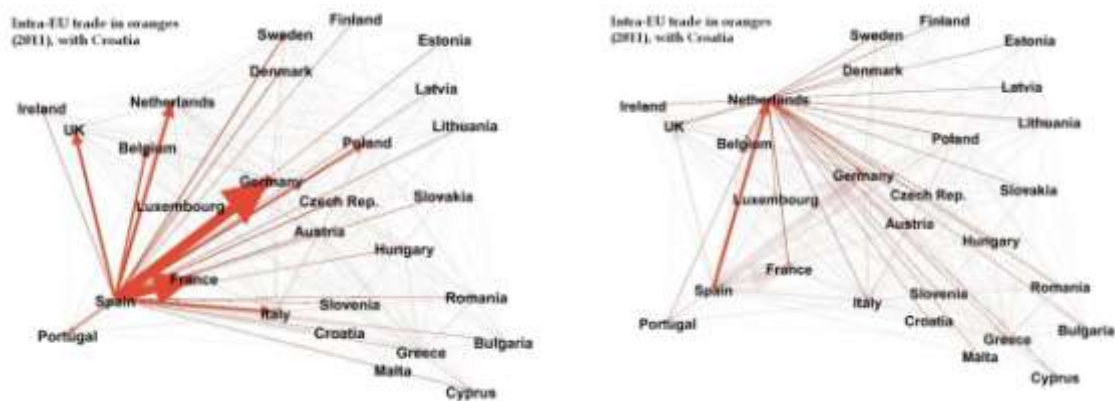
Seven countries export sweet oranges to at least 20 other countries (Spain and the Netherlands (27), Italy (26), Greece (24), Germany and France (22), and Belgium (20)). This is not the case for imports: the maximum number of countries from which sweet oranges are imported is 17 (this is true for Denmark, Germany, Italy and Poland). Some countries are more connected than others, as shown by Figure 39.



**Figure 39:** The intra-EU trade in sweet oranges (2011): . Left: network visualisation (the weight of the links is proportional to trade volume). Right: correlation between the number of incoming links (countries from which sweet oranges were imported) and the number of outgoing links (countries to which sweet oranges were exported)

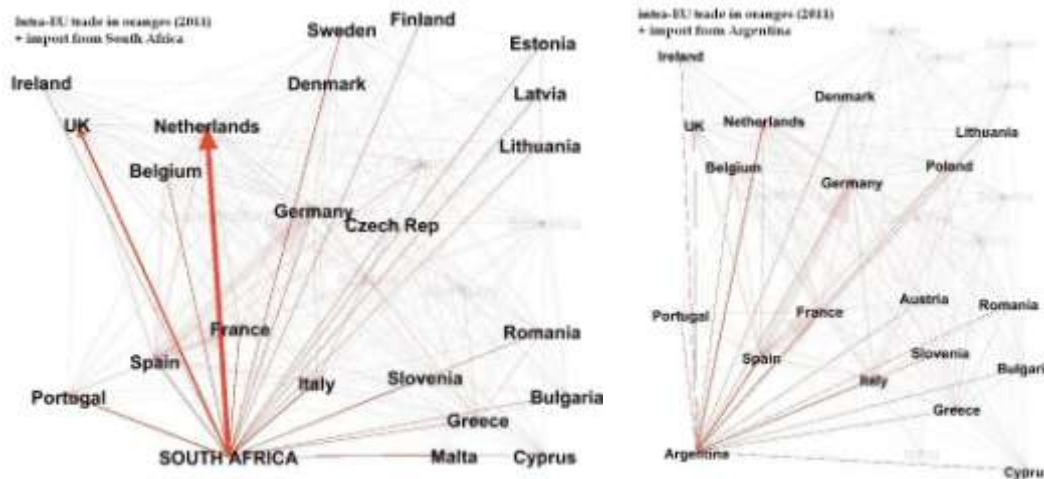
There is no correlation between the number of incoming and outgoing links. Such a correlation, at least in theory, and other things being equal, would make it easier for a pathogen to spread (as reviewed by Moslonka-Lefebvre et al., 2011). Nonetheless, there are some countries that import sweet oranges from many countries and also export them to many countries (e.g. the Netherlands, Italy, Germany and Poland). These countries are more likely to contribute to disease spread than countries importing from many countries but not exporting to many countries (e.g. Denmark, Slovenia, Slovakia and Romania). With the exception of Cyprus (3), Portugal (6) and Luxembourg (7), all EU countries import sweet oranges from at least eight different countries.

There is considerable variability in the weight of the connections, with just two links (from Spain to Germany and to France) making up about one-third of the whole sweet orange trade between European countries. On its own, Spain is responsible for nearly two-thirds of the intra-EU trade in sweet oranges (Figure 40). About 80 % of the Spanish export goes to just six countries (in decreasing order of imported sweet oranges: Germany, France, the Netherlands, the UK, Italy and Poland). Sweet orange imports are also uneven between countries, but less so than exports. Germany imports about 24 % of the total intra-EU trade, France 18 % and a further 20 % is imported by the Netherlands (7 %), Poland (7 %) and the UK (6 %). The network from the point of view of the Netherlands is shown in Figure 40.



**Figure 40:** Trade of sweet oranges with other EU countries in 2011, by Spain (left) and by the Netherlands (right), a country that exports more sweet oranges to EU countries than it imports

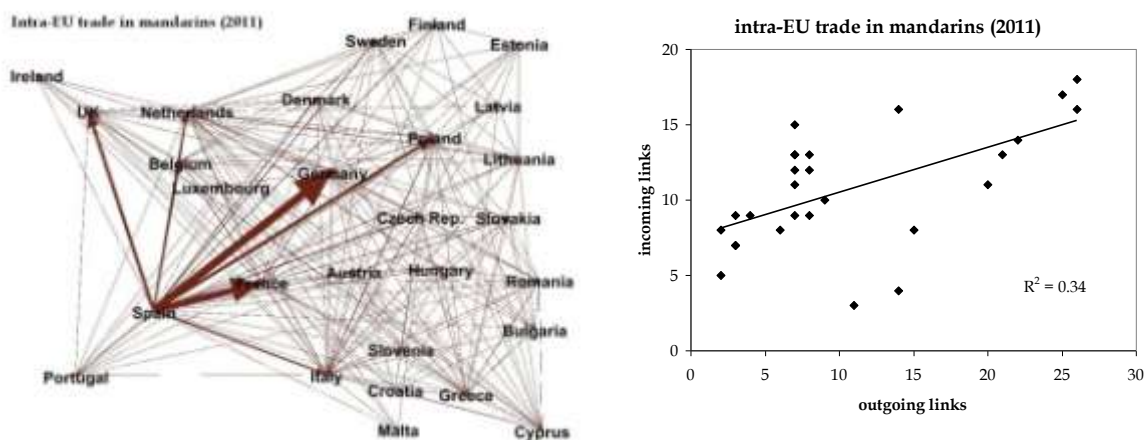
In 2011, the Netherlands imported more sweet oranges from South Africa than from the rest of the EU. South Africa is well connected to the EU sweet orange trade network, as shown in Figure 41. In 2011, South Africa exported about 0.3 million tons of sweet oranges to 22 EU countries, with nearly 50 % of South African sweet oranges being imported by the Netherlands. Argentina is another major exporter of sweet oranges to the EU (Figure 41). In 2011, like South Africa, Argentina exported sweet oranges to all the citrus-producing countries of the EU.



**Figure 41:** Imports of sweet oranges by EU countries (2011), from South Africa (left) and from Argentina (right)

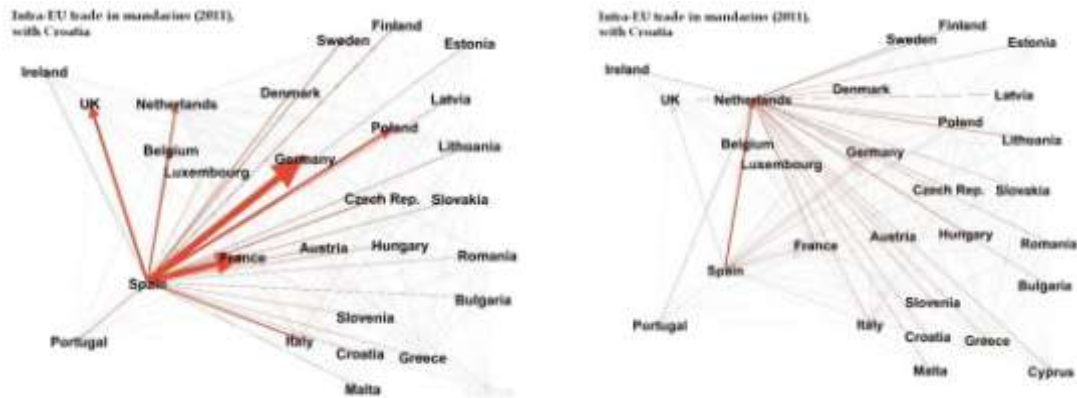
#### 3.4.2.1. Mandarins

The network of the intra-EU trade in mandarins (2011) is shown in Figure 42 (with the addition of Croatia). There are fewer trade links than for sweet oranges (300 instead of 320) and hence a slightly lower connectance level (0.38 instead of 0.41). In addition, the volume of traded mandarins is lower than that of sweet oranges (~ 1.6 vs. 2 million tons). There are six countries exporting mandarins to at least 20 EU countries: the Netherlands (to 27 countries), Spain and Italy (26), Germany and France (22), and Greece (21). There is a weak positive correlation between the number of countries from which mandarins are imported and the number of countries to which mandarins are exported (Figure 42). No EU country imports mandarins from 20 or more EU countries, with Italy importing them from 17 countries and Spain and Poland from 16.



**Figure 42:** The intra-EU trade in mandarins (2011). Left: network visualisation. Right: correlation between the number of countries to which mandarins were exported and from which mandarins were imported

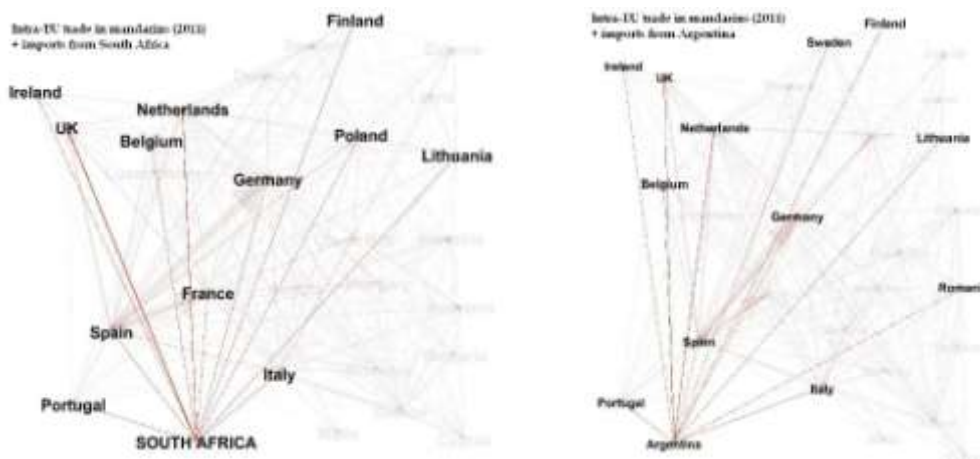
As for sweet oranges, Spain is the major EU mandarin exporter (three-quarters of exports), whereas France and Germany are the main EU importers (together accounting for 42 % of imports). About three-quarters of exported EU mandarins come from Spain. Approximately half of the Spanish export to EU countries goes to Germany and France (Figure 43).



**Figure 43:** The intra-EU mandarin trade network (2011) from the point of view of Spain (left) and the Netherlands (right).

In contrast to sweet oranges, the Netherlands import fewer mandarins from EU countries than it exports to them, but the Netherlands is still a major re-exporter (to all other EU countries, including Spain) (Figure 43).

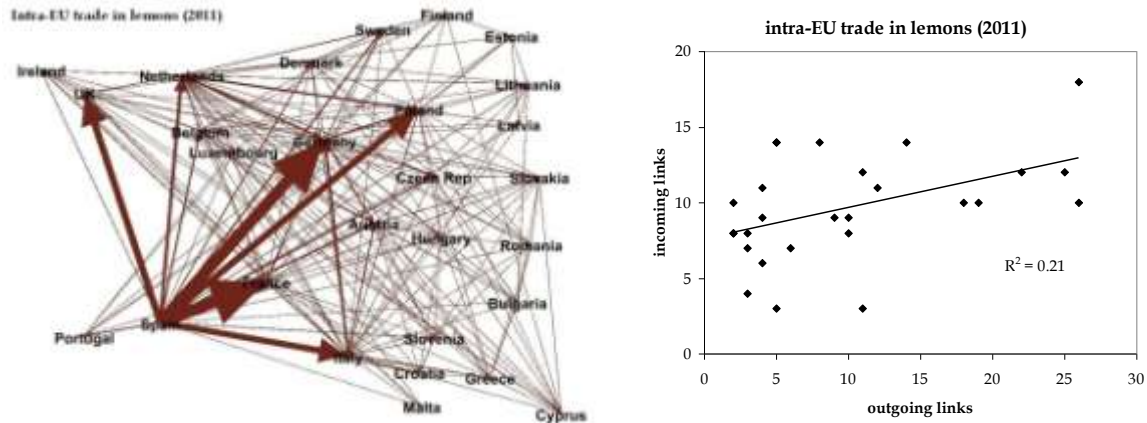
South Africa exports a smaller volume of mandarins (0.05 million tons) than of sweet oranges (one-sixth of that figure) and they go to 12 countries (mostly the UK and the Netherlands) (Figure 43). The exports of mandarins to EU countries from Argentina for the same year are shown as a comparison in Figure 44.



**Figure 44:** Import of mandarins (2011) by EU countries: from South Africa (left) and from Argentina (right)

### 3.4.2.2. Lemons

The intra-EU trade in lemons (2011) is shown in Figure 45 (with the addition of Croatia). The network is slightly less connected than for mandarins (269 instead of 290 links with a connectance level of 0.36). The volume of traded lemons is also lower than for mandarins (~ 0.5 vs. 1.6 million tons).



**Figure 45:** The intra-EU trade in lemons (2011). Left: network visualisation. Right: correlation between the number of EU countries from which lemons were imported and the number of EU countries to which lemons were exported

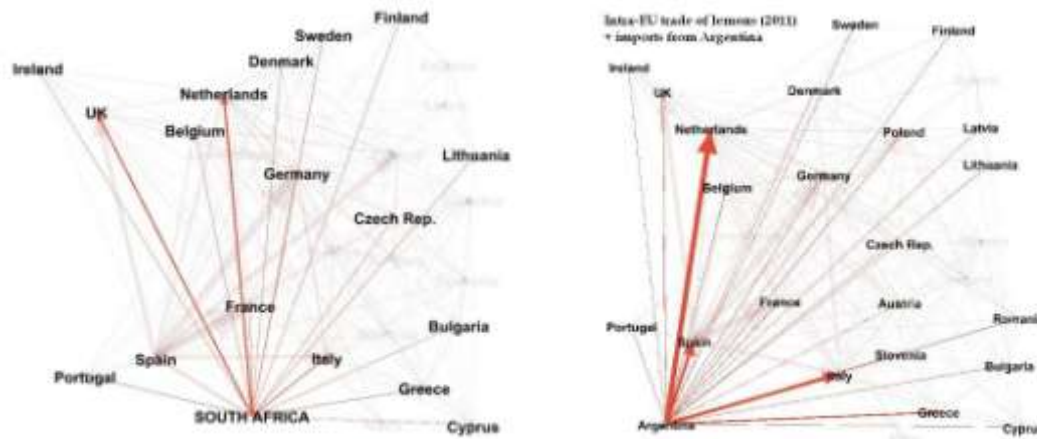
In 2011, only four EU countries exported lemons to at least 20 EU countries: Spain, the Netherlands (26), Italy (25), and Germany (22). Import sources are less diverse, with Poland importing lemons from 18 countries, and Denmark, Estonia, Portugal and Slovenia from 14 (Figure 45).

Spain is the major EU exporter of lemons (Figure 46). About one-third of imported EU lemons go to Germany and France. The Netherlands imports few lemons from EU countries (Figure 46) (fewer than, for example, Austria), but the Netherlands exports to EU countries more than twice as many lemons as it imports from EU countries.



**Figure 46:** The intra-EU network in lemons (2011) from the point of view of (left) Spain and (right) the Netherlands

South Africa exported about 0.04 million tons of lemons to EU countries in 2011 (Figure 47). This is more than the volume of lemons exported by Italy to EU countries in 2011. South African lemons are directly imported by 16 EU countries, including Greece, Italy, Spain and Portugal. Most South African lemons exported to the EU went to the UK and the Netherlands. Argentina is a more important exporter of lemons to the EU than South Africa, with most lemons exported to the Netherlands, Spain and Italy, as shown by Figure 47.



**Figure 47:** Import of lemons (2011) by EU countries, from South Africa (left) and from Argentina (right)

#### 3.4.2.3. Conclusions on spread by human assistance: trade of citrus fruit

1. On the whole, the citrus trade data for 2011 show that the EU market for sweet oranges, mandarins and lemons is closely integrated. On average, each EU MS imports from (or exports to) 9, 10 and 11 other EU countries (for lemons, mandarins and sweet oranges, respectively).
2. There are strong variations in connectivity between countries, in terms of both the number of links and traded volumes. Heterogeneities in the contact structure have been shown to reduce thresholds for disease spread and persistence in networks (Jeger et al., 2007), although for directed networks this is the case only in the presence of a positive correlation between incoming and outgoing links (Moslonka-Lefebvre et al., 2009). This is weak here, despite the presence of the Netherlands, which plays a strong role as importer and re-exporter and not only from European countries.
3. The trade data indicate that sweet oranges, mandarins and lemons from countries where CBS is present (e.g. Argentina and South Africa) can reach citrus-growing EU countries both directly and via the Netherlands and, potentially, through other re-exporting EU countries. However, the available Eurostat data provide information only on direct links between EU countries and thus do not allow an examination of how likely it is that imports of citrus fruit by EU MSs will be re-exported to citrus-growing EU MSs.
4. Authenticated records of introductions of CBS by means of trade of citrus fruit have not been found in the literature. However, there are no precedents of comparable large amounts of citrus fruit imported from CBS-affected areas into CBS-free areas in a scenario of absence of phytosanitary regulations.

#### 3.4.3. Spread by human assistance: trade in citrus plants for planting

Infected plants for planting are stated to be the main pathway of introduction of CBS into new areas (Kiely, 1948; Kotzé, 1981), but authenticated reports of introductions by means of propagating plant material have not been found in the literature. Once introduced in an area, trees from nurseries located in the affected area may also spread the disease to new locations. As with other plant diseases, spread dynamics of CBS are likely to be affected by epidemiological factors such as host demography, connectivity, spatial heterogeneity, epidemic threshold, etc. (Jeger and van der Bosch, 1994; Gubbins et al., 2000; Brown and Bolker, 2004; Margosian et al., 2009). Owing to their microscopic nature, the movement of *P. citricarpa* ascospores is unlikely to be prevented by screening. Leaf wetness, which is necessary for spore germination and infection, can be reduced in greenhouses by avoiding overhead



sprinkler irrigation. However, condensation of water on the plants as a result of soil water evaporation and the cooling of leaves below the dewpoint cannot be completely prevented (Jarvis, 1992; Wei et al., 1995). Foliar lesions of CBS are rare, especially in young vigorous plants (Kotzé, 1981). Since plants for planting produced in an affected area may carry latent infections, the efficacy of visual inspections in the nurseries is very low.

#### **3.4.4. Containment of the pest within the risk assessment area**

CBS has never disappeared or declined after the epidemic stage has been reached (Kotzé, 1981), and successful disease eradication has not been achieved anywhere. Whiteside (1967) proposed drastic pruning, involving the removal and destruction of all wood and leaves leaving only the framework limbs of the tree, as a method to eradicate CBS in affected orchards in Zimbabwe. However, this method has not been put in practice and no reports of its efficacy are available. Field surveillance for CBS eradication is challenging, considering that *P. citricarpa* may be present as latent mycelia in asymptomatic citrus fruit and leaves and there is a long lag phase between the first establishment and subsequent epidemic development (Kotzé, 1981).

In 2010, CBS was first detected in the USA in commercial citrus-growing areas in the Collier and Hendry Counties of south Florida (USDA APHIS, 2010b and c). After this first outbreak, a programme for the effective eradication of CBS was set up according to the recommendations of the Florida Citrus Health Response Program Working Group (2010). This is possibly the only documented attempt to eradicate CBS anywhere. The programme included measures to suppress CBS in affected orchards as well as contiguous areas by monthly applications of fungicides and inoculum suppression by enhancing leaf litter decomposition with irrigation and the application of urea, dolomite lime or ammonium nitrate. Other measures, such as avoiding off-season blooms, increasing air flow in the orchards, planting clean nursery stock and maintaining appropriate tree nutrient status, were also recommended. However, none of the recommended measures includes removal and destruction of trees and/or systematic plant debris elimination in affected orchards in the quarantined area. The report by the CBS technical working group (USDA-APHIS, 2010e) also questioned the feasibility of extensive tree removal for CBS eradication in Florida.

In addition to the recommended practices for disease suppression, regulatory measures have also been implemented in Florida. The movement of citrus plant material from quarantine areas is currently regulated (USDA APHIS, 2012b). Citrus plants may not be moved to other states from the quarantine areas. Initially, fruits from the quarantined area were eligible for movement interstate to states other than commercial citrus-producing states east of the Mississippi River, but fruit had to be free of CBS symptoms based on packing house inspections and treated with post-harvest chemicals (USDA-APHIS, 2010d). Later on, disease freedom was compulsory only for regulated organic fruit (USDA-APHIS, 2011a). Finally, fruits were allowed to move to all states under certificate after receiving a treatment with specific post-harvest chemicals including sodium hypochlorite, sodium o-phenyl phenate, peroxyacetic acid, imazalil or thiabendazole. Even with the same post-harvest treatments, fruits cannot be moved under limited permit to commercial citrus-producing States (USDA-APHIS, 2011b, 2012b). Under the federal domestic plant quarantine programmes of the USA, there is a difference between the use of certificates and limited permits. Certificates are issued when, because of certain conditions, a regulated article can be moved safely from a quarantined area without spreading the disease or pest. Limited permits are issued for regulated articles when, because of a possible pest risk, the articles may be moved inter-state only to a specified destination, for specified handling, processing or utilisation.

In the case of intra-state movement, fruit should be transported in vehicles properly covered by a screen mesh or tarpaulin to prevent the loss of fruit, leaves or plant debris while in transit. After being emptied and cleaned of plant debris, trailers, field boxes and bins must be disinfested using sodium hypochlorite, quaternary ammonium chloride or peroxyacetic acid. Plant debris cleaned from trailers must be treated at 82 °C for at least one hour, incinerated, buried at an approved disposal site or used

as livestock feed. Processors and packers receiving fruit from quarantine areas must also follow specific sanitation measures.

Intensive disease surveys were established along the fruit transportation corridors originating from the CBS regulated area in the South of Florida to the central areas of the State, where most juice factories and packing-houses are located (Riley, 2013). Nevertheless, despite all the measures described above, the disease expanded to new locations in south Florida in 2011 and 2012, and spread to Polk County in central Florida in 2013, about 150 km away from the initial outbreak in the south (USDA APHIS, 2013).

### 3.4.5. Conclusion on the probability of spread

**Table 12:** Assessment of the components of the probability of spread

| Rating for spread | Justification   |
|-------------------|---|
| Moderately likely | <p>Natural spread of <i>P. citricarpa</i> is known to mainly happen by airborne ascospores. The distance it can spread by natural means is poorly known. The pathogen is very likely to move with human assistance along commercial fruit and plants for planting pathways. However, because spread is defined as the expansion of the geographical distribution of a pest within an area, the rate of spread depends not only on the rapidity of movement and the number of spread pathways, but also on the likelihood of finding a suitable environment for establishment. When the proportion of the citrus-growing areas identified as potentially suitable for <i>P. citricarpa</i> is taken into account, the Panel considered that a rating of moderately likely is most appropriate for spread.</p> <p>The managed citrus orchards as well as home gardens, parks and abandoned citrus cultivations along all the EU citrus-growing MSs will probably provide a continuum for the spread of CBS. The intra-European trade in citrus fruit is closely integrated, with an average of 9, 10 and 11 trade connections (for lemons, mandarins and sweet oranges, respectively) from each EU MS. The Netherlands play a key role in re-exporting citrus fruit from citrus-producing countries (both within the EU and elsewhere) to other citrus-producing and non-citrus-producing EU MSs. In the absence of regulation, infected plants for planting is the main pathway of CBS spread. This is because plants for planting produced in a CBS-infected area can carry latent infections. As a consequence, the efficacy of visual inspections in the nurseries, wholesale traders and retailers is limited.</p> |

### 3.4.6. Uncertainties on the probability of spread

Uncertainty on the probability of spread is rated as low.

**Table 13:** Uncertainties on the probability of spread

| Rating for uncertainty | Justification   |
|------------------------|---|
| Low                    | <ul style="list-style-type: none"> <li>There is uncertainty about the potential natural spread of ascospores carried by wind over long distances, but this uncertainty does not concern the two main pathways of spread (intra-European trade of commercial fruit and plants for planting). There is uncertainty about the structure of the EU intra-trade network for citrus plants for planting owing to lack of data; however, this does not influence the conclusions above.</li> </ul> |

### 3.5. Conclusion regarding endangered areas

The risk assessment has identified parts of the EU where host plants are present and where, based on simulation results, the climatic conditions are suitable for ascospore maturation and release followed by infection (see Figures 34 and 35).

Conclusions from simulations of the release of ascospores based on gridded interpolated climate data of the EU citrus-producing areas show that, in almost all years (for the 95<sup>th</sup> percentile), ascospore release in the EU citrus growing areas will start early enough to coincide with climatic conditions that are conducive to infection in September and October. However, the simulations indicate that the onset of ascospore release in most areas will start too late to coincide with the climatic conditions conducive to infection in spring. Therefore, early-maturing citrus varieties might generally be infected in late summer and early autumn, which is when the availability of inoculum coincides with suitable conditions for infection. Owing to the long incubation time, fruits from these early varieties will be harvested before symptoms appear. The late-maturing oranges varieties and lemons are expected under such scenario to show CBS symptoms.

There are some areas, however, such as locations in Portugal, southern Italy, Cyprus, the Greek islands, Malta and southern Spain, where development of ascospores is expected also in late spring and early summer months in part of the years simulated (Fig. 56 and 57 in Appendix F). In those years, it is expected that symptoms can develop on the fruit before harvest, and therefore have an impact on the fruit quality.

The uncertainty is high as indicated in the establishment section.

### 3.6. Assessment of consequences

#### 3.6.1. Direct pest effects

In most of the area of its current distribution, *P. citricarpa* is reported to cause severe quality and yield losses to citrus production. Because of its quarantine status in some countries, *P. citricarpa* is not specifically listed in the International Standards for citrus fruit of the OECD. However, for other diseases, such as *Alternaria* brown spot, with fruit symptoms similar to some of CBS, the presence of more than one lesion per fruit is considered detrimental to quality and fruits with more than six depressed necrotic lesions in the rind are considered out of grade (OECD, 2010). In Sao Paulo, Brazil, fruits with more than three CBS lesions are considered unacceptable for the fresh market (Goes, 2002). Premature fruit drop due to CBS causes significant yield loss in Brazil, and probably in other citrus-producing regions of the world (Reis et al., 2006). In Sao Paulo, Brazil, the yield of mature sweet orange trees was reduced, due to premature fruit drop caused by CBS, from 161.31 kg/tree in fungicide-treated plots to 83.38 kg/tree in untreated plots (Araújo et al., 2013).

In order to obtain quantitative estimates of CBS impact and disease control levels in its current area of distribution, the Panel undertook a meta-analysis of recorded disease incidence in control (untreated) plots and in plots treated with fungicides from CBS experiments on fungicide evaluation trials. This meta-analysis aimed at (i) describing the variability of disease incidence in untreated citrus orchards based on published data and (ii) assessing the effectiveness of different groups of fungicides in reducing disease incidence. Meta-analysis is commonly used to compare of the effects of different chemical and biological treatments for managing plant diseases (Ojiambo and Scherm, 2006; Paul et al., 2008; Ngugi et al., 2011; Scheepmaker and van de Kastelee, 2011). The principle is to perform a systematic literature review and to collect the available experimental data measuring the effect of treatments on a given plant disease. The collected data are then analysed in order to estimate disease incidence in untreated plots and effectiveness of the considered treatments. In our study, a total of 46 experimental plots (site-years) from 16 papers were included in the dataset (Table 14). Fungicide evaluations trials are generally optimised towards displaying treatment effects, and it can be assumed that the experimental plots will be located in orchards severely affected by CBS. Because of a generally higher disease pressure than the average for the region, the disease incidence in untreated

plots should be interpreted as the estimates of the highest potential loss (losses occurring in absence of control measures) and the incidence in fungicide-treated plots as estimates of the highest primary loss (direct crop losses in presence of control measures) (Zadoks and Schein, 1979).

Directive 2000/29/CE (Annex IV, Part A, Section I 16:4) lists several requirements for the introduction of citrus fruit into the EU territory. One of these requirements is that the fruits originate in a place of production subjected to appropriate treatments against *P. citricarpa*. Fungicide schedules currently applied for CBS control are mainly based on the results obtained from field trials conducted in affected areas. Therefore, a meta-analysis of these experiments may help to evaluate the efficacy of the different spray programmes worldwide. Moreover, it may determine what level of disease control may be achieved by implementing the appropriate treatments required in the Directive 2000/26/CE and would help to devise the most appropriate fungicides to be used if an outbreak of CBS occurred in the EU.

**Table 14:** CBS fungicides control trials considered in the meta-analysis

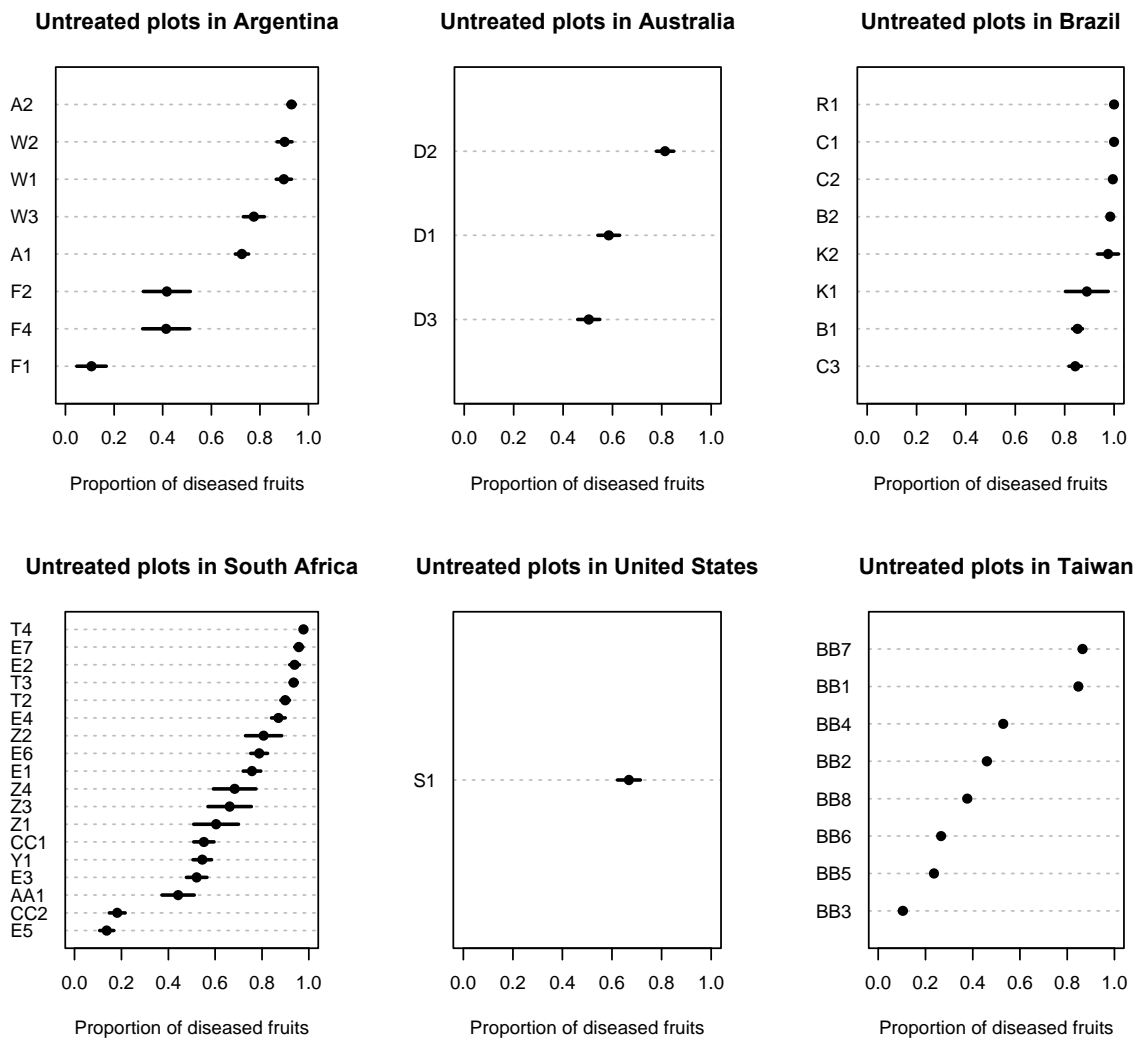
| Country       | Citrus species | Number of experiments | Reference                   |
|---------------|----------------|-----------------------|-----------------------------|
| Argentina     | Lemon          | 2                     | Fogliata et al., 2001       |
| Argentina     | Lemon          | 1                     | Agostini et al., 2006       |
| Argentina     | Sweet orange   | 1                     | Agostini et al., 2006       |
| Argentina     | Sweet orange   | 1                     | Agostini et al., 2006       |
| Argentina     | Sweet orange   | 3                     | Rodriguez et al., 2010      |
| Australia     | Mandarin       | 1                     | Miles et al., 2004          |
| Australia     | Sweet orange   | 2                     | Miles et al., 2004          |
| Brazil        | Sweet orange   | 2                     | Kupper et al., 2006         |
| Brazil        | Sweet orange   | 3                     | Goes, 2002                  |
| Brazil        | Sweet orange   | 2                     | Bernardo and Bettioli, 2010 |
| Brazil        | Sweet orange   | 1                     | Goes et al., 2000           |
| South Africa  | Sweet orange   | 4                     | Schutte et al., 2003        |
| South Africa  | Sweet orange   | 2                     | Schutte et al., 2012        |
| South Africa  | Sweet orange   | 1                     | Schutte, 2002               |
| South Africa  | Sweet orange   | 4                     | Schutte, 2006               |
| South Africa  | Sweet orange   | 1                     | Schutte et al., 1997        |
| South Africa  | Sweet orange   | 4                     | Kellerman and Kotzé, 1977   |
| Taiwan        | Mandarin       | 5                     | Tsai, 1981                  |
| Taiwan        | Sweet orange   | 3                     | Tsai, 1981                  |
| United States | Sweet orange   | 1                     | Hendricks et al., 2013      |

### 3.6.1.1. Pest effects on citrus crop in the areas of current distribution

#### Disease incidences in untreated plots

The proportions of diseased fruits are shown in Figure 48 for several untreated plots (site-years) located in six different countries (Argentina, Australia, Brazil, South Africa, the USA and Taiwan). The mean proportion of diseased fruits ranged from 0.46 (Taiwan) to 0.98 (Brazil) (Table 15). The proportion of diseased fruits varied strongly between plots in a given country. For example, in South Africa, the minimum proportion of diseased fruits was equal to 0.14 and the maximum proportion was 0.98. Statistical tests performed with a generalised linear mixed-effect model showed significant differences between countries; compared with Argentina, the mean proportion of diseased fruits was significantly higher in Brazil ( $p < 0.01$ ). It is important to note that the untreated plots reported in the literature are likely to correspond to heavily infected citrus orchards because these plots were primarily selected to test the effectiveness of fungicide treatments. For this reason, the values of disease incidence reported in Figure 48 and Table 15 should not be considered as representative of

average situations, but rather as worst cases. However, these values reveal that very high disease incidence levels can be reached in countries where CBS is present.



**Figure 48:** Proportion of CBS-affected fruits in untreated plots in Argentina, Australia, Brazil, South Africa, the USA and Taiwan. Plot names are given on the y-axis. Bars indicate 95 % confidence intervals (missing for Taiwan)

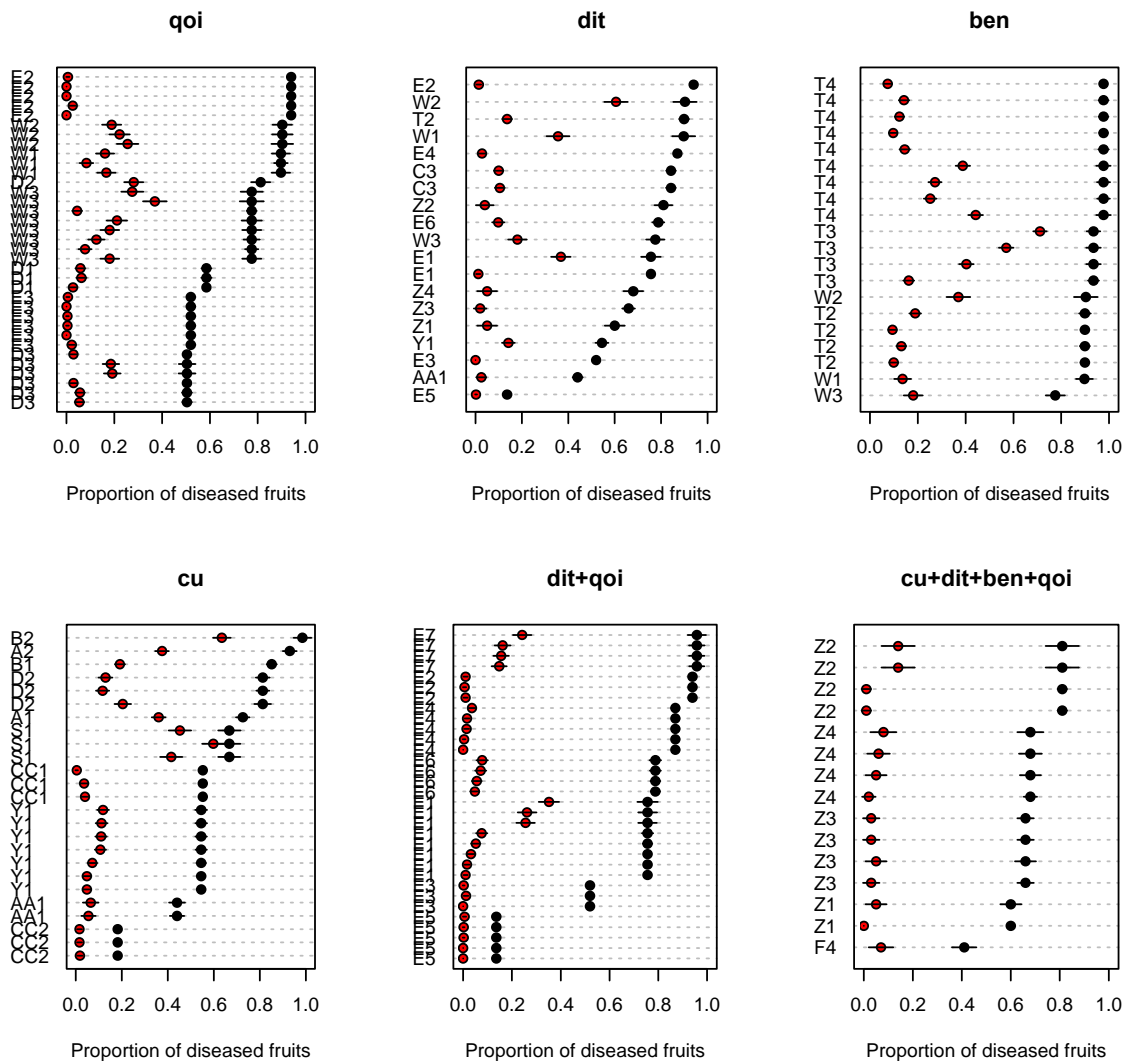
**Table 15:** Proportions of CBS-affected fruits in untreated plots. Estimated median proportion, odds (ratio of the proportion of diseased fruits to the proportion of healthy fruits), and 95 % confidence intervals between brackets (missing for Taiwan). Country effect was statistically significant ( $p < 0.01$ )

| Country      | Number of plots | Estimated proportion of diseased fruits | Odds               |
|--------------|-----------------|---|--------------------|
| Argentina    | 8               | 0.69 (0.44, 0.87)                       | 2.26 (0.8, 6.42)   |
| Australia    | 3               | 0.65 (0.48, 0.79)                       | 1.84 (0.91, 3.7)   |
| Brazil       | 8               | 0.98 (0.93, 0.99)                       | 63.99 (13.04, 314) |
| South Africa | 18              | 0.74 (0.59, 0.85)                       | 2.87 (1.45, 5.68)  |
| USA          | 1               | 0.67 (0.62, 0.72)                       | 2.01 (1.63, 2.47)  |
| Taiwan       | 8               | 0.46 (-)                                | 0.85 (-)           |

### Effects of fungicide treatments on disease incidence

Fungicide schedules were classified according to the chemical groups of the products evaluated and their combinations (FRAC, 2013): copper (“cu”), dithiocarbamates (“dit”), quinone outside inhibitors or strobilurines (“qoi”) and benzimidazoles (“ben”) (section 2.2.2). The proportions of diseased fruits in plots treated with fungicide and in untreated plots are compared in Figure 49 and Table 16, for six different types of fungicide. The results show that fungicide treatments were systematically able to reduce the proportion of diseased fruits. However, the effectiveness of fungicide treatment varied a markedly between plots (Figure 49). In some plots, disease incidence was only slightly reduced by fungicide treatment, while the proportion of diseased fruits was reduced to zero in other plots. We tried to explain part of the between-plot variability of the fungicide effect by the number of sprays of fungicide using generalised linear mixed-effect model including the number of sprays as a covariable (Figure 50). However, this model did not perform better (in terms of AIC and BIC) than a model including a fungicide effect but no covariable related to the number of sprays. The between-plot variability of the fungicide effect is thus probably not related to the number of sprays.

Odds ratios reported in Table 16 show that some types of fungicide were more efficient than others. The type of fungicide with the highest odd ratio (i.e. the least efficient fungicide type) was copper-based compounds (“cu”), while the types of fungicide showing the lowest odd ratios (i.e. the most efficient) were dithiocarbamates (“dit”), dithiocarbamates + strobilurines (“dit+qoi”), and copper + dithiocarbamates + benzimidazoles + strobilurines (“cu+dit+ben+qoi”). Fungicide treatments were all able to significantly reduce average disease incidence ( $p < 0.001$ ) (Table 16), and the mean proportion of diseased fruits in treated plots ranged from 2.2 % to 23 % depending on the fungicide type (Table 16).



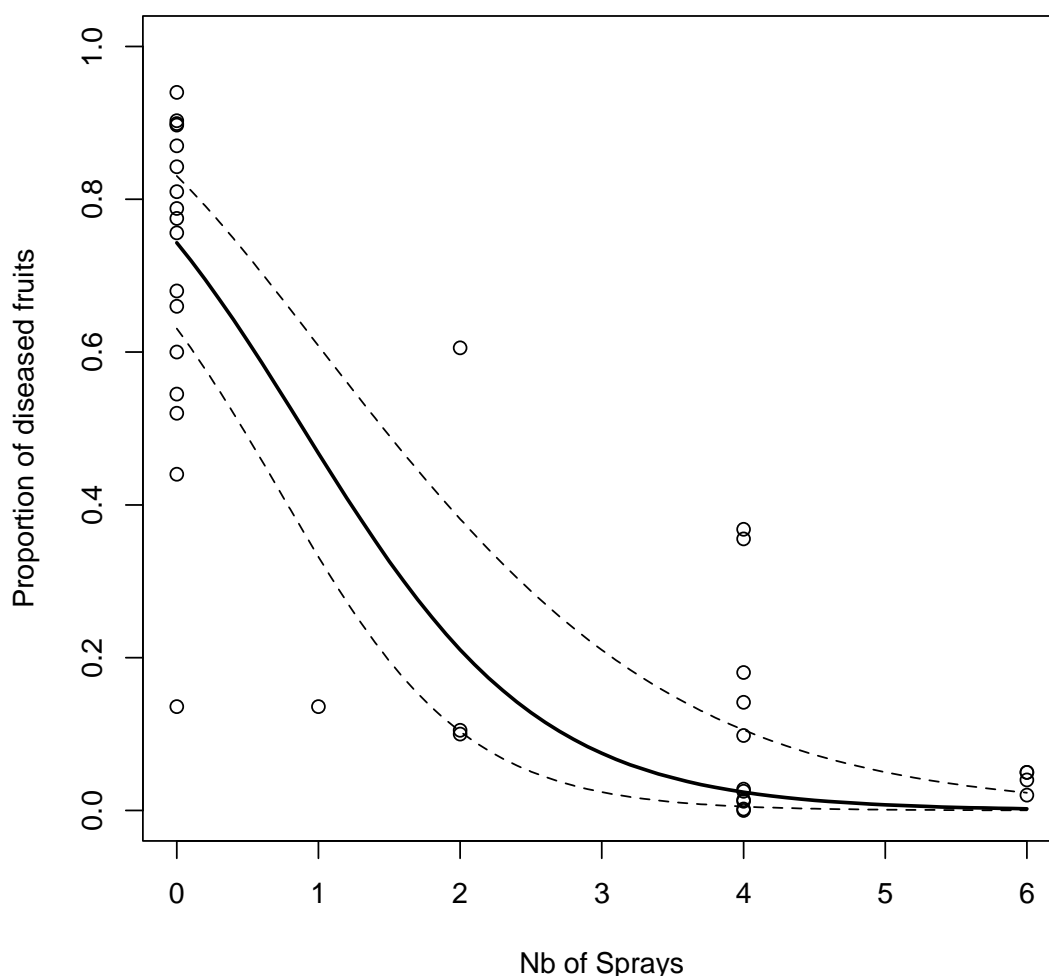
**Figure 49:** Observed proportions of CBS-affected fruits in untreated (black) and treated (red) trees for different types of fungicide (qoi, dit, cu, ben, dit+qoi, cu+dit+ben+qoi) and different plots. Bars indicate 95 % confidence intervals. Plot names are given on the y-axis

cu: copper  
dit: dithiocarbamates  
qoi: quinone outside inhibitors (strobilurines)  
ben: benzimidazoles

**Table 16:** Estimated proportions of CBS-affected fruits in untreated plots and in plots treated with fungicides. Proportion of diseased fruits, odds (ratio of the proportion of diseased fruits to the proportion of healthy fruits) and odds ratio (ratio of the odds in treated plots to the odds in untreated plots) were estimated for six types of fungicide using a generalised mixed-effect model including a fungicide effect but no covariable related to the number of sprays. 95 % confidence intervals are given between brackets. Effects of all types of fungicide were statistically significant ( $p < 0.001$ )

| Fungicide type     | Number of plots | Untreated            |                      | Treated                 |                         | Odds ratio               |
|--------------------|-----------------|----------------------|----------------------|-------------------------|-------------------------|--------------------------|
|                    |                 | Estimated proportion | Odds                 | Estimated proportion    | Odds                    |                          |
| qoi                | 8               | 0.78<br>(0.64, 0.88) | 3.6<br>(1.81, 7.16)  | 0.068<br>(0.03, 0.17)   | 0.07<br>(0.03, 0.21)    | 0.02<br>(0.01, 0.04)     |
| dit                | 17              | 0.74<br>(0.63, 0.83) | 2.89<br>(1.71, 4.9)  | 0.055<br>(0.02, 0.12)   | 0.058<br>(0.03, 0.14)   | 0.02<br>(0.016, 0.03)    |
| dit+qoi            | 7               | 0.77<br>(0.51, 0.92) | 3.34<br>(1.04, 10.7) | 0.022<br>(0.006, 0.07)  | 0.022<br>(0.006, 0.08)  | 0.0066<br>(0.004, 0.011) |
| ben                | 6               | 0.91<br>(0.86, 0.95) | 10.7<br>(5.9, 19.4)  | 0.23<br>(0.15, 0.33)    | 0.3<br>(0.18, 0.51)     | 0.028<br>(0.02, 0.036)   |
| cu                 | 10              | 0.74<br>(0.52, 0.87) | 2.77<br>(1.1, 6.97)  | 0.16<br>(0.07, 0.32)    | 0.19<br>(0.08, 0.48)    | 0.069<br>(0.059, 0.08)   |
| cu+dit+ben<br>+qoi | 5               | 0.64<br>(0.52, 0.75) | 1.79<br>(1.06, 3.0)  | 0.047<br>(0.035, 0.063) | 0.049<br>(0.036, 0.067) | 0.027<br>(0.025, 0.03)   |





**Figure 50:** Proportion of CBS-affected fruits as a function of the number of sprays with dithiocarbamate fungicides. Points are data, continuous line shows the fitted curve and the dashed lines indicate the 95 % confidence intervals. The fitted model did not perform better in terms of AIC and BIC than the model including a fungicide effect but no covariable related to the number of sprays

### 3.6.1.2. Pest effects on citrus crops in the risk assessment area

To estimate the potential impact of CBS in the EU citrus-growing areas, the surface of commercial citrus production per NUTS3 region was taken from national statistics on agriculture for the eight citrus-producing countries in the EU, namely Croatia (HR), Cyprus (CY), France (FR), Greece (GR), Italy (IT), Malta (MT) and Portugal (PT). For consistency between the different national datasets, scattered non-commercial citrus trees (private/public gardens, backyard plots, etc.) were not considered. The monthly averages of weather suitable for infection by *P. citricarpa* pycnidiospores or ascospores for the period 1989–2009 simulated by the generic model by Magarey et al. (2005) (sections 3.3.2.5 and 3.3.2.6) were calculated per NUTS3 region. In the case of pycnidiospores, a requirement of at least one rain event per day was set for splash dispersal. For ascospores, a requirement of > 1 % ascospore release was set for inoculum availability (Fourie et al., 2013) (sections 3.3.2.4 and 3.3.2.6). These NUTS3 regions were then classified according to increasing intervals in the time suitable for infection.

Infections by *P. citricarpa* pycnidiospores during rainy events were simulated for nearly all the citrus production areas of the EU for each month of the year (Table 17). When only longer periods of suitable weather, greater than > 0.5 % of the time, are taken into account, the proportion of the citrus-growing area at risk of infection ranged from 46.4 % in September to 34.2 % in November. Pycnidiospores are of biological relevance, specially those produced in affected twigs in the canopy

(Whiteside, 1967; Spósito et al., 2011). Considering that the simulated potential infection period for pycnidiospores in the PRA area is substantially longer than for ascospores (Tables 17 and 18), the role of pycnidiospores produced in twigs to supplement the sporadic occurrence of ascospore infections in spring and early summer (see Figures 56 and 57 in Appendix F) cannot be completely overlooked. However, due to the lack of quantitative data, it was not possible to estimate the potential periods of pycnidiospore availability (section 3.3.2.1). Therefore, in this present PRA pycnidiospores are considered relevant only for establishing the first infection following the entry of CBS-affected fruit into the PRA area (section 3.3.2.6), and it is implicitly assumed that the development of epidemics and the magnitude of the impact of CBS mainly depends on ascospores. Table 18 shows that the citrus-growing areas in the EU are virtually free of infection by ascospores from January to May. Infections are then predicted in more than 50 % of the citrus-growing area in the EU, but with extremely low periods of suitable weather (< 0.1 % of time). For longer periods of suitable weather (e.g. > 0.5 % of time), infections are strongly concentrated in late summer and early autumn. For instance, infections were predicted in 38.1 % and 11.7 % of the citrus-growing area in the EU in September and October, respectively. Although of great interest, it is important to stress that none of the models used for simulation (Magarey et al., 2005; Fourie et al., 2013) establishes a relationship between model output and CBS severity; therefore, it is not possible to define reliable thresholds for low, medium or high disease impact scenarios and only overall interpretations can be made.

In order to estimate the potential impact of CBS in relation to the size and the distribution of the citrus-growing area in each country, the proportion of time suitable for infection was assessed according to the area of citrus production in each country. The highest values were obtained for Portugal and Malta, for both pycnidiospores and ascospores (Table 19). The lower values were obtained for Cyprus and Spain in the case of pycnidiospores and for Cyprus and France when considering infections by ascospores.

**Table 17:** Area of citrus production (ha) in the EU according to the average length of time in each month when the weather is suitable for dispersal and infection by *Phyllosticta citricarpa* pycnidiospores from 1989 to 2009, calculated according to the Magarey et al. (2005) model ( $D_{50} = 3$  h,  $T_{min} = 10$  °C) with the additional requirement of a rain event for splash dispersal

| Time suitable for infection (%) | Jan                           | Feb               | Mar               | Apr               | May               | Jun               | Jul               | Aug               | Sep               | Oct               | Nov               | Dec               |
|---------------------------------|-------------------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|-------------------|
| 0                               | 3,590<br>(0.7) <sup>(a)</sup> | 7,984<br>(1.4)    | 34,995<br>(6.3)   | 5<br>(0.0)        | 9,508<br>(1.7)    | 5,801<br>(1.1)    | 286<br>(0.1)      | 117,631<br>(21.3) | 6,463<br>(1.2)    | 1<br>(0.0)        | 2<br>(0.0)        | 3<br>(0.0)        |
| 0–0.1                           | 291,221<br>(52.8)             | 300,558<br>(54.5) | 268,846<br>(48.7) | 4,066<br>(0.7)    | 168,322<br>(30.5) | 36,999<br>(6.7)   | 201,952<br>(36.6) | 124,156<br>(22.5) | 2,293<br>(0.4)    | 6,464<br>(1.2)    | 12<br>(0.0)       | 826<br>(0.1)      |
| 0.1–0.2                         | 82,020<br>(14.9)              | 128,612<br>(23.3) | 169,670<br>(30.8) | 159,435<br>(28.9) | 347,483<br>(63.0) | 183,153<br>(33.2) | 98,593<br>(17.9)  | 176,452<br>(32.0) | 39,230<br>(7.1)   | 49,502<br>(9.0)   | 1<br>(0.0)        | 158,833<br>(28.8) |
| 0.2–0.5                         | 95,716<br>(17.4)              | 96,454<br>(17.5)  | 63,055<br>(11.4)  | 315,652<br>(57.2) | 25,284<br>(4.6)   | 272,639<br>(49.4) | 194,240<br>(35.2) | 111,455<br>(20.2) | 246,969<br>(44.8) | 463,407<br>(84.0) | 164,475<br>(29.8) | 119,210<br>(21.6) |
| 0.5–1                           | 61,312<br>(11.1)              | 3,508<br>(0.6)    | 14,952<br>(2.7)   | 58,606<br>(10.6)  | 1,022<br>(0.2)    | 50,400<br>(9.1)   | 54,734<br>(9.9)   | 20,364<br>(3.7)   | 255,934<br>(46.4) | 32,244<br>(5.8)   | 188,502<br>(34.2) | 109,204<br>(19.8) |
| 1–2                             | 16,161<br>(2.9)               | 14,402<br>(2.6)   | 100<br>(0.0)      | 13,854<br>(2.5)   |                   | 2,465<br>(0.4)    | 1,189<br>(0.2)    | 1,559<br>(0.3)    | 730<br>(0.1)      |                   | 160,504<br>(29.1) | 116,868<br>(21.2) |
| 2–5                             | 1,599<br>(0.3)                | 100<br>(0.0)      |                   |                   |                   | 163<br>(0.0)      | 625<br>(0.1)      | 3<br>(0.0)        |                   |                   | 38,122<br>(6.9)   | 32,904<br>(6.0)   |
| > 5                             |                               |                   |                   |                   |                   |                   | 1                 |                   |                   |                   |                   | 13,770<br>(2.5)   |

(a): Values in brackets represent the percentage with respect to the total area cultivated with citrus in the EU (551 619 ha).

**Table 18:** Area of citrus production (ha) in the EU according to the average length of time in each month when the weather is suitable for infection by *Phyllosticta citricarpa* ascospores from 1989 to 2009, calculated according to the Margarey et al. (2005) model ( $D_{50} = 3$  hours,  $T_{min} = 15$  °C) with the additional requirement for ascospore availability according to the Fourie et al. (2013) models (average cumulative ascospore release  $\geq 1$  %)

| Time suitable for infection (%) | Jan           | Feb           | Mar           | Apr           | May            | Jun            | Jul            | Aug            | Sep            | Oct            | Nov            | Dec            |
|---------------------------------|---------------|---------------|---------------|---------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|----------------|
| 0                               | 551,619 (100) | 551,619 (100) | 551,619 (100) | 551,619 (100) | 250,594 (45.4) | 8,729 (1.6)    | 1,623 (0.3)    | 1,86 (0.0)     | 4 (0.0)        | 78 (0.0)       | 3295 (0.6)     | 322,382 (58.4) |
| 0-0.1                           |               |               |               |               | 300,453 (54.5) | 391,789 (71.0) | 252,808 (45.8) | 164,593 (29.8) | 6,463 (1.2)    | 1,463 (0.3)    | 37,1974 (67.4) | 229,236 (41.6) |
| 0.1-0.2                         |               |               |               |               | 572 (0.1)      | 112,112 (20.3) | 191,283 (34.7) | 103,268 (18.7) | 3,795 (0.7)    | 307,400 (55.7) | 94,343 (17.1)  |                |
| 0.2-0.5                         |               |               |               |               |                | 38,988 (7.1)   | 96,143 (17.4)  | 220,517 (40.0) | 300,395 (54.5) | 149,428 (27.1) | 6,6830 (12.1)  |                |
| 0.5-1                           |               |               |               |               |                |                | 9,753 (1.8)    | 60,194 (10.9)  | 209,995 (38.1) | 64,376 (11.7)  | 13,925 (2.5)   |                |
| 1-2                             |               |               |               |               |                |                | 2 (0.0)        | 2,854 (0.5)    | 30,966 (5.6)   | 28,774 (5.2)   | 1,252 (0.2)    |                |
| 2-5                             |               |               |               |               |                |                |                |                |                |                |                |                |
| > 5                             |               |               |               |               |                |                |                |                |                | 100 (0.0)      |                |                |

Note: Values in brackets represent the percentage with respect to the total area cultivated with citrus in the EU (551 619 ha).

**Table 19:** Weighted averages of percentage of time within a year suitable for infection by *Phyllosticta citricarpa* spores from 1989 to 2009, calculated according to Margarey et al. (2005) model ( $D_{50} = 3$  hours,  $T_{min} = 10$  °C for pycnidiospores and  $T_{min} = 15$  °C for ascospores) with additional requirement for ascospore availability according to the Fourie et al. (2013) models (average cumulative ascospore release  $\geq 1$  %) and additional requirement of a rain event for splash dispersal

| Country | Pycnidiospores      | Ascospores |
|---------|---------------------|------------|
| EU      | 0.97 <sup>(a)</sup> | 0.195      |
| CY      | 0.43                | 0.051      |
| ES      | 0.83                | 0.149      |
| FR      | 1.09                | 0.146      |
| GR      | 1.12                | 0.238      |
| HR      | 1.58                | 0.277      |
| IT      | 1.08                | 0.248      |
| MT      | 1.61                | 0.372      |
| PT      | 2.50                | 0.494      |

<sup>(a)</sup>: Weighted with the citrus production area to obtain the weighted average of the total citrus production area per country or in the EU.

Based on the integrated results from the simulation of inoculum production and ascospore infection based on historical weather data, the Panel found that epidemics will develop only sporadically in time and space during spring in EU citrus production areas. During the summer months, inoculum availability and weather conditions for infection continues and tends to increase towards the end of the summer and in the early autumn, when the highest values of infection events are predicted. These results should be interpreted by noting that shifts in the infection and host susceptibility periods have

also been described for other fungal pathogens of fruit crops that have adapted to develop epidemics in semi-arid areas (Vicent et al., 2004, 2012; Bassimba et al., 2014).

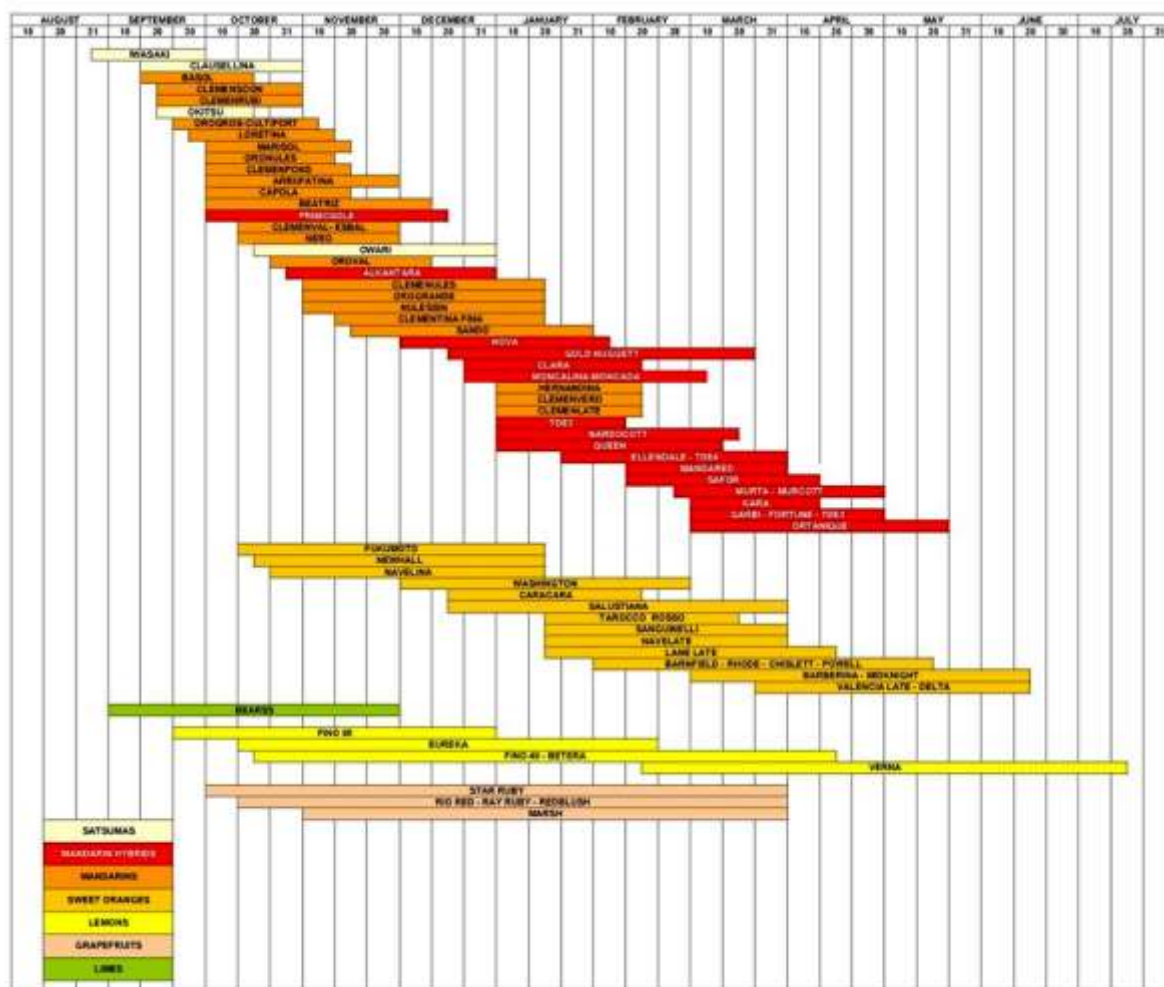
As described previously, *P. citricarpa* is known to have a relatively long incubation time, which means that it will take several weeks from when the infection takes place, until symptoms become visible on the fruit. After infection, the duration of the incubation period until symptom appearance is approximately 2–12 months, depending on environmental factors, tree age and condition (Brodrick and Rabie, 1970; Kotzé, 1963, 1981; Ninin et al., 2012). In South Africa, fungicide sprays are seldom applied from four months after fruit set onwards because sweet oranges are then assumed to be resistant to *P. citricarpa* (McOnie, 1964b, c; Kotzé, 1981). However, ontogenic resistance has not been confirmed experimentally, and the lack of fungicide sprays may also be driven by low inoculum levels and unfavourable weather conditions during the later stages of fruit development. Studies conducted in Brazil and Ghana under non-limiting inoculum and weather conditions for infection indicated a susceptibility period of six and seven months after fruit set, respectively (Reis et al., 2003; Baldassari et al., 2006; Brentu et al., 2012), but longer periods were not evaluated. Fruit age at the time of infection has a considerable influence on the duration of the incubation period. Sweet orange fruits infected by *P. citricarpa* when they are 3 cm in diameter needed about eight months to develop symptoms. However, fruits infected at 7 cm needed only two months to express symptoms (Aguiar et al., 2012). Therefore, the timing of epidemics compared with the harvesting calendar of the different citrus fruit varieties is a key factor for the assessment of direct pest effects.

Figure 51 provides the harvesting calendar for citrus fruit in the EU citrus production areas. It is apparent that fruit infected in late summer and early autumn would eventually develop symptoms in the field only if they hang on the tree for some months after infection. Early-maturing mandarins such as satsumas, which are usually harvested in September and October, may not have enough time for symptom development in the field and will already be harvested and even consumed before symptoms become visible. Nevertheless, latent infections might develop after harvest during transport and storage. Mid-season and late-maturing mandarins and sweet oranges would stay on the tree for several months after infection in late summer and early autumn, especially the sweet orange cultivars of the ‘Valencia’ group that is harvested as late as May or June. Cultivar field trials conducted in Brazil as well as studies comparing the rate of disease progress have indicated that cultivar reaction to the disease is more linked to the interaction of environmental factors with the dynamics of fruit maturation (Spósito et al., 2004; Sousa and de Goes, 2010), than cultivar resistance. Lemons have several flowerings per season, so both young and mature fruits would be present at the time of potential infection by *P. citricarpa*. These conclusions are in line with the available literature, which indicates that lemons and late-maturing sweet orange cultivars are most affected by CBS (Kotzé, 1981; Timmer, 1999; Spósito et al., 2004).

For the rarer events of disease development starting in spring these epidemics will be most damaging, because there will be sufficient time for disease symptoms to develop on the fruits on both the early, and the late maturing citrus-varieties and lemons. Owing to this differentiation, the Panel has decided also to provide different risk ratings. There are minor consequences for early-maturing citrus varieties, while the risk of direct pest effects is characterised as moderate for late-maturing citrus.

The EU citrus industry is strongly oriented towards the production of fruit for the fresh market (Agustí, 2012; Cutuli et al., 1985). According to international quality standards (section 3.1.1.5), the presence of more than one necrotic spot per fruit is considered detrimental to the quality, and fruits with more than six necrotic spots are considered to be out of grade (OECD, 2010). Thus, even relatively low disease severities would cause significant negative impacts in the fresh fruit industry. The presence of fruit spots is not important for the citrus processing industry, so CBS is not expected to cause impacts to this sector. Under high disease pressure conditions in Brazil, CBS also induces premature fruit drop (Reis et al., 2006; Araújo et al., 2013), which impacts both the fresh fruit and processing industries. However, the information available on CBS does not suggest that the disease will reach such high levels of disease intensity in the EU endangered areas.

In EU citrus-growing areas, early- and late-maturing cultivars are generally grown together in the same regions, so the areas indicated in Figures 34 and 35 can be considered to show the potential geographical limits to impacts. In Spain, lemons are mainly grown in the south of the Alicante and Murcia provinces (Agustí, 2012). In Italy, lemon production is mainly concentrated in the island of Sicily (Cutuli et al., 1985). As this citrus species is considered to be the most susceptible to CBS, these regions might experience higher impacts than other citrus-growing areas in the EU. Nevertheless, new cultivars are being introduced by the European citrus industry to extend the harvesting period, so the diversity of cultivated genotypes in the EU is changing and new varietal scenarios may be expected in the future (Aleza et al., 2012).



**Figure 51:** Harvesting calendar of citrus fruit in the EU citrus production areas (Pardo et al., 2013)

### 3.6.2. Control

Agronomic practices, such as leaf litter and soil cover management, irrigation and early fruit harvesting, are used to some extent for CBS control in the areas where the disease is present (Kotzé, 1981; Timmer, 1999; Miranda-Bellote et al., 2013). However, chemical control involving the use of protective and curative fungicides is generally necessary for economic disease management (Kotzé, 1981; Spósito et al., 2011). In order to obtain a summary of the fungicide schedules used for CBS control elsewhere and to quantify their efficacy, data were obtained from fungicide control trials conducted in areas of Argentina, Australia, Brazil, South Africa, Taiwan and USA where CBS is present (section 3.6.1.1). Results from the meta-analysis showed that copper-based compounds were the least efficient fungicides for the control of CBS. The highest disease control levels were obtained with fungicide schedules including dithiocarbamates, benzimidazoles and strobilurines (QoI).

Copper compounds and mancozeb (dithiocarbamate) are the only fungicides currently registered for citrus in the EU (Directive 91/414/CEE) than may have some effect against *P. citricarpa*. Strobilurin fungicides (QoI) and benzimidazoles, which are highly effective for CBS control (Goes, 2002; Goes et al., 2000; Schutte et al., 2003; Miles et al., 2004), are not currently labelled for citrus in the EU, and their future use will depend on private or public funding resources to cover the registration costs. Repeated use of benzimidazoles in South Africa led to the proliferation of *P. citricarpa* strains resistant to this group of fungicides (Schutte et al., 2003). Although the risk of resistance to QoIs is considered to be high (FRAC, 2013), molecular studies have suggested a low QoI resistance risk in *P. citricarpa* populations (Possiede et al., 2009; Stammler et al., 2013; Hincapie et al., 2014). Assuming a potential infection period of about two months in September and October, between one and two fungicide sprays would be necessary to protect the fruit, depending on rainfall and other meteorological factors. For some cultivars, compliance with maximum residue limits (MRLs) will be challenging considering the time lapse between the timing of fungicide application and harvest. In years having an infection period also in spring and early summer, between one and two additional fungicide sprays would be needed during this season for effective disease control.

### 3.6.3. Environmental consequences

In addition to the economic costs of the fungicides and their application in the orchards, environmental side-effects should be also considered. Environmental consequences are envisaged owing to the additional fungicide treatments required for the control of *P. citricarpa* once the pathogen is established (Cunha et al., 2013). Copper compounds and mancozeb have been associated with environmental concerns (Alva et al., 1993; Houeto et al., 1995) and, in fact, the use of copper in organic production in the EU is strictly limited (Regulation EC/473/2002) to reduce environmental pollution of soil and changes in microbial communities (Zhou et al., 2011). Moreover, as it is described above, the effective life of fungicide is shortened if they are used more frequently, reducing their effectiveness for management of other diseases and jeopardising the effectiveness and sustainability of integrated pest management (IPM) approaches (van den Bosch and Gilligan, 2008).

### 3.6.4. Indirect pest effects

In the event of *P. citricarpa* establishment in the EU, indirect pest effects could be linked to the need to implement eradication and/or containment measures to prevent establishment and spread to other EU citrus-growing areas (section 3.6.4.1, below), as well as additional fungicide treatments and/or quality controls in packing houses for citrus fruit exported to non-EU citrus-growing areas.

#### 3.6.4.1. Indirect pest effects: eradication and/or containment

Although eradication has never been proved successful for this disease, containment measures may be needed to prevent or limit the spread of *P. citricarpa* (section 3.4.4) to other citrus-growing areas, if the disease became established in an EU location. These measures are described in detail in section 3.4.4 and further evaluated in section 4 of this opinion.

#### 3.6.4.2. Indirect pest effects: additional fungicides treatments and quality controls for export of citrus fruit

During the last six years (2007–2012), citrus fruit were exported from the EU (EU-27) to 38 countries where CBS is not present and where citrus is also cultivated in commercial orchards (Table 20). Of these 38 countries, five (Bosnia and Herzegovina, the United Arab Emirates, Montenegro, Russia and Algeria) imported from the EU more than 3 500 tonnes of citrus fruit per year.

**Table 20:** List of countries where EU citrus fruit are exported. Only those countries where citrus cultivation exists and citrus black spot is not present are listed. Data are yearly average values for the period 2007 to 2012

| Country                | Average yearly export of citrus fruit from EU-27 (tonnes/year) |
|------------------------|--|
| Russia                 | 129 789  |
| Bosnia and Herzegovina | 25 951   |
| Algeria                | 10 749   |
| United Arab Emirates   | 5 322  |
| Montenegro             | 3 ,563   |
| Angola                 | 793  |
| Turkey                 | 653  |
| Philippines            | 588  |
| Malaysia               | 538  |
| Korea                  | 492  |
| Costa Rica             | 470  |
| Kuwait                 | 301  |
| Japan                  | 276  |
| Mexico                 | 236  |
| Colombia               | 199  |
| Liberia                | 198  |
| Azerbaijan             | 196  |
| Georgia                | 172  |
| Libya                  | 156  |
| Cote d'Ivoire          | 140  |
| Panama                 | 108  |
| Jordan                 | 103  |
| Afghanistan            | 101  |
| Mali                   | 98   |
| Honduras               | 75   |
| Senegal                | 62   |
| El Salvador            | 55   |
| Egypt                  | 45   |
| Mauritius              | 30   |
| Congo                  | 27   |
| Dominican Republic     | 27   |
| Israel                 | 23   |
| Morocco                | 21   |
| Iraq                   | 20   |
| Madagascar             | 16   |
| Lebanon                | 12   |
| Eritrea                | 3  |
| Chile                  | 1  |

Among these 38 countries, Turkey, Jordan, Israel and Chile are currently regulating *P. citricarpa* as a quarantine organism in citrus fruit commodities (see section 3.1.4). In addition, *P. citricarpa* is in the A1 List of the CPPC and in the A2 Lists of the APPC, the COSAVE, the IAPSC and the PPPO. Thus, if *P. citricarpa* became established in the EU, to export citrus fruit, additional fungicide treatments in the orchards, official inspections, quality controls in packing houses and/or establishment of pest-free areas might be needed to meet the phytosanitary requirements of these countries.

### 3.6.5. Conclusion of the assessment of consequences

**Table 21:** Assessment of consequences

| Rating   | Justification  |
|--|--|
| <i>Moderate for fresh fruit of late-maturing citrus varieties and lemons</i> | <p>Owing to the required incubation (a minimum of two months) and to results from the simulations showing more frequent late summer and early autumn infection, late-maturing citrus varieties and lemons are likely to express more symptoms in the field.</p> <p>The main impact will be on quality for the fresh market (fruit with more than one lesion are reduced in quality and with more than six lesions are not suitable for the fresh market). There would a potential for reduction in disease incidence by chemical treatment, but this would cause environmental impacts because in most EU citrus-growing areas fungicides are not widely applied and the most effective fungicide products are not currently registered for use in citrus by the EU MSs. In addition, to export citrus fruit to areas where CBS is regulated, additional fungicide treatments in the orchards, official inspections, quality controls in packing houses and/or establishment of pest-free areas might be needed to meet the phytosanitary requirements of these countries.</p> |
| <i>Minor for fresh fruit of early-maturing citrus varieties</i>              | <p>The impact on early-maturing varieties would be sporadic in time and space, limited to years with rainy springs and/or to specific locations. However, the impact could be higher in areas where late spring infection, based on simulation results, is expected to be more frequent, such as some locations in southern Spain, southern Italy, Portugal, Cyprus, Malta and the Greek islands.</p>  |
| <i>Minimal for citrus for processing</i>                                     | <p>External lesions or spots on citrus fruit are not a quality issue for citrus for processing.</p>  |

### 3.6.6. Uncertainties on the assessment of consequences

**Table 22:** Uncertainty on the assessment of consequences

| Rating      | Justification  |
|-------------|--|
| <b>High</b> | <p>High uncertainties about the time from infection to symptom expression (incubation period)</p> <p>High uncertainties owing to the lack of information on key parameters in the epidemiological models, the lack of knowledge about the rate of disease dynamics and inoculum build-up for this pathogen, especially in semi-arid areas within the current area of distribution, e.g. Eastern Cape Province of South Africa, where environmental conditions are more similar to those in the risk assessment area, and the limited information available about the impact and the fungicides treatments in these marginal areas.</p> |



### 3.7. Conclusion and uncertainties of the pest risk assessment

Under the scenario of absence of specific phytosanitary regulations against *P. citricarpa*, the conclusions of the pest risk assessment are as follows:

#### Entry

The probability of entry is rated as:

- moderately likely for the citrus fruit trade pathway (medium uncertainty)
- very unlikely for the Tahiti lime (*Citrus latifolia*) fruit trade pathway (high uncertainty)
- unlikely for citrus fruit import by passenger traffic pathway (medium uncertainty)
- likely for the citrus fruit with leaves trade pathway (medium uncertainty)
- likely for the citrus plants for planting trade pathway (low uncertainty)
- likely for the Tahiti lime (*Citrus latifolia*) plants for planting trade pathway (high uncertainty)
- likely for the citrus plants for planting import by passengers traffic (medium uncertainty)
- unlikely for the citrus leaves (medium uncertainty).

#### Establishment

The probability of establishment is rated as moderately likely because of:

- the widespread availability of suitable hosts (no uncertainty)
- the suitability for ascospore maturation, dispersal and infection of the climate of many EU citrus-growing areas in September and October and of specific locations also in May (high uncertainty)
- cultural practices (fungicides) not preventing establishment (low uncertainty)
- sprinkle and micro-sprinkle irrigation (still used in some EU citrus-growing areas) favouring establishment (low uncertainty)
- the simultaneous occurrence of host susceptibility and of weather conditions suitable for ascospore production and release and weather conditions for ascospore germination and infection (high uncertainty).

Overall, uncertainty on the probability of establishment is rated as high, mainly because of the lack of knowledge on how *P. citricarpa* will respond under the EU climatic conditions. Although it is known which environmental factors are important for the organism in the various stages of the life cycle, there is a lack of scientific evidence to precisely determine the exact threshold values the organism require, e.g. for temperature and wetness levels and durations. Further validation of the models applied by the Panel, especially for marginal areas within the current distribution of the citrus black spot disease, would be needed to reduce the uncertainty on the probability of establishment of *P. citricarpa* in the EU.

#### Spread

Natural spread of *P. citricarpa* is known to occur by airborne ascospores. The distance the pathogen can spread by natural means is poorly known, The pathogen is very likely to spread with human

assistance along the commercial fruit and plants for planting pathways. However, because spread is defined as the expansion of the geographical distribution of a pest within an area, the rate of spread depends not only on the rapidity of movement and the number of spread pathways but also on the likelihood of finding a suitable environment for establishment. When the proportion of the citrus-growing areas identified as potentially suitable for *P. citricarpa* is taken into account, the Panel considered that a rating of moderately likely is most appropriate for spread.

There is uncertainty about the potential natural spread of ascospores carried by wind over long distances, but this uncertainty does not concern the two main pathways of spread (intra-European trade of commercial fruit and plants for planting). There is uncertainty about the structure of the EU intra-trade network for the citrus plants for planting owing to lack of data; however, this does not influence the conclusions above.

#### Endangered area

The risk assessment has identified parts of the EU where host plants are present and where, based on simulation results, the climatic conditions are suitable for ascospore maturation and release followed by infection.

Conclusions from simulations of the release of ascospores based on gridded interpolated climate data of the EU citrus-producing areas show that, in almost all years (for the 95th percentile), ascospore release in the EU citrus growing areas will start early enough to coincide with climatic conditions that are conducive to infection in late summer and early autumn, as simulated by EFSA (2008). However, the same simulations indicate that the onset of ascospore release in most areas will start too late to coincide with the climatic conditions conducive to infection in spring. Therefore, in the areas where infections occur in late summer and early autumn only, owing to the long incubation period of CBS, fruits of early-maturing varieties are expected to be harvested before symptoms appear. In contrast, under this scenario, late-maturing orange varieties and lemons are expected to show CBS symptoms.

There are some areas however, such as locations in Cyprus, Greek islands, Malta, Portugal, southern Italy and southern Spain where infections may start in May. In those locations, it is expected that symptoms can develop on the fruit before harvest in all susceptible cultivars, and therefore have an impact on the fruit quality.

The results from the simulations on interpolated (grid-based) weather data are consistent with the simulations run on weather data measured by agrometeorological stations. The uncertainty is high, as indicated in the establishment section.

#### Consequences

The results from the simulation of ascospore maturation, release and infection show that citrus black spot will develop and express symptoms mainly in late-maturing sweet orange varieties and lemons grown within the endangered area. The expected consequences will be moderate for fresh fruit of late-maturing citrus varieties and lemons. There is the potential to reduce disease incidence by chemical treatments, but this would have an environmental impact because in most EU citrus-growing areas fungicides are not widely applied and the most effective fungicide products are not currently registered for use in citrus in the EU MSs. In addition, to export citrus fruit to areas where CBS is regulated, additional fungicide treatments in the orchards, official inspections, quality controls in packing houses and/or establishment of pest-free areas might be needed to meet the phytosanitary requirements of these countries.

The consequences for fresh fruit of early-maturing citrus varieties are assessed as minor. The impact on early-maturing varieties would be sporadic in time and space, limited to years with rainy springs and/or to specific locations. However, the impact could be higher in areas where spring infection, based on simulation results, is expected to be more frequent, such as some locations in southern Spain, southern Italy, Portugal, Cyprus, Malta and the Greek islands.

The consequences would be minimal for citrus for processing, as external lesions or spots on citrus fruit are not a quality issue in the case of citrus for processing.

As for establishment, the uncertainties about consequences are high owing to the lack of information on key parameters in the epidemiological models and on the incubation period, the lack of knowledge about the rate of disease build-up for this pathogen; the limited information available about the impact and the fungicides treatments in semi-arid areas within the current CBS area of distribution, e.g. Eastern Cape, where environmental conditions are more similar to those in the pest risk assessment area.

#### **4. Identification of risk reduction options and evaluation of their effect on the level of risk and of their technical feasibility**

This section assesses the effectiveness of options for reducing the risk of entry, establishment and spread of *P. citricarpa* following the ‘Guidance on methodology for evaluation of the effectiveness of options to reduce the risk of introduction and spread of organisms harmful to plant health in the EU territory’ (EFSA PLH Panel, 2012). Section 4.1 first presents a systematic evaluation of options for reducing the probability of entry. This considers all the entry pathways analysed in sections 3.2.2–3.2.9. Section 4.2 evaluates the options for reducing the probability of establishment while section 4.3 evaluates options for reducing the probability of spread. Section 4.4 discusses the effectiveness of combining risk reduction options. The effectiveness of current EU phytosanitary measures is evaluated in section 4.5.

##### **4.1. Systematic identification and evaluation of options to reduce the probability of entry**

In this section, options to reduce the probability of entry of *P. citricarpa* are systematically identified and evaluated. Each of the eight introduction pathways described in the entry part of this opinion (sections 3.2.2–3.2.8) is considered and the citrus fruit commercial trade (section 4.2.1) and citrus plants for planting (section 4.2.5) pathways are analysed in detail. For these pathways, 14 potential risk reduction options (hereinafter abbreviated as RROs) identified by the EFSA Panel on Plant Health (EFSA PLH Panel, 2012) have been evaluated as a stand-alone measure, assuming that other RROs are not in force for that pathway or for the other pathways. The RROs considered are listed in Table 23. This checklist has been followed to ensure that no options are overlooked and consistency and objectivity is maximised between opinions. For each RRO, the Panel assessed its *effectiveness* and *technical feasibility* together with the *uncertainty* in the ratings given. The effectiveness of a systems approach, integrating two or more independent RROs, is discussed in section 4.4.

Prohibiting the import of consignments in theory closes a pathway, making all other RROs for that pathway redundant. The *effectiveness* of this RRO is **very high** for all pathways. The *technical feasibility* is **high** for all pathways because it already is, or can be, implemented in customs operations and phytosanitary import procedures. The level of *uncertainty* is **low**, for all pathways.

The effectiveness of individual RROs in one pathway on the overall probability of entry (via all pathways) is not discussed, nor is the effectiveness of an individual RRO in one pathway compared with RRO(s) in one or more other pathways. To undertake such a complex evaluation, ideally a fully quantitative probabilistic pathway model would be required. For example, the effectiveness of the treatment of consignments of citrus fruit in commercial trade in reducing the overall probability of *P. citricarpa* entry has not been compared with the effectiveness of post-entry quarantine for citrus plants for planting. However, it should be kept in mind that the overall reduction of probability of entry of *P. citricarpa* is determined by the combined set of RROs for all pathways.

**Table 23:** Potential risk reduction options, listed by the EFSA PLH Panel (2012) and used for this opinion

|   |
|---|
| <b>Options for consignments</b>   |
| Prohibition   |
| Prohibition of parts of the host or of specific genotypes of the host   |
| Pest freedom of consignments: inspection or testing   |
| Pre- or post-entry quarantine system  |
| Preparation of the consignment  |
| Specified treatment of the consignment/reducing pest prevalence in the consignment  |
| Restriction on end use, distribution and periods of entry   |
| <b>Options preventing or reducing infestation in the crop at the place of origin</b>  |
| Treatment of the crop, field or place of production in order to reduce pest prevalence                                      |
| Resistant or less susceptible varieties   |
| Growing plants under exclusion conditions (glasshouse, screen, isolation)   |
| Harvesting of plants at a certain stage of maturity or during a specified time of year                                      |
| Certification schemes   |
| <b>Options ensuring that the area, place or site of production at the place of origin remains free from the pest</b>        |
| Limiting import of host plant material to material originating in pest-free areas (PFAs)                                    |
| Limiting import of host plant material to material originating in pest-free production places or pest-free production sites |

#### 4.1.1. RROs to reduce entry along the citrus fruit commercial trade (pathways I, II and IV)

This section deals with the identification and evaluation of RROs to reduce the probability of entry of *P. citricarpa* along the three pathways of citrus fruit commercial trade described in the entry section: the pathway (I) of commercial trade of citrus fruit (excluding Tahiti lime and citrus fruit with leaves), the pathway (II) of commercial trade of Tahiti lime fruit and the pathway (IV) of commercial trade of citrus fruit with leaves and peduncles (for a detailed description and analysis of these pathways, see sections 3.2.2, 3.2.3 and 3.2.5, respectively).

The results of this evaluation are summarised in Table 24.

##### A. Options for consignments

###### 4.1.1.1. Prohibition

The prohibition of the import of all citrus fruit along the three pathways of commercial trade would prevent the entry of *P. citricarpa* into the EU along these pathways. The *effectiveness* is assessed as **very high**. The *technical feasibility* is **very high**, because it can be implemented in customs operations and phytosanitary procedures. The *uncertainty* on these ratings is assessed as **low**.

###### 4.1.1.2. Prohibition of parts of the host or of specific genotypes of the host

###### a) Prohibition of parts of the host

This option would prohibit the presence of all other plant material than fruit (potentially carrying *P. citricarpa*, such as leaves and peduncles) in the consignment. The *effectiveness* is assessed as **high**. *P. citricarpa* reproduces through pycnidiospores in fruit and ascospores in leaves, with a much broader dispersal potential for ascospores. Thus, prohibiting the presence of leaves can greatly reduce the introduction potential of *P. citricarpa*. The *technical feasibility* is **very high**, because it is already

implemented in customs operations and phytosanitary procedures. The *uncertainty* on these ratings is assessed as **low**.

#### b) Prohibition of specific genotypes of the host

All commercial citrus species and cultivars are considered to be susceptible to CBS, except for sour orange (*C. aurantium*) and Tahiti lime (*C. latifolia*) (see section 3.1.1.4). The host status of pomelo (*C. maxima*) is still uncertain (see section 3.1.1.4). The prohibition of import of fruit of susceptible citrus varieties would therefore default to a general prohibition of all citrus fruit except for Tahiti lime and sour orange fruit.

The *effectiveness* in reducing the probability of entry of *P. citricarpa* via this pathway is **very high**, as, considering the current trade flows, it is almost equivalent to the general prohibition of import of fruit from all citrus species. The *technical feasibility* is **moderate**, because fruits of sour orange and Tahiti limes cannot be clearly identified at import inspection unless inspectors are well trained or equipped with tools for fruit analysis. The *uncertainty* for these ratings is **medium** because some publications indicate that sour orange and Tahiti lime, although pycnidia have never been observed on their fruit, can be colonised by *P. citricarpa* (see section 3.1.14).

#### 4.1.1.3. Pest freedom of consignments: inspection or testing

The detection of *P. citricarpa* in consignments is based on visual inspection, sampling and laboratory testing. Inspection and sampling of the consignment should be performed according to guidelines in the IPPC Standards ISPM No 23 Guidelines for inspection (FAO, 2005) and ISPM No 31 Methodologies for sampling of consignments (FAO, 2009). For laboratory testing, *P. citricarpa*-specific detection methods have been developed (see section 3.1.1.3). Inspection or testing of consignments may be applied at the time of export and/or at the time of import. At export, inspection or testing may serve as a stand-alone measure without other official measures for production, harvest and packaging or as a measure to verify that other measures have been effective. At import, inspection generally serves to verify phytosanitary measures taken by the exporting country.

The CBS disease is characterised by a long incubation period (50–200 days; see section 3.1.1.2). Fruit symptoms become visible only several months after infection and may not yet have appeared at the time of inspection (at export or at import). Infected but asymptomatic fruit may pass these inspections unnoticed, limiting the effectiveness of visual inspection. Techniques to induce symptom expression and laboratory testing using molecular procedures are generally required for the detection of latent infections and for accurate identification of the pathogen. Following the recent discovery of new *Phyllosticta* species on citrus, the performance of diagnostic protocols needs to be reassessed (see section 3.1.1.3). The effectiveness of both visual inspection and laboratory testing for detection of *P. citricarpa* in consignments of citrus fruit depends on the sampling method and the sample size. No method will provide 100 % effectiveness of detection. The effectiveness of visual inspection alone is further limited by the possible presence of latent or mildly infected fruits escaping detection in the sample. The *effectiveness* of visual inspection as stand alone measure is assessed as **low**, with **low uncertainty**.

The visual inspection of consignments combined with laboratory testing is effective in reducing the probability of entry of *P. citricarpa* along the citrus fruit commercial trade pathways, provided that up to date diagnostic protocols are used. A sampling procedure that gives high confidence in detecting low disease incidence should be employed (e.g. > 90 % effectiveness), but no method will provide 100 % effectiveness of detection. Owing to the difficulties in implementing the most effective sampling procedures, the *effectiveness* of visual inspection combined with laboratory testing is assessed as **moderate**.

The *technical feasibility* of visual inspection is **moderate**, owing to the huge volumes of imported citrus fruit that would have to be inspected to give a high confidence. Also some EU MSs may be following a reduced check regime on citrus under 1756/2004/EC (this is a voluntary system that can

be applied by MSs if interceptions have only been found at a very low level in a large number of consignments inspected over the previous three years). The technical feasibility of routine laboratory testing for exported and imported consignments is **moderate** owing to the relatively long duration of some laboratory procedures, although new methods available may reduce the time required (Tomlinson et al., 2013).

The *uncertainty* for these ratings is **medium** because of the lack of knowledge on the proportion of CBS latent infection in citrus fruit consignments (see section 4.1.1.1, below), the lack of an estimate of the incidence of CBS in imported consignments and the lack of a detailed analysis of the practical implementation of the inspections in all points of entry in the EU together with the recent description of new *Phyllosticta* species associated with citrus, for which some molecular detection methods appear to be insufficiently sensitive.

#### 4.1.1.4. Pre- or post-entry quarantine system

Pre- or post-entry quarantine systems are not applicable to citrus fruit commercial trade at the ports of the exporting or importing country because of the size of the consignments and to the difficulty of storing citrus fruit for long period to make the expression of symptoms possible.

Regarding pre-harvest inspections, Baldassari et al. (2007) have shown that treating asymptomatic fruit of orange ‘Pêra-Rio’, aged between 20 and 28 weeks, after flowering by immersion in a solution of ethephon (2.10 g/l, 1 minute) induces precocious symptom expression (assessed 28–35 days after treatment) of *P. citricarpa* in proportions equivalent to those observed in fruit matured on trees. This system applied to field samples of asymptomatic citrus fruit allows the detection of latent infections of *P. citricarpa* in advance in the country of origin before harvest and export. This technique could be applied in the country of origin before harvest with **high effectiveness** and **high technical feasibility**, and with **medium uncertainty** owing to the lack of information on the field sampling protocol applied.

#### 4.1.1.5. Preparation of the consignment

The preparation of the consignment includes several stages, beginning with the handling of harvested fruit and transport to the packing station, to the closing of boxes or other packaging material prior to export. Specific conditions and procedures, particularly culling, may be implemented during this process to reduce the presence of *P. citricarpa* infected units in the consignment. Management procedures at citrus fruit packing stations can play an important role in reducing the incidence of CBS-infected fruit in consignments. Packing stations should be registered and employ a system of record keeping, enabling quality control of packing house operations and the tracking and tracing of consignments from the production site and the recording of information on the disease management programme. Fruit originating from official pest-free areas and official pest-free places of production should be packed at dedicated packing stations where handling of fruit from other places of production is not allowed.

The culling and cleaning of fruit may allow the removal of leaves, peduncles, other debris and many (but not all) symptomatic fruits. However, the *effectiveness* of this option when applied alone is assessed as **low**, because of the existence of latent infections and the similarity of unspecific CBS symptoms to those caused by other citrus pathogens as well as by mechanical or insect damage (see section 3.2.2.1). The *technical feasibility* is **very high** since such measures are currently implemented in citrus-producing countries. The *uncertainty* of these ratings is **medium** because knowledge on the proportion of CBS latent infection in citrus fruit consignments is limited.

#### 4.1.1.6. Specified treatment of the consignment/reducing pest prevalence in the consignment.

During the preparation of consignments of citrus fruit, several treatments, such as waxing or hot water and fungicide treatments, may be applied that can reduce the viability and delay the post-harvest development of *P. citricarpa*, but they are unlikely to eliminate the pathogen (see section 3.2.2.3). Methods that eliminate *P. citricarpa* from infected fruit are not available.

It is recommended that registered packing houses have an approved system in place to limit the build-up in the treatment tank of extraneous organic matter, including leaves, twigs, grass, weed, soil, slime or any other material that would interfere with the treatment.

The *effectiveness* of post-harvest chemical treatments alone is **low**, the *technical feasibility* is **very high**, since such treatments are currently implemented in fruit packing houses, and the *uncertainty* is **low**.

#### 4.1.1.7. Restriction on end use, distribution and periods of entry

##### **Restriction on period of entry**

It is not possible to identify periods of the year when the harvested citrus fruit in the country of origin is uninfected. Therefore, the *effectiveness* of a restriction in the period of import of citrus fruit, to reduce the probability of entry of the pathogen, is assessed as **negligible**, although the *feasibility* is **very high**, with **low uncertainty**.

##### **Restriction on distribution of imported citrus fruit within the pest risk assessment area**

Plants susceptible to CBS are not grown throughout the EU, and climatic conditions are not suitable for the disease throughout the EU. Therefore, a restriction of the distribution of imported consignments of citrus fruit potentially infected with CBS to the parts of the EU where host plants of CBS are absent, or climatic conditions inhibit the development of CBS, could be investigated. The basis for this RRO would be the demarcation of endangered and non-endangered areas of the EU with respect to CBS.

However, the EU treaties allow for the free trade of citrus fruit in the entire territory of the EU. Consignments of citrus fruit imported in a MS without citrus production and subjected to phytosanitary inspection in that MS may subsequently be traded to citrus-producing areas of the EU without further inspections. For example, in 2009 the Netherlands imported around 450 kt of sweet orange and 170 kt of grapefruit from various countries (including CBS-affected countries such as the USA, Argentina, Brazil and Uruguay) and re-exported almost 200 kt of sweet orange and 115 kt of grapefruit to other EU countries, including citrus-producing countries (Eurostat, online).

Specific plant health risks associated with the free internal market of the EU may, under conditions of Council Directive 2000/29/EC, be managed with the concept of 'Protected Zone'. Protected Zones may be established with respect to (a) pests listed in 2000/29/EC that are established in one or more parts of the EU but are not established in the Protected Zone despite favourable conditions for establishment there; and (b) pests that are not endemic or established in the EU, but for which there is a danger of establishment, given propitious ecological conditions, on particular crops.

Since CBS would qualify according to option (b), the endangered area of the EU with respect to this pest might be designated a Protected Zone. The introduction into and movement within this endangered area of specified commodities may be prohibited completely or may be restricted according to special requirements. Within the non-endangered area there would be no restriction on the introduction and movement of citrus commodities.

Several scenarios could be envisaged to restrict the introduction of citrus fruit potentially infected by *P. citricarpa* into the endangered area of the EU, ranging from a full prohibition of import of citrus fruit to combinations of special requirements for such commodities. This would have to cover the introduction from third countries and from the non-endangered area of the EU. Under all scenarios specific procedures need to be developed to prevent the high rate of movement of consignments of citrus fruit from the non-endangered area (where there would be no requirements with respect to CBS) to the endangered area (see examples of these volumes presented above), taking into account the fact that internal frontiers and designated border inspection points between endangered and non-endangered areas do not exist in the EU. Should the possibilities for trade between non-endangered

and endangered areas be maintained, then in the non-endangered area of the EU the import of consignments satisfying special requirements with respect to CBS would have to be distinguished from import of other consignments. The trade of consignments possibly infected by CBS from the non-endangered to the endangered area of the EU would have to be prohibited. Imported consignments satisfying the special import requirements would have to be officially labelled and their movement officially monitored and registered throughout the traffic within the EU to their final destination, to prevent mixing or repacking of consignments, subject to the complex internal pathways and market structure for citrus fruit within the EU (section 3.4.2).

The *effectiveness* of restricting the distribution of consignments of fruit potentially infected by *P. citricarpa* to the non-endangered area of the EU would be **high**. The *technical feasibility* is assessed as **low** because of the difficulties to establish and maintain the required control and monitoring systems, associated with the designation of protected zones with respect to CBS, as explained above. The *uncertainty* on these ratings is **low**.

### **Restriction on end use of imported consignments of citrus fruit**

Part of the imported citrus fruit consignments is destined for industrial processing (mainly juice but also marmalade, etc.). In the non-endangered area of the EU, the officially controlled import, immediate movement to the processing facility and processing of consignments of citrus fruit potentially infected by *P. citricarpa* would strongly reduce the probability of transfer to a suitable host. Elements of such official control are, for example, the regular monitoring of storage and processing premises, specification of points of entry in the EU, and supervised transport of imported consignments. Those fruit processing facilities should nevertheless have the capacity to prove that no fruit or any other citrus by-product escape the processing lines and should employ adapted traceability, containment and waste processing measures (according to the guidelines for handling of such biowaste in EPPO Standard PM 3/66(2)). This approach is conceivable on the basis of a derogation from official special import requirements for citrus fruit with respect to absence of *P. citricarpa*, for officially registered and approved processing facilities.

In the endangered area of the EU, citrus processing plants are located within or near citrus-producing areas and therefore even more stringent containment, traceability and control measures would be required to reduce the probability of transfer of *P. citricarpa* from fruit processing facilities to suitable hosts to an acceptable level. The *effectiveness* of a derogation approach is assessed as **high**, based on the arguments presented above. The *technical feasibility* of a derogation approach is assessed as **high** for the non-endangered area and as **low** for the endangered area of the EU, owing to the difficulties in implementing the required levels of containment and control measures in the endangered area. The *uncertainty* on these ratings is **low**.

## **B. Options preventing or reducing infestation in the crop at the place of origin**

### 4.1.1.8. Treatment of the crop, field or place of production in order to reduce pest prevalence.

Fungicide treatments against *P. citricarpa* infestation in orchards at the place of origin can reduce the incidence of CBS, but do not eliminate it (see the meta-analysis of CBS control trials in section 3.6.1.1). Culling and cleaning of fruit at harvest removes leaves, peduncles and many (but not all) symptomatic fruits, but is not effective in reducing the presence of asymptomatic latent infections. The *effectiveness* of treatments of the crop and orchards at the origin is thus **moderate**. The *technical feasibility* is **very high**, given that these treatments are already applied. The *uncertainty* for these ratings is **low**.

### 4.1.1.9. Resistant or less susceptible varieties.

All citrus species and cultivars grown for fresh fruit production are susceptible to CBS caused by *P. citricarpa*, except for sour orange (*C. aurantium*) and Tahiti lime (*C. latifolia*). The host status of pomelo (*C. maxima*) is still uncertain (see section 3.1.1.4). The *effectiveness* of the use of cultivars that are resistant or tolerant to *P. citricarpa* would be **high** for pathway II (Tahiti lime fruit commercial



trade without leaves and peduncles), but would be **low** overall because currently Tahiti lime and sour orange constitute only a small fraction of the total import of citrus fruit. This rating could be increased in the future if citrus varieties genetically modified for CBS resistance traits (Kava-Cordeiro et al., 2012) became available. The *technical feasibility* is very high for Tahiti lime and sour orange but is **low** overall given the current lack of resistant or tolerant varieties of sweet oranges, mandarins, grapefruit or lemons. The *uncertainty* for these ratings is **high**, owing to some publications indicating that sour orange and Tahiti lime fruit can be colonised by *P. citricarpa* and that ascospores of this pathogen are produced in the leaf litter of Tahiti lime.



**Figure 52:** Citrus orchards for commercial fruit production under nets for protection from hailstorms

#### 4.1.1.10. Growing plants under exclusion conditions (glasshouse, screen, isolation).

Growing commercial citrus orchards for fruit production under exclusion could theoretically limit infection by reducing the introduction of external inoculum but may require screening with extremely fine mesh nets and forced ventilation. Very early-maturing varieties are sometimes grown under nets to protect them from hailstorms and other meteorological factors, but this kind of net is not fine enough to impede the dispersal of *P. citricarpa* spores (Figure 52). Complete exclusion conditions are applicable to plant propagation material (Figure 53), but not to commercial citrus orchards on a large scale.

The *effectiveness* is likely to be **low**, since, owing to the microscopic dimensions of *P. citricarpa* spores and because of the difficulty of securely excluding the pathogen in close proximity to outdoor grown citrus that may be infected, infection cannot be completely excluded. The *technical feasibility* is **negligible**, because of the difficulty of implementation in citrus orchards for fruit production over large areas. The *uncertainty* of these ratings is **medium**, owing to the lack of data on the effectiveness of exclusion and the dispersal potential of the pathogen.

#### 4.1.1.11. Harvesting of plants at a certain stage of maturity or during a specified time of year

Citrus fruit are susceptible to infection by *P. citricarpa* for several months after petal fall (Reis et al., 2003; Brentu et al., 2012). Following fruit infection, the latent period can last between 50 and 200 days (McOnie, 1967; Kellerman and Kotzé, 1977; Kotzé, 1981; Aguiar et al., 2012), depending on the citrus variety and growing conditions.

The *effectiveness* of harvesting citrus fruit during a specified time of the year is **negligible**, owing to the long susceptibility period and also the time that has elapsed between fruit infection and symptoms development.

The *technical feasibility* is **low**, because of the need to harvest citrus fruit at commercial maturity.

The *uncertainty* for these ratings is **low**.

#### 4.1.1.12. Certification scheme

Citrus plants for planting, produced under a certification scheme, will initially be free from *P. citricarpa*. However, these plants can become infected when planted in a citrus-growing area where *P. citricarpa* occurs. The prevalence of *P. citricarpa* in the orchard will then become dependent on the measures discussed in section 4.1.1.8. In areas where *P. citricarpa* occurs, the *effectiveness* of this option in reducing the probability of entry via the fruit pathway is likely to be **low**, the *technical feasibility* is **low** and the *uncertainty* is **medium**, owing to the lack of data on the local incidence of the pathogen in CBS-infested countries.

### **C. Options ensuring that the area, place or site of production at the place of origin remains free from the pest**

#### 4.1.1.13. Limiting the import of host plant material to material originating in pest-free areas

A pest-free area is defined as an area in which a specific pest does not occur as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained (ISPM No 4; FAO, 1995). A pest-free area may be an entire country, a non-infested part of a country in which a limited infested area is present or a non-infested part of a country situated within a generally infested area. Pest freedom of the area must be supported by general surveillance, delimiting surveys to demarcate the area and detection surveys to demonstrate the absence in the area and its buffer zone (for guidance on surveys and surveillance, see EFSA PLH Panel, 2012). Phytosanitary measures must be in place to prevent the movement of potentially infested material into the area and to prevent natural spread of the pest into the area.

Surveys for CBS to demonstrate the pest-free status of a region within a CBS-infested country are not without their limitations, because of the likelihood of latently infected plants or *P. citricarpa* populations at low incidence being undetected in surveys. In the areas where the pathogen is currently distributed, CBS is usually first detected on lemons. Therefore, lemon trees should be the first to be inspected in an area for the detection of the pathogen. Based on the slow rate of spread and the frequent occurrence of latent infection, effective buffer zones are difficult to implement.

When the import of citrus fruit is restricted to material originating in pest-free areas, the probability of introduction of *P. citricarpa* into the risk assessment area is reduced. The *effectiveness* is assessed as **high**, but this depends on the frequency and the confidence level of detection surveys to confirm the absence of *P. citricarpa* in the pest-free area and on the intensity of phytosanitary measures to prevent entry of plant material (including fruit) infected by *P. citricarpa* into the pest-free area. The design and frequency of surveys to confirm absence of *P. citricarpa* in the area should take into account the scattered presence of unmanaged citrus plants in private gardens, public areas or in uncultivated areas and the possible presence of latently infected plants in order to reach the required confidence level of the surveys.

The *technical feasibility* of the establishment and maintenance of a pest-free area for *P. citricarpa* is **high** in countries where *P. citricarpa* is absent. The *feasibility* of establishment and maintenance of pest-free areas in proximity to CBS-infested areas is assessed as **moderate**, owing to the difficulties of detecting latent infections and low disease incidence in combination with the long lag phase observed between the first establishment and the development of CBS epidemics (see sections 3.1.1.2, 3.3.4 and 3.4.4). The *uncertainty* for these ratings is **low**.

#### 4.1.1.14. Limiting import of host plant material to material originating in pest-free production places or sites of production

The designation and maintenance of pest-free places or sites of production with respect to CBS is limited because of the presence of latent infections of *P. citricarpa* in citrus fruits and the difficulties in distinguishing CBS symptoms from those caused by other citrus pests and diseases. Also, as stated above (section 4.1.1.3), growing citrus orchards under exclusion conditions has **low feasibility**.

The *effectiveness* of this option is **low**. The *technical feasibility* is **low**, given the difficulties in maintaining the pest-free status of places and sites of production within CBS-infested countries owing to latent fruit infections, the rarity of foliar symptoms and the postulated long lag phase between the first establishment and the development of the epidemics (see sections 3.1.1.2, 3.3.4. and 3.4.4).

The *uncertainty* is **medium**, owing to the lack of detailed information on the incidence or absence of the pathogen at local level, as well as the lack of knowledge on the development of CBS epidemics at its inception in new sites.

#### 4.1.1.15. Systems approaches integrating individual RROs

Systems approaches combining individual RROs may further reduce the probability of entry of *P. citricarpa* along this pathway. The following combinations are proposed:

- For fruit originating from pest-free areas or pest-free production places, harvest and transport to packing stations should be done using clean boxes free of other plant materials. Packing should be carried out only in designated packing houses registered for packing of fruit from areas and production places free of *P. citricarpa*. The *effectiveness* and *feasibility* is **high** with **low uncertainty**.
- For fruit originating from areas where *P. citricarpa* is present, cultural measures and fungicide treatments to prevent *P. citricarpa* infections in the orchards should be combined with handling procedures and post-harvest treatments for fruit during packing to suppress the pathogen during handling and packing. Packing houses should keep a register of all processed fruit lots to allow tracking and tracing of infestations. Detection of latent infections in fruit prior to harvest by using ethephon dips and incubation will reduce the possibility of further symptom development during transport and storage. The *effectiveness* of each of these measures individually is assessed as **low**, except for the treatment in the orchard, which has **moderate effectiveness**, and etephon detection, which has **high effectiveness**. The *effectiveness* of the integrated approach combining these measures with appropriate official inspections is assessed as **high**. The *technical feasibility* is **high**, and the *uncertainty* is assessed as **medium**.

**Table 24:** Summary of the risk reduction options identified and evaluated to reduce the entry along the citrus fruit commercial trade (pathways I, II and IV)

| Category of options  | Type of measure (for details, see EFSA PLH Panel, 2012)  | Position in the pathway          | Existing measure | Effectiveness   | Technical feasibility | Uncertainty |
|--|--|----------------------------------|------------------|---|-----------------------|-------------|
| <b>Options for consignments</b>                                    | Prohibition  | Before shipment                  | No               | Very high   | Very high             | Low         |
|  | Prohibition of parts of the host   | Before shipment                  | Yes              | High  | Very high             | Low         |
|  | Prohibition of specific genotypes of the host  | Before shipment                  | No               | Very high   | Moderate              | Medium      |
|  | Pest freedom of consignments; inspection   | Before shipment and/or at import | Yes              | Low   | Moderate              | Low         |
|  | Pest freedom of consignments; inspection combined with testing   | Before shipment and/or at import | No               | Moderate  | Moderate              | Medium      |
|  | Pre- or post-entry quarantine system: at harbours of exporting or importing country  | Before shipment and/or at import | No               |   | Not applicable        |             |
|  | Pre- or post-entry quarantine system; in the country of origin at the orchard before harvest (induction of precocious symptoms expression in citrus fruit samples) | Before shipment and/or at import | No               | High  | High                  | Medium      |
|  | Preparation of consignment   | Before shipment                  | No               | Low   | Very high             | Medium      |
|  | Specified treatment of consignment   | Before shipment                  | Yes              | Low   | Very high             | Low         |
|  | Restriction on end use, distribution and periods of entry: period of entry   | Before shipment and/or at import | No               | Negligible  | Very high             | Low         |
| Restriction on end use, distribution and periods of entry: end use | After import   | No                               | High             | High (non-endangered areas)<br>Low (endangered areas) | Low                   |             |

| Category of options   | Type of measure (for details, see EFSA PLH Panel, 2012)  | Position in the pathway | Existing measure | Effectiveness | Technical feasibility                                 | Uncertainty |
|---|--|-------------------------|------------------|---------------|---|-------------|
|   | Restriction on end use, distribution and periods of entry: distribution  | After import            | No               | High          | High (non-endangered areas)<br>Low (endangered areas) | Low         |
| <b>Options for the crop at the place of origin</b>  | Treatment of the crop, field or place of production  | Before shipment         | No               | Moderate      | Very high   | Low         |
|   | Resistant or less susceptible varieties  | Before shipment         | No               | Low           | Low   | High        |
|   | Growing plants under exclusion conditions  | Before shipment         | No               | Low           | Negligible  | Medium      |
|   | Harvesting of plants during a certain period   | Before shipment         | No               | Negligible    | Low   | Low         |
|   | Certification scheme   | Before shipment         | Yes              | Low           | Low   | Medium      |
| <b>Options ensuring that the area, place or site of production at the place of origin, remains free from the pest</b> | Limiting import of host plant material to material originating in pest-free areas  | Before shipment         | Yes              | High          | Moderate to high                                      | Low         |
|   | Limiting import of host plant material to material originating in pest-free production places or pest-free production sites  | Before shipment         | Yes              | Low           | Low   | Medium      |
| <b>Systems approaches</b>   | Pest-free areas and production places combined with dedicated packing stations   | Before shipment         | No               | High          | High  | Low         |
|   | Infested production places: measures in orchards combined with: handling procedures and treatments during packing; detection of latent infections in fruit prior to harvest by using ethephon dips and incubation; visual inspection and testing | Before shipment         | No               | High          | High  | Medium      |
|   | Combined restriction on end use and distribution of imported citrus fruit  | After import            | No               | High          | Low   | Medium      |
| <b>Options for</b>  | Prohibition  | Before shipment         | No               | Very high     | Very high   | Low         |

| Category of options                                | Type of measure (for details, see EFSA PLH Panel, 2012)  | Position in the pathway          | Existing measure | Effectiveness | Technical feasibility | Uncertainty |
|--|--|----------------------------------|------------------|---------------|-----------------------|-------------|
| <b>consignments</b>                                | Prohibition of parts of the host   | Before shipment                  | Yes              | High          | Very high             | Low         |
|  | Prohibition of specific genotypes of the host  | Before shipment                  | No               | Very high     | Moderate              | Medium      |
|  | Pest freedom of consignments; inspection or testing  | Before shipment and/or at import | Yes              | Moderate      | Moderate              | Medium      |
|  | Pre- or post-entry quarantine system: at harbours of exporting or importing country  | Before shipment and/or at import | No               |               | Not applicable        |             |
|  | Pre- or post-entry quarantine system; in the country of origin at the orchard before harvest (induction of precocious symptoms expression in citrus fruit samples) | Before shipment and/or at import | No               | High          | High                  | Medium      |
|  | Preparation of consignment   | Before shipment                  | No               | Low           | Very high             | Medium      |
|  | Specified treatment of consignment   | Before shipment                  | Yes              | Low           | Very high             | Low         |
|  | Restriction on end use, distribution and periods of entry: period of entry   | Before shipment and/or at import | No               | Negligible    | Very high             | Low         |
|  | Restriction on end use, distribution and periods of entry: end use   | After import                     | No               | Negligible    | Negligible            | Medium      |
|  | Restriction on end use, distribution and periods of entry: distribution  | After import                     | No               | High          | Negligible            | Low         |
| <b>Options for the crop at the place of origin</b> | Treatment of the crop, field or place of production  | Before shipment                  | No               | Moderate      | Very high             | Low         |
|  | Resistant or less susceptible varieties  | Before shipment                  | No               | Low           | Low                   | High        |
|  | Growing plants under exclusion conditions  | Before shipment                  | No               | Low           | Low                   | Medium      |
|  | Harvesting of plants during a certain period   | Before shipment                  | No               | Negligible    | Low                   | Low         |
|  | Certification scheme   | Before shipment                  | Yes              | Low           | Low                   | Medium      |

| Category of options   | Type of measure (for details, see EFSA PLH Panel, 2012)  | Position in the pathway | Existing measure | Effectiveness | Technical feasibility | Uncertainty |
|---|--|-------------------------|------------------|---------------|-----------------------|-------------|
| <b>Options ensuring that the area, place or site of production at the place of origin, remains free from the pest</b> | Limiting import of host plant material to material originating in pest-free areas  | Before shipment         | Yes              | High          | Moderate to high      | Low         |
|   | Limiting import of host plant material to material originating in pest-free production places or pest-free production sites  | Before shipment         | Yes              | Low           | Low                   | Medium      |
| <b>Systems approaches</b>   | Pest-free areas and production places combined with dedicated packing stations   | Before shipment         | No               | Very high     | High                  | Low         |
|   | Infested production places: measures in orchards combined with: handling procedures and treatments during packing; detection of latent infections in fruit prior to harvest by using ethephon dips and incubation; visual inspection and testing | Before shipment         | No               | High          | High                  | Medium      |
|   | Combined restriction on end use and distribution of imported citrus fruit  | After import            | No               | High          | Low                   | High        |

#### 4.1.2. RROs to reduce entry along the citrus fruit import by passenger traffic (pathway III)

The RROs for this pathway are similar to those of the previous section. The results are summarised in Table 25.

##### A. Options for consignments

###### 4.1.2.1. Prohibition

The prohibition of import of citrus fruit by passengers would prevent the entry of *P. citricarpa* into the EU along this pathway. Such a prohibition requires compliance by passengers, which can be influenced by the intensity and clarity of communication and by the frequency of passenger checks. The *effectiveness* is **high**, although it would depend on proper communication and the level of compliance by passengers. As an example, results from audits performed in Australia, where such a prohibition is implemented, show that interceptions of fruit carried by passengers are a regular occurrence, despite the communication campaigns. Moreover, there is a need for a high frequency of the passenger checks. The *technical feasibility* is therefore **low**. The *uncertainty* is **medium** owing to the lack of EU data on the frequency of citrus fruit transport by passengers.

###### 4.1.2.2. Prohibition of parts of the host or of specific genotypes of the host

Not applicable.

###### 4.1.2.3. Phytosanitary certificates and other compliance measures

Not applicable.

###### 4.1.2.4. Pest freedom of consignments: inspection or testing

The *effectiveness* of visual inspection of citrus fruit, carried by passengers, for symptoms of *P. citricarpa* is **moderate** owing to the presence of asymptomatic latent infections and confusion with symptoms of other injuries and pests. Testing is not applicable, since passengers cannot reasonably be expected to wait for the results of the test. The *technical feasibility* of inspection of citrus fruit carried by passengers as an option to reduce the risk of entry of *P. citricarpa* is **low**. With an estimated 0.1 % of passengers carrying, on average, one citrus fruit and thousands of passengers arriving daily in the EU, the frequency of passenger checks would have to be high. The *uncertainty* on these ratings is **low**.

###### 4.1.2.5. Pre- or post-entry quarantine system.

Not applicable.

###### 4.1.2.6. Preparation of the consignment

Not applicable.

###### 4.1.2.7. Specified treatment of the consignment/reducing pest prevalence in the consignment.

Not applicable.

###### 4.1.2.8. Restriction on end use, distribution and periods of entry

Not applicable.

##### B. Options preventing or reducing infestation in the crop at the place of origin

Such options are not applicable to citrus fruit carried by passengers.

##### C. Options ensuring that the area, place or site of production at the place of origin remains free from the pest

Such options are not applicable to citrus fruit carried by passengers.



**Table 25:** Summary of applicable risk reduction options identified and evaluated for the pathway III: citrus fruit import by passenger traffic

| Category of options      | Type of measure (for details, see EFSA PLH Panel, 2012) | Position in the pathway | Existing measure | Effectiveness | Technical feasibility | Uncertainty |
|--------------------------|---|-------------------------|------------------|---------------|-----------------------|-------------|
| Options for consignments | Prohibition   | During customs checks   | No               | High          | Low                   | Medium      |
|                          | Visual inspection for pest freedom                      | During customs checks   | No               | Moderate      | Low                   | Low         |

#### 4.1.3. RROs to reduce entry along the commercial trade of citrus plants for planting, excluding seeds (pathways V and VI)

This section deals with the identification and evaluation of RROs to reduce the probability of entry of *P. citricarpa* along the two pathways of commercial trade of citrus plants for planting, excluding seeds, described in the entry section: the pathway (V) of commercial trade of citrus plants for planting and the pathway (VI) of commercial trade of Tahiti lime plants for planting (for a detailed description and analysis of these pathways see sections 3.2.6 and 3.2.7, respectively). The plants for planting of Tahiti lime are dealt with here together with the general pathway of citrus plants for planting because Baldassarri et al. (2008) demonstrated that *P. citricarpa* colonises and produces ascospores on leaves of Tahiti lime (see section 3.1.1.4). There is therefore no difference in RROs along pathways V and VI.

Seeds are not included as they are not considered to be a potential entry pathway for *P. citricarpa* (see section 3.2.1).

The results of the evaluation are summarised in Table 26.

#### A. Options for consignments

##### 4.1.3.1. Prohibition

The *effectiveness* of prohibition is **very high**. The prohibition of imports of citrus plants for planting would be likely to prevent the introduction of the organism into the EU territory on citrus plant material for propagation purposes as well as on ornamental citrus plants for planting, particularly when these are latently infected.

The *technical feasibility* is **very high**, because it can be implemented in phytosanitary import procedures and customs operations and is already implemented in the EU (Council Directive 2000/29/EC, Annex III, point 16).

The *uncertainty* is assessed as **low**.

##### 4.1.3.2. Prohibition of parts of the host or of specific genotypes of the host

###### *Prohibition of specific genotypes*

As far as citrus species grown for propagating purposes (e.g. rootstocks) are concerned, sour orange (*C. aurantium*) has traditionally been considered resistant to CBS, but experimental studies would be needed to demonstrate whether it can still carry the pathogen if imported (section 3.1.1.4). The *effectiveness* of prohibiting plants for planting of all citrus species apart from sour orange is **high**,

especially as sour orange rootstocks are mostly propagated by seed (sour oranges for ornamental purpose can also be vegetatively propagated). The *technical feasibility* of limiting the prohibition of citrus propagating material imports to specific genotypes is, however, **low**, given the expertise required to distinguish between plants for planting of different citrus genotypes. The *uncertainty* is **high** because *P. citricarpa* has been isolated from asymptomatic leaves of sour orange in Brazil (Wickert et al., 2009), although *P. citricarpa* has not been observed to reproduce on this host.

#### *Prohibition of parts of the host*

Citrus vegetative plant propagation material always includes leaves or buds, which are likely to transport the pathogen if infected, so this option is not applicable.

#### 4.1.3.3. Pest freedom of consignments: inspection or testing

As far as citrus species grown for propagating purposes (e.g. rootstocks) are concerned, sour orange (*C. aurantium*) has traditionally been considered resistant to be CBS, but experimental studies would be needed to demonstrate whether it can still carry the pathogen if imported (section 3.1.1.4). The *effectiveness* of prohibiting plants for planting of all citrus species apart from sour orange is **high**, especially as sour orange rootstocks are mostly propagated by seed (sour oranges for ornamental purposes can also be vegetatively propagated). The *technical feasibility* of limiting the prohibition of citrus propagating material imports to specific genotypes is, however, **low**, given the expertise required to distinguish between plants for planting of different citrus genotypes. The *uncertainty* is **high** because *P. citricarpa* has been isolated from asymptomatic leaves of sour orange in Brazil (Wickert et al., 2009), although *P. citricarpa* has not been observed to reproduce on this host.

#### 4.1.3.4. Pre- or post-entry quarantine system.

*P. citricarpa*-infected citrus seedlings, scions and budwood are likely to remain asymptomatic and there is no validated method reported in the literature for inducing CBS symptom expression on living plants. Since latent infection of plants for planting may occur, post-entry quarantine measures may be applied. Post-entry quarantine is applied for import of citrus nursery stock in EU MSs (Council Directive 2008/61/EC) and in other citrus producing countries (e.g. Biosecurity New Zealand, 2010; Vidalakis et al., 2010). For example, in New Zealand, the imported propagation material must be grown for a minimum period of 6–16 months in a post-entry quarantine facility, where it will be inspected, treated and/or tested for regulated pests (Biosecurity New Zealand, 2010).

The effectiveness of pre- and post-entry quarantine systems depends on the level of containment established by the quarantine facilities and their distance to citrus-growing areas, the quarantine period, and the methods and intensity of inspection and performance of diagnostic protocols during the quarantine period. The *effectiveness* is **high** and the *technical feasibility* is **high**, as this option is already in place (Directive 95/44/CE), but for limited import frequency of small consignments only. The *uncertainty* is **medium**, owing to the lack of data on the specific effectiveness of such a scheme for *P. citricarpa*.

#### 4.1.3.5. Preparation of the consignment

Culling of citrus planting material in the nursery is unlikely to detect plants infected by *P. citricarpa*, as young citrus seedlings/rootstocks/scions remain asymptomatic (see section 3.6.1.1).

The *effectiveness* is thus **negligible**, although the *technical feasibility* is **high**. The *uncertainty* is **low**.

#### 4.1.3.6. Specified treatment of the consignment/reducing pest prevalence in the consignment.

Fungicide sprays applied to consignments of citrus planting material following their harvest may reduce CBS incidence and severity but they cannot eliminate the pathogen.

The *effectiveness* is thus **low**, although the *technical feasibility* is **high**.

The *uncertainty* is **medium**, because there is no information on the use of fungicide sprays on citrus plant propagating material following harvest and before dispatch from the nursery.

#### 4.1.3.7. Restriction on end use, distribution and periods of entry

Such restrictions are not applicable to citrus plants for planting: host plants of *P. citricarpa* may carry the pathogen year around, the end use is planting by definition, and the distribution is by definition to areas with host plants.

### **B. Options preventing or reducing infestation in the crop at the place of origin**

#### 4.1.3.8. Treatment of the crop, field or place of production in order to reduce pest prevalence.

Fungicide sprays in nurseries may reduce CBS incidence and severity, but the pathogen is unlikely to be completely eliminated (see section 3.6.1.1). The *effectiveness* in reducing the probability of entry with plants for planting is thus **low**, although the *technical feasibility* is **high**, with **low uncertainty**.

#### 4.1.3.9. Resistant or less susceptible varieties.

Given the lack of resistant or tolerant cultivars, this option is not yet applicable.

#### 4.1.3.10. Growing plants under exclusion conditions (glasshouse, screen, isolation).

To limit the introduction of inoculum, growing citrus plants for planting in nurseries under exclusion conditions may require plastic or glass greenhouses or screening with extremely fine mesh nets combined with forced ventilation to avoid the entry of *P. citricarpa* spores into the facility.

The *effectiveness* is likely to be **low**, as, due to the microscopic dimensions of the spores of *P. citricarpa* and because of the difficulty of securely excluding the pathogen in close proximity to outdoor grown citrus that may be infected, infection cannot be completely excluded.

The *technical feasibility* is **high** because it is regularly applied in nurseries against vectors of plant diseases (see Figure 53). The *uncertainty* is **high**, owing to the lack of data on the effectiveness of exclusion and the dispersal potential of *P. citricarpa*.



**Figure 53:** Citrus plant propagation material grown in a plastic greenhouse

#### 4.1.3.11. Harvesting of plants at a certain stage of maturity or during a specified time of year

Given the year-round infectiousness and susceptibility of host plants, this option is not applicable.

#### 4.1.3.12. Certification scheme of plant propagation material.

For plants for planting of citrus, certification schemes have been developed worldwide (see, for example, Navarro, 1986; Von Broembsen and Lee, 1988; Passos et al., 2000; Vidalakis et al., 2010; Australian Citrus Propagation Association Inc., undated). Citrus plants for planting produced according to such a scheme are, however, unlikely to be completely free from *P. citricarpa*, unless they are produced in a pest-free area. The *effectiveness* of this RRO is **low**, unless this option is combined with a pest-free area (then the *effectiveness* is **high**). The *technical feasibility* is **very high**. The *uncertainty* is **low**.

### **C. Options ensuring that the area, place or site of production remains free from the pest**

#### 4.1.3.13. Limiting import of host plant material to material originating in pest-free areas

This is a viable RRO, but the long period of asymptomatic latent infection can reduce the *feasibility* of this option when pest-free areas are in proximity to CBS-affected areas. The *effectiveness* is **high**. Owing to the difficulties of detecting latent infection and low CBS incidence, the *technical feasibility* of maintaining pest-free areas in proximity to CBS-infested areas is **moderate**.

The *uncertainty* is **medium**, due to the difficulties in detecting latent infections and to lack of studies on the maximum distance of ascospore dispersal.

#### 4.1.3.14. Limiting import of host plant material to material originating in pest-free production places or pest-free production sites

The *effectiveness* of establishing pest-free production places/sites for plants for planting is **low**, owing to the spread potential of the disease (see section 4.1.1.7). The *technical feasibility* is **moderate**. The *uncertainty* is **medium**, owing to the lack of knowledge on long-distance dispersal of *P. citricarpa* spores.

#### 4.1.3.15. Systems approaches integrating individual RROs.

A possible systems approach for the production of plants for planting is the application of a certification scheme in citrus nurseries in pest-free areas, including regular testing for *P. citricarpa* at different production stages, and the preparation and sealing of consignments at the nursery. The *effectiveness* of this approach is assessed as **high**, with **very high technical feasibility** and **low uncertainty**.

**Table 26:** Summary of the risk reduction options identified and evaluated for the commercial trade of citrus plants for planting (pathways V and VI)

| Category of options                                | Type of measure  | Position in the pathway          | Existing measure | Effectiveness  | Technical feasibility | Uncertainty |
|--|--|----------------------------------|------------------|----------------|-----------------------|-------------|
| <b>Options for consignments</b>                    | Prohibition  | Before shipment                  | Yes              | Very high      | Very high             | Low         |
|  | Prohibition of specific genotypes                        | Before shipment                  | No               | High           | Low                   | High        |
|  | Prohibition of parts of the host                         | Before shipment                  | No               | Not applicable |                       |             |
|  | Visual inspection /testing for pest freedom              | Before shipment and/or at import | No               | Low            | Low                   | Low         |
|  | Pre- or post-entry quarantine systems                    | Before/after shipment            | No               | High           | High                  | Medium      |
|  | Preparation of consignment                               | Before shipment                  | No               | Negligible     | High                  | Low         |
|  | Specified treatment of consignment                       | Before shipment                  | No               | Low            | High                  | Medium      |
|  | Restriction on end use, distribution and period of entry | After shipment                   | No               | Not applicable |                       |             |
| <b>Options for the crop at the place of origin</b> | Treatment of the crop, field or place of production      | Before shipment                  | Yes              | Low            | High                  | Low         |

| Category of options  | Type of measure   | Position in the pathway | Existing measure | Effectiveness                                    | Technical feasibility | Uncertainty |
|--|---|-------------------------|------------------|--|-----------------------|-------------|
|  | Growing plants under exclusion conditions (glasshouse, screen, isolation)   | Before shipment         | No               | Low  | High                  | High        |
|  | Harvesting of plants during a specific time of the year   | Before shipment         | No               | Not applicable                                   |                       |             |
|  | Certification scheme  | Before shipment         | No               | Low<br>High (in combination with pest-free area) | Very high             | Low         |
| <b>Options ensuring that the area, place or site of production at the place of origin remains free from the pest</b> | Limiting import of host plant material to material originating in pest-free areas   | Before shipment         | No               | High   | Moderate to high      | Medium      |
|  | Limiting import of host plant material to material originating in pest-free production places or pest-free production sites | Before shipment         | No               | Low  | Moderate              | Medium      |

**Table 27:** Summary of applicable risk reduction options identified and evaluated for the pathway of citrus plants for planting by passenger traffic (pathway VII)

| Category of options             | Type of measure (for details, see EFSA PLH Panel, 2012) | Position in the pathway | Existing measure | Effectiveness | Technical feasibility | Uncertainty |
|---------------------------------|---|-------------------------|------------------|---------------|-----------------------|-------------|
| <b>Options for consignments</b> | Prohibition   | During customs checks   | No               | Moderate      | Low                   | High        |
|                                 | Visual inspection for pest freedom                      | During customs checks   | No               | Low           | Negligible            | Medium      |

**Table 28:** Summary of the risk reduction options identified and evaluated for the commercial trade of citrus leaves (pathway VIII)

| Category of options             | Type of measure  | Position in the pathway          | Existing measure | Effectiveness   | Technical feasibility | Uncertainty |
|---------------------------------|--|----------------------------------|------------------|---|-----------------------|-------------|
| <b>Options for consignments</b> | Prohibition  | Before shipment                  | Yes              | Very high   | Low                   | Low         |
|                                 | Prohibition of parts of the host                         | Before shipment                  | No               | Not applicable  |                       |             |
|                                 | Prohibition of specific genotypes                        | Before shipment                  | No               | Not applicable  |                       |             |
|                                 | Visual inspection/testing for pest freedom               | Before shipment and/or at import | No               | Low   | Negligible            | Medium      |
|                                 | Pre- or post-entry quarantine systems                    | Before/after shipment            | No               | Not applicable  |                       |             |
|                                 | Preparation of consignment                               | Before shipment                  | No               | Low   | High                  | Low         |
|                                 | Specified treatment of consignment                       | Before shipment                  | No               | No information available, not evaluated   |                       |             |
|                                 | Restriction on end use, distribution and period of entry | After shipment                   | No               | Restriction on period not applicable. Restriction in distribution to EU MSs where citrus is not grown: effective but feasibility negligible. Uncertainty high |                       |             |



|  |   |                 |     |                |                  |        |
|--|---|-----------------|-----|----------------|------------------|--------|
| <b>Options for the crop at the place of origin</b>   | Treatment of the crop, field or place of production   | Before shipment | Yes | Moderate       | High             | Medium |
|  | Resistant or less susceptible varieties   | Before shipment | No  | Not applicable |                  |        |
|  | Growing plants under exclusion conditions (glasshouse, screen, isolation)   | Before shipment | No  | Low            | High             | High   |
|  | Harvesting of plants during a specific time of the year   | Before shipment | No  | Not applicable |                  |        |
|  | Certification scheme  | Before shipment | No  | Low            | High             | Low    |
| <b><u>Options ensuring that the area, place or site of production at the place of origin, remains free from the pest</u></b> | Limiting import of host plant material to material originating in pest-free areas   | Before shipment | No  | High           | High             | Medium |
|  | Limiting import of host plant material to material originating in pest-free production places or pest-free production sites | Before shipment | No  | Low            | Moderate to high | High   |
| <b>Systems approaches</b>  | Certification scheme + pest-free area + preparation and sealing of consignment on nursery                                   | Before shipment | No  | High           | Very high        | Low    |

#### 4.1.4. Risk reduction options to reduce entry along the pathway of import of citrus plants for planting by passenger traffic (pathway VII)

The RROs for this pathway are similar to those of the general citrus plants for planting pathway, although there is very little information on the frequency of transport of citrus plants for planting along this pathway. The results of the evaluation are summarised in Table 27.

##### A. Options for consignments

###### 4.1.4.1. Prohibition

The prohibition of import of citrus plants for planting for citrus fruit production by passenger traffic would prevent the entry of *P. citricarpa* into the EU along this pathway. Such a prohibition requires compliance by passengers, which can be influenced by the intensity and clarity of communication of this measure to passengers and the intensity of passenger checks. Results of audits performed in Australia for citrus fruit show that interceptions of fruit carried by passengers are a regular occurrence, despite communication and inspection. There are no specific data on interception of citrus plants for planting for citrus fruit production carried by passengers, but the frequency of passengers carrying such material is assumed to be lower than the frequency of passengers carrying fruit for consumption. The *effectiveness* is assessed as **moderate**, although it would depend on the level of compliance by passengers. The *technical feasibility* is **low**, because of the need to implement it on a very large volume of passenger luggage at all entry points over the whole year and because it would require the technical ability to identify citrus plants for planting at the border (e.g. citrus plants, rootstocks or buds). The *uncertainty* of these ratings is **high**, owing to the lack of data on the frequency of transport of citrus plants for planting along this pathway and on the compliance by passengers.

###### 4.1.4.2. Prohibition of parts of the host or of specific genotypes of the host

Not applicable.

###### 4.1.4.3. Pest freedom of consignments: inspection or testing

The *effectiveness* of visual inspection of citrus plants for planting carried by passengers for symptoms of *P. citricarpa* is **low**, mainly because of the presence of latent infections. The leaves of young citrus plants infected by *P. citricarpa* are usually asymptomatic and, thus, not detectable by visual inspection. Sample testing could be applicable; however, the plants for planting should be stored until the results of the test are available before further customs procedures. Therefore, the *technical feasibility* of inspection of citrus plants carried by passengers as an option to reduce the risk of entry of *P. citricarpa* is **negligible**. The fraction of passengers carrying such planting material is likely to be much lower than the estimated 0.1 % of passengers carrying on average one citrus fruit (see section 4.1.2.4), and therefore a very large volume of passenger luggage would need to be inspected to detect citrus plants for planting. The *uncertainty* on these ratings is **low**.

###### 4.1.4.4. Pre- or post-entry quarantine system.

Not applicable.

###### 4.1.4.5. Preparation of the consignment

Not applicable.

###### 4.1.4.6. Specified treatment of the consignment/reducing pest prevalence in the consignment.

Not applicable.

###### 4.1.4.7. Restriction on end use, distribution and periods of entry

Not applicable.

## **B. Options preventing or reducing infestation in the crop at the place of origin**

Such options are not applicable to plants for planting carried by passengers.

## **C. Options ensuring that the area, place or site of production at the place of origin remains free from the pest**

Such options are not applicable to plants for planting carried by passengers.

### **4.1.5. Risk reduction options to reduce entry along the pathway of import of citrus leaves (pathway VIII)**

The results of the evaluation of RROs for this pathway are summarised in Table 28.

#### **A. Options for consignments**

##### 4.1.5.1. Prohibition

The prohibition of import of fresh or dry citrus leaves via commercial trade would prevent the entry of *P. citricarpa* into the EU along this pathway. The *effectiveness* is assessed as **very high**. The *technical feasibility* is **low**, because citrus leaves could be sent in non-declared packages, escaping customs operations and phytosanitary procedures. The *uncertainty* on these ratings is assessed as **low**.

##### 4.1.5.2. Prohibition of parts of the host

Not applicable to citrus leaves commercial trade.

##### 4.1.5.3. Prohibition of specific genotypes

The prohibition of specific genotypes is not applicable to commercial trade of fresh or dry citrus leaves. The host status of *C. hystrix* and other exotic citrus species for *P. citricarpa* is highly uncertain and there is no information on the use for cooking purposes of other citrus species apparently not affected by CBS, such as sour orange or Tahiti lime. In addition, it has been reported that *P. citricarpa* can colonise leaves of both citrus species and, in the case of Tahiti lime, even reproduce on them (see section 3.1.1.4).

##### 4.1.5.4. Pest freedom of consignments: inspection or testing

The detection of *P. citricarpa* in consignments is based on visual inspection, sampling and laboratory testing. Inspection or testing of consignments may be applied at the time of export and/or at the time of import. At export, inspection or testing may serve as a stand-alone measure, without other official measures for production, harvest and packaging, or as a measure to verify that other measures have been effective. At import, inspection generally serves to verify phytosanitary measures by the exporting country.

The *effectiveness* of visual inspection of citrus leaves for symptoms of *P. citricarpa* is **low**, mainly because of the presence of latent infections. The leaves of citrus infected by *P. citricarpa* are usually asymptomatic and, thus, not detectable by visual inspection. Sample testing could be applicable, but, without a reliable detection of symptoms on leaves, very large sample sizes would be required. Therefore, the *technical feasibility* of inspection of citrus leaves as an option to reduce the risk of entry of *P. citricarpa* is **negligible**. The *uncertainty* on these ratings is **medium**, owing to lack of data on the amounts, origin and end use of citrus leaves along this pathway, as well as the unknown host status of *C. hystrix* and other exotic citrus species for *P. citricarpa*.

##### 4.1.5.5. Pre- or post-entry quarantine system.

Not applicable to citrus leaves.

#### 4.1.5.6. Preparation of the consignment

The preparation of the consignment involves several stages, including handling and transport of harvested leaves and packing prior to export. Culling and cleaning of leaves may theoretically allow the removal of leaves infected by *P. citricarpa*, but leaves with asymptomatic infections or with small lesions will not be detected and eliminated by these procedures. The *effectiveness* is assessed as **low**. The *technical feasibility* is assessed as **high**. The *uncertainty* on these ratings is **low**.

#### 4.1.5.7. Specified treatment of the consignment/reducing pest prevalence in the consignment.

No information is available on treatments against *P. citricarpa* on fresh or dry citrus leaves for flavouring, cooking or ornamental purposes. This RRO has therefore not been evaluated.

#### 4.1.5.8. Restriction on end use, distribution and periods of entry

It is not possible to identify periods of the year when citrus leaves are not infected, nor periods of the year when host plants are not susceptible to infection. Therefore, a restriction on the period of entry of citrus leaves is not applicable.

A restriction on the distribution of the imported citrus leaves for flavouring to the EU MSs where citrus is not grown could potentially be effective; however, the entry through this pathway is considered unlikely. The *feasibility* is **negligible** because of the free internal market of the EU. *Uncertainty* is **high** owing the lack of information on the trade of citrus leaves for flavouring,

Since the end use is human consumption and ornamental uses only, a restriction on end use is also not applicable. Further to the assessment in section 4.1.1.8, the technical feasibility of restricting the distribution of commercially traded citrus leaves would be negligible.

### **B. Options preventing or reducing infestation in the crop at the place of origin**

#### 4.1.5.9. Treatment of the crop, field or place of production in order to reduce pest prevalence

Treatments against *P. citricarpa* to reduce the incidence of CBS in citrus plants may be routinely applied by citrus producers in the absence of official phytosanitary requirements, although the efficacy of different fungicide programmes in combination with cultural and other control methods may vary among producers. However, these measures will not eliminate *P. citricarpa* in production places and the harvest of infected leaves cannot be prevented owing to the scarcity of CBS leaf symptoms. The incidence of *P. citricarpa* in harvested leaves remains variable, depending on the citrus species, the efficacy of control programmes and the weather conditions during the growing season. The *effectiveness* of control programme is assessed as **moderate**. The *technical feasibility* is assessed as **moderate**, because fungicide treatments may conflict with the purpose of consumption of the leaves. The *uncertainty* on these ratings is **medium**.

#### 4.1.5.10. Resistant or less susceptible varieties

The host status of *C. hystrix* and other exotic citrus species for *P. citricarpa* is highly uncertain and there is no information on the use for cooking or ornamental purposes of other citrus species apparently not affected by CBS, such as sour orange or Tahiti lime. In addition, it has been reported that *P. citricarpa* can colonise leaves of both citrus species and, in the case of Tahiti lime, even reproduce on them (see section 3.1.1.4). This RRO is not applicable to citrus leaves.

#### 4.1.5.11. Growing plants under exclusion conditions (glasshouse, screen, isolation)

This RRO may be applicable to production places producing citrus leaves, if the plants are kept sufficiently small so that they can grow in plastic or glass greenhouses or screened with extremely fine mesh nets and forced ventilation to avoid the entry of spores of *P. citricarpa*. The *effectiveness* is likely to be **low** owing to the microscopic dimensions of the spores of *P. citricarpa* and the difficulty of securely excluding the pathogen in close proximity to outdoor-grown citrus that may be infected.

The *technical feasibility* is **moderate**, as this option is regularly applied in nurseries against vectors of plant diseases and could be used also for small citrus trees (Figure 42). The *uncertainty* is **high**, owing to the lack of data on the effectiveness of exclusion and the dispersal potential of the pathogen.

#### 4.1.5.12. Harvesting of plants at a certain stage of maturity or during a specified time of year.

This option is not applicable since citrus leaves are susceptible to *P. citricarpa* for 8–10 months and new leaves are produced year-round (see section 3.3.1.1).

#### 4.1.5.13. Certification scheme

Plants for production of citrus leaves, produced under a certification scheme, will initially be free from *P. citricarpa*. However, these plants can become infected when planted in areas where *P. citricarpa* occurs. The *effectiveness* of a certification scheme is **low**. The technical feasibility is assessed as **high**. The *uncertainty* on these ratings is assessed as **low**.

### **C. Options ensuring that the area, place or site of production at the place of origin, remains free from the pest**

#### 4.1.5.14. Limiting import of host plant material to material originating in pest-free areas

The different aspects of this RRO are discussed in section 4.1.1.14.

The *effectiveness* of pest-free areas is assessed as **high**, on the condition that procedures for maintaining the pest-free area and its buffer zone are documented and the area is regularly officially evaluated and the results reported.

The establishment and maintenance of a pest-free area for *P. citricarpa* is technically feasible, but surveys considering the possibility of asymptomatic infections, with adequate attention to the distribution of managed and unmanaged host plants in the pest-free area should be performed when designating the pest-free area and its buffer zone. The *technical feasibility* is assessed as **high**. The *uncertainty* is **medium**, owing to the difficulties in detecting latent infections and to the lack of studies on the maximum distance of airborne ascospore dispersal.

#### 4.1.5.15. Limiting import of host plant material to material originating in pest-free production places or pest-free production sites

The *effectiveness* of this measure to establish CBS-free production sites for production of citrus leaves is assessed as **low**, but depends on the intensity of monitoring. As infections may be asymptomatic, the *technical feasibility* is **moderate to high**. The *uncertainty* is **high**.

### **4.2. Systematic identification and evaluation of options to reduce the probability of establishment and spread**

This section analyses the RROs that can be applied in the EU to prevent the establishment and spread of *P. citricarpa*. However, some of the RROs to reduce the probability of transfer to a suitable host in the entry pathways are the same as those that can reduce spread and are therefore also included in this section. The results are summarised in Table 29.

#### **4.2.1. Pruning**

The trade in citrus fruit has been considered as a pathway for both the entry and spread of *P. citricarpa*. In both steps the transfer of *P. citricarpa* to a citrus plant depends on the splash dispersal of pycnidiospores from culled fruit, peel or citrus by-products. The transfer may be favoured by low-hanging citrus branches in commercial orchards, private gardens, roadsides and parks; therefore, pruning the lower branches of citrus trees could theoretically reduce the probability of transfer. However, a requirement for pruning the low branches of citrus trees in parks, roadsides and private gardens is difficult to implement. This measure is not feasible in commercial orchards, because low-

hanging branches are the most productive and they are more easily harvested, so citrus trees in the EU are trained and pruned to maximise this part of the canopy (see section 3.3.3.3). This option is considered as having a **low effectiveness** and a **negligible technical feasibility**, with **low uncertainty**.

#### 4.2.2. Irrigation and other cropping practices

There is a trend to move away from the irrigation systems that use large amounts of water (see sections 3.3.3.1 and 3.3.3.2). A wider use of drip irrigation can reduce the risk of establishment as this method does not wet citrus leaf surfaces. Instead, micro-sprinkler irrigation uses spray jets under the tree canopy that wet not only the soil but also the leaves in the lower canopy of the tree, thereby significantly increasing leaf wetness and aiding *P. citricarpa* establishment. In addition, *P. citricarpa* pseudotecia production in leaf litter is favoured by alternating leaf wetting and leaf dryness. When micro-sprinkler irrigation is applied, the number of dead leaves with pseudothecia in the orchard floor is reported as apparently much higher (10×) than with drip or flood irrigation (Feichtenberger E, Citrus black spot and its management in Brazil, ppt at Packinghouse Day & The Indian River Postharvest Workshop). However, these data should be interpreted together with leaf litter decomposition rates, which may differ depending on the irrigation system used. Cover crops and mulching of the orchard floor with grass cuttings after the leaf drop can accelerate the decomposition of the citrus leaves bearing the perithecia, limiting ascospore dispersal and thus reducing the inoculum (Miranda-Bellote et al., 2013). In addition, since citrus trees in poor conditions are more susceptible to CBS, it is important to maintain tree vigour (Schutte, 2009). Therefore, the *effectiveness* of the application of drip and flood irrigation to reduce the probability of CBS establishment is assessed as **moderate**, with **high technical feasibility** and **low uncertainty**.

#### 4.2.3. Hygiene measures: waste management

However, adopting hygiene practices specific to CBS in citrus waste management mainly in citrus packing houses and citrus processing plants is likely to be more effective and easier to implement than cultural practices in orchards, gardens, roadsides and parks. The implementation of strict containment and waste processing measures (according to the guidelines for handling such biowaste in EPPO Standard PM 3/66(2)) at citrus packing houses or processing industries handling citrus fruit imported from areas where CBS occurs would reduce the probability of transfer to a suitable host, and thus establishment and spread. However, large amounts of culled fruit and waste are produced by citrus packing houses and processing plants located in citrus-producing areas of the EU (section 3.2.2.4). Most of this waste is solar dried in open-air facilities located in the citrus-growing area and then used for livestock feeding (Bampidis and Robinson, 2006; Caparra et al., 2007). High bio-safety standards would have to be set for these facilities. Moreover, a considerable proportion of citrus fruit imported in the EU is destined for direct consumption via retail markets ranging from supermarkets to small outdoor markets, where standards for waste management cannot be controlled other than by making consumers aware of the phytosanitary risk. Therefore, the effectiveness and feasibility are limited by the scattered distribution of the numerous points of potential transfer in the citrus-growing EU MSs. The *effectiveness* of such a measure is assessed as **high**, but with **low technical feasibility** owing to the need for the application of specific measures for strict citrus waste management in all citrus-growing EU MSs. *Uncertainty* is **high**, particularly on the feasibility of the practical implementation and due to the lack of studies on survival of *P. citricarpa* in citrus waste and in the compost derived from citrus waste.

#### 4.2.4. Eradication

Following the discovery of an outbreak of *P. citricarpa*, eradication measures should be implemented immediately. An eradication programme includes surveys to determine the limits of the outbreak, eradication actions to eliminate a pest from an area and containment action to prevent pest spread and surveys to verify absence of that pest (ISPM 9 by FAO, 1998). CBS has never disappeared or declined after being introduced, and successful disease eradication has never been achieved in any of the humid and semi-arid regions where it is currently present. Field surveillance for CBS eradication is challenging, considering that *P. citricarpa* may be present as latent mycelia in asymptomatic citrus

fruit and leaves and there is a long lag phase between the first establishment and subsequent epidemic development (section 3.4.4).

The *effectiveness* of CBS eradication is **low**, because there are no reports of successful eradication of CBS: once established, the disease is reported to expand slowly but relentlessly. The *technical feasibility* is **low**, and the *uncertainty* on these ratings is **low**.

#### 4.2.5. Containment

Once the disease has established, RROs to reduce the spread of *P. citricarpa* include containment measures in infested areas (e.g. cultural/fungicide control measures in orchards); preventative measures in areas suitable for new infection foci (e.g. the adoption of drip instead of sprinkler irrigation and the avoidance of citrus mono-cultures; Bellotte et al., 2013); targeted surveys at high-risk nodes in the trade network of fruit and plants for planting; and information campaigns aimed at local growers, stakeholders and the public to raise awareness of the disease and increase the likelihood of implementation of containment and preventative measures. With regard to the fungicide programmes, the results of the meta-analysis (section 3.6.1) indicated that strobilurin fungicides (QoI) and benzimidazoles are the most effective for CBS control, but they are not currently authorised for use in citrus in the EU. Given the unavailability of these compounds, dithiocarbamate fungicides may be the preferred post-entry containment fungicide treatments to be implemented after a possible introduction of CBS into the EU territory.

The *effectiveness* of containment is assessed to be **low**, because there is little evidence from other regions that CBS can be successfully contained. Once established, the disease is reported to expand slowly but relentlessly. The *technical feasibility* is **moderate**, and the *uncertainty* on these ratings is **low**.

#### 4.2.6. Surveillance

A surveillance programme including regular detection surveys in commercial citrus orchards, abandoned citrus orchards and public areas, in areas with production of citrus fruit and/or plants for planting would contribute to eradication and containment. The *effectiveness* is determined by the intensity of the surveys including sampling, visual inspection and laboratory testing; however, it is assessed as **moderate** owing to latent asymptomatic infections and to the reported long lag phase between first introduction and development of the epidemic. The *technical feasibility* is **moderate**, owing to the difficulty of organising surveys in public areas, and the *uncertainty* is **medium**.

**Table 29:** Summary of risk reduction options identified and evaluated to reduce the probability of establishment and spread

| Type of measure (for details, see EFSA Panel, 2012) | Existing measure | Effectiveness | Technical feasibility | Uncertainty |
|---|------------------|---------------|-----------------------|-------------|
| Pruning   | No               | Low           | Negligible            | Low         |
| Irrigation and other cropping practices             | No               | Moderate      | High                  | Low         |
| Hygiene measures: waste management                  | No               | High          | Low                   | High        |
| Eradication   | No               | Low           | Low                   | Low         |
| Containment   | No               | Low           | Moderate              | Low         |
| Surveillance  | No               | Moderate      | Moderate              | Medium      |

### 4.3. Systems approach of risk reduction options

With the exception of prohibition and of limiting the import from pest-free areas, the effectiveness of the RROs evaluated is generally low to moderate (see summary tables of RROs, Tables 24–29). Combining ineffective RROs may slightly increase their overall effectiveness, but is unlikely to result in a significant risk reduction in the case of *P. citricarpa*.

The only RROs with high effectiveness were found to be:

- prohibition: prohibition of parts of the host; prohibition of specific genotypes of the host
- citrus fruit consignment testing using the method to induce precocious symptom expression in latent infections (see section 4.1.1.4) together with validated diagnostic protocols, which should thus be further developed and adopted in conjunction with the other options
- and pest-free areas.

The *effectiveness* of a systems approach to RROs is assessed as **moderate**, with a **moderate technical feasibility** and **high uncertainty**.

### 4.4. Evaluation of the current phytosanitary measures to prevent the introduction and spread of *P. citricarpa*

#### 4.4.1. General remarks

“*Guignardia citricarpa* Kiely all strains pathogenic to Citrus” is listed in Annex II, Part A, Section I (c), of Directive 2000/29/EC as a harmful organism whose introduction into and spread within all EU MSs should be banned if present on plants of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf. and their hybrids, other than seeds.

“*Guignardia citricarpa* Kiely all strains pathogenic to Citrus” is listed in Annex II, Part A, Section I, and Annex IV, Part A, Section I (see section 3.1.3.3), of the EU Plant Health Directive (Directive 2000/29/EC). However, following recent nomenclatural changes, the correct name for the causal agent of CBS is now *Phyllosticta citricarpa* (McAlpine) Van der Aa and *Guignardia citricarpa* Kiely is considered to be a synonym. While new knowledge on the *Phyllosticta* species associated with citrus is continuously emerging, the current knowledge supports the conclusion that only *P. citricarpa* has proven to be pathogenic to citrus and a threat to citrus cultivation in regions that are suitable for this pathogen (see section 3.1.1.1); therefore, the specification “all strains pathogenic to Citrus” is not further needed when using the updated name *Phyllosticta citricarpa* (McAlpine) Van der Aa.

The subject of contamination is indicated as “plants,,,,, other than seeds”. The term “plants” as described in Article 2 1 (a) of Directive 2000/29/EC includes the following items of relevance for citriculture: living plants, fruit, cut flowers, branches with twigs and foliage, cut trees retaining twigs and foliage, plant tissue culture. Of these items, living plants, fruit and any plant part bearing leaves are entry pathways for *P. citricarpa*. Flowers do not harbour *P. citricarpa* unless attached to a branch with foliage. Seeds are correctly excluded as they are not considered an entry pathway for this pathogen.

With regard to the botanical genera, generally all *Citrus* species are susceptible to *P. citricarpa*. The only possible exceptions are:

- *Citrus latifolia* Tanaka (Tahiti lime) leaves and fruit can be colonised by *P. citricarpa*. The pathogen does not produce symptoms/pycnidia on Tahiti lime fruit, but it produces ascospores on leaves, and therefore entry is unlikely with fruit (with high uncertainty) but is more likely with plants and plant parts with leaves.



- *Citrus aurantium* L. (sour orange) is considered resistant although *P. citricarpa* has occasionally been isolated from asymptomatic sour orange.
- *Citrus maxima* (Burm.) Merr. (pomelo) is reported to be resistant based on field surveys. However, the results of experimental confirmatory pathogenicity testing and re-isolation have not been published so far and are needed to exclude this citrus species as a potential host of *P. citricarpa*.

With regard to *Fortunella* Swingle (kumquat), this species was recorded by Kiely (1948) as moderately susceptible to CBS under conditions of natural infection, but no further experimental information is available.

No definitive information has been found on the susceptibility of *Poncirus* Raf. (trifoliate orange) to CBS.

The combination of the requirements listed in Directive 2000/29/EC for all citrus pathways could be considered as being highly effective in preventing the introduction of *P. citricarpa* into the EU because there have been no outbreaks of CBS. However, it has also been argued that successful introductions have not taken place, despite very large shipments of citrus into Europe over many decades from areas where the disease is present, because the climate in the risk assessment area is unsuitable for *P. citricarpa* (Kotzé, 2000). In this respect, it is important to note that, until 1993, when the EC phytosanitary directive established common quarantine requirements for import of citrus fruit to prevent the entry of *P. citricarpa* into the EU, most citrus-growing EU countries had a very strict national quarantine for citrus, with a general prohibition of citrus fruit import (see section 3.1.3.1). For this reason the trade in citrus fruit from CBS areas in the world to EU citrus-growing areas has been very limited, with the only exception being Italy, where the import of grapefruit was allowed (although still in limited quantities) from countries where CBS is present (see section 3.1.3.2). Responding to the requirements of the EU directive, imports of citrus fruit were first allowed in 1993 and 1999 by Spain and Italy, respectively, but since then such import complied with current EU phytosanitary measures for *P. citricarpa*.

#### 4.4.2. Effectiveness and uncertainty of the present phytosanitary measures.

The effectiveness and uncertainty of the present phytosanitary measures of the EU against introduction into and spread within the EU of *P. citricarpa* (see section 3.1.3) are summarised in Table 30.

A limited quantitative pathway analysis conducted for the citrus fruit pathway for Spain (Appendix E) indicated that, under current regulations, the number of fruit infected with *P. citricarpa* entering the citrus-growing regions of Spain is likely to be small. Thus, under current regulations, entry via the citrus fruit trade pathways is very unlikely, with low uncertainty, owing to the minor amounts of inoculum that may reach the trees from the small number of infected fruit moving along the pathway. The same analysis indicated that scenarios in the absence of regulation showed major increases, by a factor of 10 000 or more, in the potential for entry.

**Table 30:** Effectiveness and uncertainty of present requirements against *P. citricarpa* as formulated in Annexes of Council directive 2000/29/EC, Commission Decision 2004/416/EC and Commission Decision 2006/473/EC

| EU phytosanitary measure       | Commodities + origin  | Countries of origin | Requirement  | Effectiveness and uncertainty assessed by PLH Panel (NB: technical feasibility is not relevant since these measures are already implemented)  |
|--------------------------------|---|---------------------|--|---|
| 2000/29/EC Annex III A (16)    | Plants of <i>Citrus</i> L., <i>Fortunella</i> Swingle, <i>Poncirus</i> Raf., and their hybrids, other than fruit and seeds                      | Third countries     | Prohibition of introduction in all EU MSs  | This requirement is discussed in sections 4.1.3.1 and 4.1.5.1.<br>The <i>effectiveness</i> is assessed as very high, with low <i>uncertainty</i> .  |
| 2000/29/EC Annex IV A I (16.1) | Fruits of <i>Citrus</i> L., <i>Fortunella</i> Swingle, <i>Poncirus</i> Raf., and their hybrids  | Third countries     | The fruits shall be free from peduncles and leaves and the packaging shall bear an appropriate origin mark   | This requirement is discussed in section 4.1.1.2.<br>The <i>effectiveness</i> is assessed as high with low <i>uncertainty</i> .   |
| 2000/29/EC Annex IV A I (16.4) | Fruits of <i>Citrus</i> L., <i>Fortunella</i> Swingle, <i>Poncirus</i> Raf., and their hybrids, other than fruits of <i>Citrus aurantium</i> L. | Third countries     | (a)<br>the fruits originate in a country recognised as being free from <i>Guignardia citricarpa</i> Kiely (all strains pathogenic to <i>Citrus</i> ), in accordance with the procedure referred to in Article 18(2)  | Country freedom is a type of area freedom, discussed in section 4.1.1.13.<br>The <i>effectiveness</i> is assessed as very high with low <i>uncertainty</i>  |
|                                |   |                     | OR (b)<br>the fruits originate in an area recognised as being free from <i>Guignardia citricarpa</i> Kiely (all strains pathogenic to <i>Citrus</i> ), in accordance with the procedure referred to in Article 18(2), and mentioned on the certificates referred to in Articles 7 and 8 of this Directive                                    | Area freedom is discussed in section 4.1.1.13.<br>The <i>effectiveness</i> is assessed as high, depending on the frequency and confidence level of detection surveys and intensity of phytosanitary measures to prevent entry into the pest-free area. The Panel notes that technical feasibility to maintain a pest-free area is affected by its proximity to CBS-affected areas and the presence of asymptomatic latent infections. The <i>uncertainty</i> is low |
|                                |   |                     | OR (c)<br>no symptoms of <i>Guignardia citricarpa</i> Kiely (all strains pathogenic to <i>Citrus</i> ) have been observed in the field of production and in its immediate vicinity since the beginning of the last cycle of vegetation,<br>AND<br>none of the fruits harvested in the field of production has shown, in appropriate official | This is a systems approach (discussed in section 4.3) and requires two components acting together: (1) pest freedom of the production site (measured as the <i>observed</i> absence of symptoms of <i>Guignardia citricarpa</i> in the field of production and its immediate vicinity), and (2) visual inspection to confirm the absence of symptoms of <i>Guignardia citricarpa</i> on the harvested fruit.  |

| EU phytosanitary measure | Commodities + origin | Countries of origin | Requirement                            | Effectiveness and uncertainty assessed by PLH Panel (NB: technical feasibility is not relevant since these measures are already implemented)  |
|--------------------------|----------------------|---------------------|--|---|
|                          |                      |                     | examination, symptoms of this organism | <p>Component (1) is discussed in section 4.1.1.14, where effectiveness is rated as low and uncertainty high (following discussion on ascospores moving in pest-free sites). The Panel notes that the procedures for the designation of pest-free production sites in areas infested by <i>P. citricarpa</i> are insufficiently specified in this requirement of Directive 2000/29/EC, because visual observation will not detect the possible presence of asymptomatic latent infections in citrus fruits, and symptoms caused by <i>P. citricarpa</i> are difficult to distinguish from those caused by other citrus pests and diseases. Also, growing commercial citrus orchards under exclusion conditions has low feasibility (section 4.1.1.14). The specification of a buffer zone as ‘immediate vicinity’ is imprecise. If the buffer zone is too small, ascospores or pycnidiospores of <i>P. citricarpa</i> may enter the production site by natural spread during the last cycle of vegetation, leading to latent infections. Although there are no precise data for <i>P. citricarpa</i> (section 3.4.1), ascospores of other fungi can spread very long distances.</p> <p>Component (2), the subsequent ‘appropriate examinations’ of consignments, would include visual inspection but not laboratory testing, since the purpose is to detect symptoms of this organism. This RRO is discussed in section 4.1.1.3. The effectiveness of visual inspection is assessed as low, with medium uncertainty. The effectiveness of this RRO would increase if appropriate examinations were required for detection of the organism, instead of only symptoms caused by organism. The use of</p> |

| EU phytosanitary measure | Commodities + origin | Countries of origin | Requirement   | Effectiveness and uncertainty assessed by PLH Panel (NB: technical feasibility is not relevant since these measures are already implemented)   |
|--------------------------|----------------------|---------------------|---|--|
|                          |                      |                     |   | <p>techniques to induce symptom expression will aid the detection of asymptomatic latent infections. However, without knowledge of the sample size there will be further concerns about the effectiveness and uncertainty of this requirement.</p> <p>The Panel assesses the <i>effectiveness</i> of this systems approach as low, with medium <i>uncertainty</i>. The lack of explicit requirements for buffer zones, of minimum procedures for designation of pest-free production sites and of explicit requirements for laboratory testing as part of the official examination of citrus orchards and consignments are important points of concern for this regulatory requirement.</p>  |
|                          |                      |                     | <p>OR (d)<br/>the fruits originate in a field of production subjected to appropriate treatments against <i>Guignardia citricarpa</i> Kiely (all strains pathogenic to <i>Citrus</i>),<br/>AND<br/>none of the fruits harvested in the field of production has shown, in appropriate official examination, symptoms of this organism</p> | <p>This RRO requires two components acting together: (1) ‘treatment of the crop, field or place of production in order to reduce pest prevalence’ and (2) visual inspection to confirm the absence of symptoms of citrus black spot on the harvested fruit.</p> <p>Component (1) is discussed in section 4.1.1.8, where <i>effectiveness</i> is rated as moderate and <i>low</i> uncertainty.</p> <p>Component (2), the subsequent ‘appropriate examinations’ of consignments, would include visual inspection but not laboratory testing, since the purpose is to detect symptoms caused by this organism. This RRO is discussed in section 4.1.1.3. The effectiveness of visual inspection is assessed as low, with medium uncertainty, and does not alter the effectiveness and uncertainty that would be realised by the appropriate field</p> |

| EU phytosanitary measure                   | Commodities + origin   | Countries of origin | Requirement   | Effectiveness and uncertainty assessed by PLH Panel (NB: technical feasibility is not relevant since these measures are already implemented)   |
|--|--|---------------------|---|--|
|  |  |                     |   | <p>treatments.</p> <p>The Panel therefore assesses the <i>effectiveness</i> of this systems approach as moderate, with medium <i>uncertainty</i>.</p> <p>The effectiveness of this RRO would increase if appropriate examinations were required for detection of the organism, instead of symptoms of the organism. The use of techniques to induce symptom expression will aid detection of asymptomatic latent infections. However, without knowledge of the sample size there will be further concerns about the effectiveness and uncertainty of this requirement.</p> <p>The lack of explicit requirements for laboratory testing as part of examination of consignments is an important point of concern for this regulatory requirement</p> |
| 2000/29/EC Annex V B I (3)                 | Fruits of <i>Citrus</i> L., <i>Fortunella</i> Swingle, <i>Poncirus</i> Raf., and their hybrids   | Non-EU countries    | Subject to a plant health inspection in the country of origin or the consignor country, before being permitted to enter the community   | The plant health inspection would have to fulfil the special requirements of Annex IV A I. The effectiveness and uncertainty of the inspection are the same as those for the option of Annex IV A I that applies according to the conditions of the place of production of these fruits.   |
| Decision 2004/416/EC                       | Consolidated legislation: amended by 2007/347/EU and 2013/67/EU  |                     |   |  |
| Decision 2004/416/EC Article 1 + Annex (2) | Fruits of <i>Citrus</i> L., <i>Fortunella</i> Swingle, <i>Poncirus</i> Raf., and their hybrids (hereinafter referred to as citrus fruits), other than <i>Citrus aurantium</i> L. | Brazil              | (a) the fruits originate in an area recognised as being free from <i>Guignardia citricarpa</i> Kiely (all strains pathogenic to <i>Citrus</i> ), in accordance with the procedure referred to in Article 18(2) of Directive 2000/29/EC, and mentioned on the certificate, | Area freedom is discussed in section 4.1.1.13. The <i>effectiveness</i> is assessed as high, depending on frequency and confidence level of detection surveys and intensity of phytosanitary measures to prevent entry into the pest-free area. The <i>uncertainty</i> is low.   |

| EU phytosanitary measure | Commodities + origin | Countries of origin | Requirement   | Effectiveness and uncertainty assessed by PLH Panel (NB: technical feasibility is not relevant since these measures are already implemented)  |
|--------------------------|----------------------|---------------------|---|---|
|                          |                      |                     | <p>OR (b)<br/>           no symptoms of <i>Guignardia citricarpa</i> Kiely (all strains pathogenic to <i>Citrus</i>) have been observed in the place of production since the beginning of the last cycle of vegetation,<br/>           AND<br/>           none of the fruits harvested in the place of production has shown, in appropriate official examination, symptoms of this organism,<br/>           AND<br/>           the place of production, the packing facilities, exporters and any other operator involved in the handling of the fruits are officially registered for this purpose.</p> | <p>This is a systems approach, requiring three components acting together: (1) pest freedom of the production site (measured as the <i>observed</i> absence of symptoms caused by <i>P. citricarpa</i> in the field of production and its immediate vicinity), (2) visual inspection and/or laboratory testing to confirm the absence of symptoms caused by <i>P. citricarpa</i> on the harvested fruit and (3) registration of place of production and any operators involved in handling of the fruits.</p> <p>Component (1) is discussed in section 4.1.1.14, where effectiveness is rated as low and uncertainty as medium. The Panel notes that the procedures for the designation of pest-free production sites in areas infested by <i>P. citricarpa</i> are insufficiently specified in this requirement of Directive 2000/29/EC, because visual observation will not detect the possible presence of asymptomatic latent infections on citrus fruits, and because of the difficulties in distinguishing symptoms caused by <i>P. citricarpa</i> from those caused by other citrus pests and diseases. Also, growing citrus orchards under exclusion conditions has low feasibility (section 4.1.1.14). Therefore, infected fruit may be harvested despite the requirement of pest freedom of the production site.</p> <p>Component (2), the subsequent ‘appropriate examinations’ (visual inspection and /or laboratory testing) of consignments, is discussed in section 4.1.1.3. Visual inspection combined with laboratory testing is rated as moderately effective, with medium uncertainty.</p> |

| EU phytosanitary measure                   | Commodities + origin  | Countries of origin | Requirement   | Effectiveness and uncertainty assessed by PLH Panel (NB: technical feasibility is not relevant since these measures are already implemented)  |
|--|---|---------------------|---|---|
|  |   |                     |   | <p>The Panel considers visual inspection by itself not an ‘appropriate official examination’. The effectiveness of visual inspection is assessed as low, with low uncertainty. Inspection combined with laboratory testing of consignments will increase the effectiveness of observations in the field of production to determine pest freedom of the production site, with effectiveness rated as moderate, with medium uncertainty.</p> <p>Component (3) would have no additional effect on reduction of the probability of entry but would facilitate tracking and tracing of intercepted infested consignments.</p> <p>The lack of explicit requirements for buffer zones, of minimum procedures for designation of pest-free production sites and of explicit requirements for laboratory testing as part of examinations of fields and consignments are important points of concern for this regulatory requirement. Therefore, the Panel assesses the <i>effectiveness</i> of this systems approach as low to moderate, with medium <i>uncertainty</i>.</p> |
| Decision 2004/416/EC Article 1 + Annex (3) | Fruits of <i>Citrus</i> L., <i>Fortunella</i> Swingle, <i>Poncirus</i> Raf., and their hybrids (hereinafter referred to as citrus fruits) | Brazil              | The movement, from their place of production to the point of export to the Community, is accompanied by documents issued under the authority of and supervised by the National Plant Protection Organisation of Brazil, as part of a documentary system on which information is made available to the Commission. | This would have no effect on the probability of entry but will increase the consistency of the implemented measures.  |
| Decision 2004/416/EC Article 2             | Fruits of <i>Citrus</i> L., <i>Fortunella</i> Swingle, <i>Poncirus</i> Raf., and their hybrids (hereinafter referred to as                | Brazil              | Each Member State importing citrus fruits originating in Brazil shall provide the Commission and the other Member States, by  | This would have no effect on the probability of entry but will increase the consistency of the implemented measures.  |

| EU phytosanitary measure         | Commodities + origin  | Countries of origin | Requirement   | Effectiveness and uncertainty assessed by PLH Panel (NB: technical feasibility is not relevant since these measures are already implemented)   |
|----------------------------------|---|---------------------|---|--|
|                                  | citrus fruits)  |                     | 31 December of each year, with a detailed technical report on the results of plant health checks carried out on those fruits in accordance with Article 13(1) of Directive 2000/29/EC between 1 May and 30 November of the same year.   |  |
| Decision 2006/473/EC             | Recognising certain third countries and certain areas of third countries as being free from <i>Xanthomonas campestris</i> (all strains pathogenic to <i>Citrus</i> ), <i>Cercospora angolensis</i> Carv. et Mendes and <i>Guignardia citricarpa</i> Kiely (all strains pathogenic to <i>Citrus</i> ). The listing of these countries and areas is based on ‘the information provided by the European and Mediterranean Plant Protection Organization and the Centre for Agriculture and Bioscience International’ (preamble of Commission Decision 1998/83/EC) and amended by subsequent Commission Decisions 199/104/EC, 2001/440/EC, 2003/129/EC. Commission Decision 1998/83/EC was replaced by Commission Decision 2006/473/EC, which has been amended by Commission Decisions 2010/134/EC and 2013/253/EC. |                     |   |  |
| Decision 2006/473/EC Article 3.1 | Fruits of <i>Citrus</i> L., <i>Fortunella</i> Swingle, <i>Poncirus</i> Raf., and their hybrids  | Non-EU countries    | <p>Pest-free countries:</p> <p>For the purposes of point 16.4 of Section I of Part A of Annex IV (of Directive 2000/29/EC), the following third countries are recognised as being free from all strains of <i>Guignardia citricarpa</i> Kiely pathogenic to <i>Citrus</i>:</p> <p>(a) all citrus-growing third countries in North, Central and South America, except Argentina, Brazil and the United States, the Caribbean and Europe;</p> <p>(b) all citrus-growing third countries in Asia, except Bangladesh, Bhutan, China, Indonesia, Philippines and Taiwan;</p> <p>(c) all citrus-growing third countries in Africa, except South Africa, Ghana, Kenya, Mozambique, Swaziland, Zambia and Zimbabwe;</p> <p>(d) all citrus-growing third countries in Oceania, except Australia and Vanuatu.</p> | <p>There are inconsistencies between the list of pest-free countries in this Article and the list of countries with reports of <i>P. citricarpa</i> as presented in Table 4 of section 3.1.2 of this opinion (derived by the Panel from EPPO-PQR database, 2013, from interceptions recorded in the Europhyt database and from records in scientific and technical literature),</p> <p>In Table 4 Cuba and Uruguay are reported to be infested by <i>P. citricarpa</i>, whereas in Article 3.1. these countries are recognized as being free from <i>Guignardia citricarpa</i>.</p> <p>In Table 4 Thailand is reported as the origin of a consignment infested with <i>P. citricarpa</i> intercepted at the EU border, but this country is recognized as being free from <i>P. citricarpa</i> by Article 3.1..</p> <p>In Table 4 Uganda is listed with few occurrences of <i>P. citricarpa</i> and Benin, Cameroon and Guinea are reported as the origin of consignments infested with <i>P. citricarpa</i> intercepted at the EU border. However, these countries are recognized as being</p> |



| EU phytosanitary measure                | Commodities + origin  | Countries of origin     | Requirement  | Effectiveness and uncertainty assessed by PLH Panel (NB: technical feasibility is not relevant since these measures are already implemented)  |
|---|---|-------------------------|--|---|
|   |   |                         |  | <p>free from <i>P. citricarpa</i> by Article 3.1.</p> <p>Because of the differences between the list in this Article 3.1 and Table 4, the effectiveness of the list in this Article is assessed as <b>low to moderate</b>, with <b>medium</b> uncertainty.</p>  |
| <p>Decision 2006/473/EC Article 3.2</p> | <p>Fruits of <i>Citrus</i> L., <i>Fortunella</i> Swingle, <i>Poncirus</i> Raf., and their hybrids</p> | <p>Non-EU countries</p> | <p>Pest-free areas:<br/>           For the purposes of point 16.4 of Section I of Part A of Annex IV IV (of Directive 2000/29/EC), the following areas are recognised as being free from all strains of <i>Guignardia citricarpa</i> Kiely pathogenic to <i>Citrus</i>:</p> <p>(a) South Africa: Western Cape; Northern Cape: magisterial districts of Hartswater and Warrenton;<br/>           (b) Australia: South Australia, Western Australia and Northern Territory;<br/>           (c) China: all areas, except Sichuan, Yunnan, Guangdong, Fujian and Zhejiang;<br/>           (d) Brazil: all areas except the States of Amazonas, Bahia, Espírito Santo, Mato Grosso, Mato Grosso do Sul, Minas Gerais, Paraná, Rio de Janeiro, Rio Grande do Sul, Santa Catarina and São Paulo;<br/>           (e) the United States: all areas except counties of Collier, Hendry and Polk located in the state of Florida.</p> | <p>In Table 4, Northern Territory (Australia) is reported to be infested by <i>P. citricarpa</i> but it is listed as pest-free area in Article 3.2.<br/>           In Table 4, Xianggang (Hong Kong) is reported to be infested by <i>P. citricarpa</i>, but is listed as pest-free area in Article 3.2.</p> <p>In addition, the list of areas infested by <i>P. citricarpa</i> for Brazil and for United States in Article 3.2 are more detailed than the list of areas in Table 4. However a detailed evaluation of the geographical features of pest-free areas of third countries is not included in this scientific opinion.</p> <p>Because of some differences between the list in this Article 3.2 and Table 4, the effectiveness of the list of pest-free areas in this Article is assessed as <b>moderate</b>, with <b>medium</b> uncertainty.</p> |

#### 4.4.3. Remarks concerning the pathway ‘citrus fruit by commercial trade’

The entry of fruit of *Citrus*, *Fortunella*, *Poncirus* and their hybrids with *P. citricarpa* is banned under Annex II A I of EU Plant Health Directive (2000/29/EC). In order for third countries to export such fruit to the EU, in brief, Annex IV A I states not only that fruits must be free from peduncles and leaves and that the packaging shall bear an appropriate origin mark, but also that the fruit must be accompanied by an official statement to confirm that they originate from either:

- a) a country that is free from the pest
- b) a pest-free area
- c) a pest-free place of production (no symptoms observed in the field of production and in its immediate vicinity since the beginning of the last cycle of vegetation, and none of the fruits harvested in the field of production has shown, in appropriate official examination, symptoms of this organism)
- d) a field of production subjected to appropriate treatments against *Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*), and none of the fruits harvested in the field of production has shown in appropriate official examination, symptoms of this organism.

Options (a) and (b) have already been evaluated in section 4.1.1.13. This section concluded that the establishment and maintenance of pest-free areas for *P. citricarpa* has high effectiveness and moderate to high technical feasibility with medium uncertainty, but such pest-free areas need to be based on surveys with adequate attention to the distribution of managed and unmanaged host plants in the pest-free area and the possible presence of asymptomatic latent infections. The uncertainty was rated as low. Examples of country freedom and pest-free areas for *P. citricarpa* include New Zealand and the Hartswater and Warrenton magisterial districts of Northern Province, South Africa, respectively (Commission Decision 2006/473/EC (OJ L 187, 8.7.2006, p. 35). Carstens et al. (2012) have recently supported area freedom for Western Cape, Northern Cape and Free State Provinces in South Africa.

Option (c) has already been partly evaluated in section 4.1.1.14 and assessed as having a low effectiveness, a low technical feasibility and medium uncertainty. Option (c) includes also an inspection that is separately evaluated in section 4.1.1.3. This inspection in Brazil can be connected with the ethephon pre-export test to induce precocious symptoms expression in latent infections (see section 4.1.1.4).

For option (d), appropriate treatments (section 4.1.1.8) are assessed as having a moderate effectiveness but with a very high technical feasibility and low uncertainty. For the second part of option (d), section 4.1.1.4 covers the “pest freedom of consignments: inspection or testing”: the visual inspection of consignments combined with laboratory testing is assessed as moderate with moderate technical feasibility and medium uncertainty.

Between 1999 and 2012, *P. citricarpa* was detected in 859 consignments by EU MS, indicating that exporting countries (see section 3.2.2 and Figure 8) have difficulties in implementing the special requirements of the EU concerning *P. citricarpa*. There is a good correlation between the amount of citrus fruit imported and the number of *P. citricarpa* interceptions. A detailed analysis of 101 interceptions done by the Netherlands in 2012 showed that

- Eighty-seven interceptions concerned non-compliance with Council Directive 2000/29/EC, Annex IV Part A, Section I, point 16d (appropriate field treatments and absence of symptoms in the consignment).

- Eleven interceptions concerned non-compliance with Council Directive 2000/29/EC Annex IV, Part A, section I, point 16c or Commission Decision 2004/416/EC Annex point 2b (pest-free place or field of production).
- Three interceptions concerned non-compliance with a general statement that plant health regulations have been observed.
- There were no interceptions for non-compliance with country or area freedom.

The analysis of the RROs in the EC Plant Health Directive utilised to prevent the entry of *P. citricarpa* on fruit also indicates that only country and area freedom are highly effective. However, from section 4.1.1.15 a system approach is also shown to have high effectiveness.

#### **4.4.4. Remarks concerning the pathway ‘lime fruit (*Citrus latifolia*) by commercial trade’**

Although no interceptions of *P. citricarpa* on Tahiti lime have been made in EU border inspections, the pathogen has been shown to colonise Tahiti lime fruit under natural conditions, but without expressing CBS symptoms in fruit. This implies a high probability of association of the pathogen with the pathway of Tahiti lime fruit without leaves and peduncles at origin as latent mycelia in asymptomatic fruits but a very unlikely probability of transfer to suitable host. The overall probability of entry with the pathway of Tahiti lime fruit, without leaves and peduncles, is rated as very unlikely, with high uncertainty.

#### **4.4.5. Remarks concerning the pathway ‘citrus fruit with passenger traffic’**

Currently, under EU legislation, measures to prevent the entry of *P. citricarpa* via citrus fruit may not be applied to citrus fruit carried by passengers since the special requirements for plants, plant products and other objects listed in Annex IV, Part A, and in Annex V B need not apply for small quantities of plants, plant products, foodstuffs or animal feedingstuffs where they are intended for use by the owner or recipient for non-industrial and non-commercial purposes or for consumption during transport, provided that there is no risk of harmful organisms spreading (Council Directive 2000/29/EC, Article 5, paragraph 4; Article 13b, paragraph 3). According to the risk assessment (section 3.2.4) the movement of *P. citricarpa* on fruit carried by passengers is very likely, but the transfer to a suitable host is unlikely, although with high uncertainty. The frequency of passengers carrying citrus fruit was estimated as 0.1 % (section 4.1.2.4) and a large sample of passengers would need to be inspected to reduce the rate of entry of citrus fruit by passengers. A combination of improved communication measures to inform incoming passengers of their obligations and incidental targeted inspection of passengers might be more effective.

#### **4.4.6. Remarks concerning pathway ‘citrus fruit with leaves by commercial trade’**

Although importation from third countries of citrus fruit with leaves is not permitted by EU legislation, a number of interceptions have been made by EU MS of consignments with citrus fruit with leaves originating from third countries during recent years (see section 3.2.5).

According to Annex IV, part A, Section I, item 16.1, fruits of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf. and their hybrids, originating in third countries, shall be free from peduncles and leaves and the packaging shall bear an appropriate origin mark. This measure has been evaluated in section 4.1.1.2 as having high effectiveness and very high technical feasibility, with low uncertainty. It has to be noted that the pathway of citrus fruit with leaves and peduncles has a likely rating for entry owing to the likely transfer to suitable host by ascospores produced on leaf litter. This also applies to Tahiti lime fruit with leaves and peduncles, since *P. citricarpa* ascospores have been reported to be produced in Tahiti lime leaf litter.

#### **4.4.7. Remarks concerning pathways ‘citrus plants for planting for citrus fruit production, commercial trade’, ‘lime (*Citrus latifolia*) plants for planting’ and ‘citrus leaves’**

Since the import of citrus plants into the EU is prohibited, no trade data are available on the volume of citrus plant propagation material from countries of current distribution of *P. citricarpa* to the EU.

According to Annex III, part A, item 16, of Directive 2000/29/EC, the introduction into the EU of plants of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf. and their hybrids, other than fruit and seeds, originating in third countries is prohibited. This measure has been evaluated in section 4.1.3.1 as having very high effectiveness and technical feasibility, with low uncertainty. It should be noted that the pathway of citrus plants for planting, as well as that of Tahiti lime plants for planting, has a likely overall rating for entry and a very likely transfer to suitable host because of the production of *P. citricarpa* ascospores in Tahiti lime leaves.

#### **4.4.8. Remarks concerning pathway ‘citrus plants for planting for citrus fruit production, passenger traffic’**

Since citrus plants for planting are subject to prohibition of import according to Annex III of Council Directive 2000/29/EC instead of special requirements of Annex IV, Part A, the exceptions of Article 5, point 4, of the Directive do not apply. The prohibition of import of plants for planting via passengers traffic is the only option for this pathway evaluated (see section 4.1.4.1) with at least moderate effectiveness, although with low feasibility.

#### **4.5. Conclusions on the analysis of risk reduction options and on the current phytosanitary measures**

For the reduction of the probability of entry of *P. citricarpa*, prohibition and import from pest-free areas have overall a high to very high effectiveness with moderate to high feasibility for all pathways. Prohibition of parts of the host also has high effectiveness and very high feasibility with regard to the prohibition of citrus fruit with leaves and peduncles. For the fruit pathway, systems approaches as well as the induction of precocious symptoms expression in latent infections also have high effectiveness and feasibility. For plants for planting, certification and pre- and post-entry quarantine systems were also found to have high effectiveness and feasibility.

For reduction of the probability of establishment and spread, the application of strict waste processing measures would be highly effective in reducing the potential transfer of *P. citricarpa* from infected citrus fruit, for both entry and spread, although with low feasibility. The effectiveness of eradication, as well as of containment, is assessed as low. The application of drip irrigation practices will moderately reduce the probability of establishment.

The effectiveness of current EU phytosanitary measures to reduce the risk of *P. citricarpa* introduction ranges from moderate to high, except for the pest free production site, for which the effectiveness is rated as low.

After establishment, *P. citricarpa* has not been eradicated anywhere and is reported to be very difficult to contain. Therefore RROs to prevent the entry of the pathogen are evaluated as most effective. Should the disease be reported from the risk assessment area, limited options are available to reduce the risk of establishment and spread. Current EU measures are overall judged to be effective in preventing the introduction of *P. citricarpa*.

## **CONCLUSIONS**

Following a request from the European Commission, the Panel had conducted a pest risk assessment and evaluation of risk reduction options for *P. citricarpa* for the EU territory and it had undertaken in the summer 2013 a public consultation on the draft Scientific Opinion. The comments received during the public consultation were taken into account and the Scientific Opinion was revised accordingly.

The Panel conducted the risk assessment following the guidance documents on a harmonised framework for pest risk assessment (EFSA PLH Panel, 2010) and on evaluation of risk reduction options (EFSA PLH Panel, 2012). The Panel conducted the risk assessment in the absence of current and potential new risk reduction measures in place. The risk assessment therefore expresses the full risk posed by *P. citricarpa* to the EU territory corresponding to a situation in which all current EU citrus requirements listed in Council Directive 2000/29/EC (in Annexes II, III, IV and V) and Commission Decisions 2004/416/EC and 2006/473/EC are lifted without being replaced by any other risk reduction measures. The Panel undertook a simplified quantitative pathway analysis exercise for the trade of commercial citrus fruit in order to examine with further detail the various steps involved in a potential pathogen entry process and to support the qualitative ratings.

The risk assessment covers *Guignardia citricarpa* Kiely, which has since been renamed *Phyllosticta citricarpa* (McAlpine) Van der Aa. Other *Phyllosticta* species are not included.

After consideration of the evidence, the Panel reached the following conclusions:

**With regard to the pest categorisation:**

*P. citricarpa* is absent from the EU and has a potential for establishment and spread and for causing consequences in the risk assessment area.

**With regard to the assessment of the risk to plant health for the EU territory:**

Under the scenario in which all current EU requirements listed in Council Directive 2000/29/EC and Commission Decisions 2004/416/EC and 2006/473/EC are lifted, the conclusions of the pest risk assessment are as follows:

Entry

The probability of entry is rated as:

- moderately likely for the citrus fruit trade pathway (medium uncertainty)
- very unlikely for the Tahiti lime (*Citrus latifolia*) fruit trade pathway (high uncertainty)
- unlikely for citrus fruit import by passengers traffic pathway (medium uncertainty)
- likely for the citrus fruit with leaves trade pathway (medium uncertainty)
- likely for the citrus plants for planting trade pathway (low uncertainty)
- likely for the Tahiti lime (*Citrus latifolia*) plants for planting trade pathway (high uncertainty)
- likely for the citrus plants for planting import by passengers traffic (medium uncertainty)
- unlikely for the citrus leaves for cooking (medium uncertainty).

Establishment

The probability of establishment is rated as moderately likely because of:

- the widespread availability of susceptible hosts (no uncertainty)
- the climate suitability for ascospores maturation, dispersal and infection of many EU citrus-growing areas in late summer and early autumn and for specific location also in late spring and early summer (high uncertainty)

- cultural practices (fungicides) not preventing establishment (low uncertainty)
- sprinkle and micro-sprinkle irrigation (still used in part of the EU citrus-growing areas) favouring establishment (low uncertainty)

Overall, the uncertainty on the probability of establishment is rated as high, mainly because of lack of knowledge of how *P. citricarpa* will respond under the EU climatic conditions. Although it is known which environmental factors are important to the organism in the various stages of the life cycle, there is insufficient scientific evidence to determine the exact thresholds of these factors required by the organism, e.g. temperature and wetness levels and durations. Further validation of the models applied by the Panel, especially for marginal areas within the current distribution of the citrus black spot disease, would be needed to reduce the uncertainty on the probability of establishment of *P. citricarpa* in the EU.

### Spread

Natural spread of *P. citricarpa* is known to mainly happen by dispersal of airborne ascospores. There is little evidence about the dispersal distances of the pathogen by natural means. The pathogen is very likely to spread with human assistance along the commercial fruit and plants for planting pathways. However, because spread is defined as the expansion of the geographical distribution of a pest within an area, the rate of spread depends not only on the rapidity of movement and the number of spread pathways but also on the likelihood of finding a suitable environment for establishment. When the proportion of the citrus-growing areas identified as potentially suitable for *P. citricarpa* is taken into account, the Panel considered that a rating of moderately likely is most appropriate for spread.

Although there is uncertainty about the potential natural spread of ascospores carried by wind over long distances, this uncertainty does not concern the two main pathways of spread (intra-European trade of commercial fruit and plants for planting).

### Endangered area

The risk assessment has identified parts of the EU where host plants are present and where, based on simulation results, the climatic conditions are suitable for ascospore maturation and release followed by infection.

Conclusions from simulations of the release of ascospores based on gridded interpolated climate data of the EU citrus-producing areas show that, in almost all years, ascospore release in the EU citrus growing areas will start early enough to coincide with climatic conditions that are conducive to infection in September and October. However, the simulations indicate that the onset of ascospore release in most areas will start too late to coincide with the climatic conditions conducive to infection in spring. Therefore, early-maturing citrus varieties might generally be infected in late summer and early autumn, which is when the availability of inoculum coincides with suitable conditions for infection. Owing to the long incubation time, fruits from these early varieties will be harvested before symptoms appear. The late-maturing oranges varieties and lemons are expected under such scenario to show CBS symptoms

There are some areas, however, such as locations in Portugal, southern Italy, Cyprus, the Greek islands, Malta and southern Spain, where development of ascospores is expected also in late spring and early summer months in part of the years simulated. In those years, it is expected that symptoms can develop on the fruit before harvest, and therefore have an impact on the fruit quality.

The uncertainty is high as indicated in the establishment section

### Consequences

The results from the simulation of ascospore maturation, release and infection show that citrus black spot will develop and express symptoms mainly in late-maturing sweet orange varieties and lemons grown within the endangered area. The expected consequences will be moderate for fresh fruit of late-maturing citrus varieties and lemons. There would be a potential for reduction in disease incidence by chemical treatments, but this would cause environmental impacts because in most EU citrus-growing areas fungicides are not widely applied and the most effective fungicide products are not currently registered for use in citrus in the EU MSs. In addition, to export citrus fruit to areas where CBS is regulated, additional fungicide treatments in the orchards, official inspections, quality controls in packing houses and/or establishment of pest-free areas might be needed to meet the phytosanitary requirements of these countries.

The consequences for fresh fruit of early-maturing citrus varieties are assessed as minor. The impact on early-maturing varieties would be sporadic in time and space, limited to years with rainy springs and summers and/or to specific locations. However, the impact could be higher in areas where late spring and early summer infection, based on simulation results, is expected to be more frequent...

The consequences would be minimal for citrus for processing, as external lesions or spots on citrus fruit are not a quality issue for citrus for processing.

As for establishment, the uncertainties about consequences are high owing to the lack of information on key parameters in the epidemiological models and on the incubation period; the lack of knowledge about the rate of disease build-up for this pathogen; and the limited information available about the impact of the disease and the programmes of fungicide treatments in semi-arid areas within the current CBS area of distribution, e.g. Eastern Cape, where environmental conditions are more similar to those in the pest risk assessment area.

**With regard to risk reduction options**, the Panel notes that, for the reduction of the probability of entry of *P. citricarpa*, prohibition and import from pest-free areas have overall a high to very high effectiveness with moderate to high feasibility for all pathways. Prohibition of parts of the host also has high effectiveness and very high feasibility with regard to the prohibition of citrus fruit with leaves and peduncles. For the fruit pathway, systems approaches as well as the induction of precocious symptoms expression in latent infections also have high effectiveness and feasibility. For plants for planting, certification and pre- and post-entry quarantine systems were also found to have high effectiveness and feasibility.

For reduction of the probability of establishment and spread, the application of strict waste processing measures would be highly effective in reducing the potential transfer of *P. citricarpa* from infected citrus fruit, both for entry and spread, although with low feasibility. The effectiveness of eradication, as well as of containment, is assessed as low. The application of drip irrigation practices will moderately reduce the probability of establishment.

The effectiveness of current EU phytosanitary measures to reduce the risk of *P. citricarpa* introduction ranges from moderate to high, except for the pest free production site, for which the effectiveness is rated as low.

After establishment, *P. citricarpa* has not been eradicated anywhere and is reported to be very difficult to contain. Therefore risk reduction options to prevent the entry of the pathogen are evaluated as most effective. Should the disease be reported from the risk assessment area, limited options are available to reduce the risk of establishment and spread.

## DOCUMENTATION PROVIDED TO EFSA

1. Request (background and term of reference) to provide a Scientific Opinion on the risk to plant health posed by *Guignardia citricarpa* (all strains pathogenic to *Citrus*) for the EU territory. SANCO.E2 GC/ap (2012) 1648697 December 2012. Submitted by European Commission, DG SANCO, Directorate-General Health and Consumers.
2. Letter with additional documents provided by the Brazilian authorities on *Guignardia citricarpa*. SANCO.E2 GC/ap (2012) 1739253 December 2012. Submitted by European Commission, DG SANCO, Directorate-General Health and Consumers, Safety of the Food Chain, Plant Health Unit.
3. Information from South Africa for the EFSA PRA on *Guignardia citricarpa*. Republic of South Africa, Department Agriculture, Forestry and Fisheries, Directorate Plant Health, May 2013. Forwarded by European Commission, DG SANCO, Directorate-General Health and Consumers, Safety of the Food Chain, Plant Health Unit.
4. Information provided by South Africa regarding the PRA on *Guignardia citricarpa* (CBS): 1) van Zyl K, 2011. Citrus disease. In: The control of fungal, viral and bacterial diseases in plants. A CropLife South Africa Compendium©. Published by AVCASA. 2) Schutte GC, 2009. Black spot. In: Citrus diseases, Chapter 6: Foliar and Fruit Diseases, Citrus Research International, 10 pp. Forwarded by European Commission, DG SANCO, Directorate-General Health and Consumers, Safety of the Food Chain, Plant Health Unit.

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## APPENDICES

### Appendix A. Rating descriptors

In order to follow the principle of transparency as described under Paragraph 3.1 of the Guidance document on the harmonised framework for risk assessment (EFSA PLH Panel, 2010)—“...*Transparency requires that the scoring system to be used is described in advance. This includes the number of ratings, the description of each rating ... the Panel recognises the need for further development...*”—the Plant Health Panel has developed specifically for this opinion rating descriptors to provide clear justification when a rating is given.

#### Ratings used in the conclusion of the pest risk assessment

In this opinion of EFSA’s Plant Health Panel on the risk assessment of *P. citricarpa* and the evaluation of the effectiveness of the RROs, a rating system of five levels with their corresponding descriptors has been used to formulate separately the conclusions on entry, establishment, spread and impact as described in the following tables.

#### Rating of probability of entry

| Rating for entry         | Descriptors for <i>P. citricarpa</i>   |
|--------------------------|--|
| <i>Very unlikely</i>     | The likelihood of entry would be very low because the pest: <ul style="list-style-type: none"> <li>• is not, or is only very rarely, associated with the pathway at the origin; and/or</li> <li>• may not survive during transport or storage; and/or</li> <li>• cannot survive the current pest management procedures existing in the risk assessment area; and/or</li> <li>• may not transfer to a suitable host in the risk assessment area.</li> </ul>                   |
| <i>Unlikely</i>          | The likelihood of entry would be low because the pest: <ul style="list-style-type: none"> <li>• is rarely associated with the pathway at the origin; and/or</li> <li>• survives at a very low rate during transport or storage; and/or</li> <li>• is strongly limited by the current pest management procedures existing in the risk assessment area; and/or</li> <li>• has considerable limitations for transfer to a suitable host in the risk assessment area.</li> </ul> |
| <i>Moderately likely</i> | The likelihood of entry would be moderate because the pest: <ul style="list-style-type: none"> <li>• is frequently associated with the pathway at the origin; and/or</li> <li>• survives at a low rate during transport or storage; and/or</li> <li>• is affected by the current pest management procedures existing in the risk assessment area;</li> </ul>   |



|                    |   |
|--------------------|---|
|                    | <p>and/or</p> <ul style="list-style-type: none"> <li>• has some limitations for transfer to a suitable host in the risk assessment area.</li> </ul>   |
| <i>Likely</i>      | <p>The likelihood of entry would be high because the pest:</p> <ul style="list-style-type: none"> <li>• is regularly associated with the pathway at the origin;</li> </ul> <p>and/or</p> <ul style="list-style-type: none"> <li>• mostly survives during transport or storage;</li> </ul> <p>and/or</p> <ul style="list-style-type: none"> <li>• is partially affected by the current pest management procedures existing in the risk assessment area;</li> </ul> <p>and/or</p> <ul style="list-style-type: none"> <li>• has very few limitations for transfer to a suitable host in the risk assessment area.</li> </ul> |
| <i>Very likely</i> | <p>The likelihood of entry would be very high because the pest:</p> <ul style="list-style-type: none"> <li>• is usually associated with the pathway at the origin;</li> </ul> <p>and/or</p> <ul style="list-style-type: none"> <li>• survives during transport or storage;</li> </ul> <p>and/or</p> <ul style="list-style-type: none"> <li>• is not affected by the current pest management procedures existing in the risk assessment area;</li> </ul> <p>and/or</p> <ul style="list-style-type: none"> <li>• has no limitations for transfer to a suitable host in the risk assessment area.</li> </ul>                 |

#### Rating of probability of establishment

| Rating for establishment | Descriptors for <i>P. citricarpa</i>   |
|--------------------------|--|
| <i>Very unlikely</i>     | The likelihood of establishment would be very low because, although the host plants are present in the risk assessment area, the environmental conditions are unsuitable and/or the host is susceptible for a very short time during the year; other considerable obstacles to establishment occur.  |
| <i>Unlikely</i>          | The likelihood of establishment would be low because, although the host plants are present in the risk assessment area, the environmental conditions are mostly unsuitable and/or the host is susceptible for a very short time during the year; other obstacles to establishment occur.   |
| <i>Moderately likely</i> | The likelihood of establishment would be moderate because, although the host plants are present in the risk assessment area, the environmental conditions are frequently unsuitable and/or the host is susceptible for short time; other obstacles to establishment may occur.   |
| <i>Likely</i>            | The likelihood of establishment would be high because the host plants are present in the risk assessment area, they are susceptible for a long time during the year, and the environmental conditions are frequently suitable; no other obstacles to establishment occur.  |
| <i>Very likely</i>       | The likelihood of establishment would be very high because the host plants are present in the risk assessment area, they are susceptible for a long time during the year and the environmental conditions are suitable for most of the host growing season; no other obstacles to establishment occur. Alternatively, the pest has already been established in the risk assessment area. |

### Rating of probability of spread

| Rating for spread        | Descriptors for <i>P. citricarpa</i>   |
|--------------------------|--|
| <i>Very unlikely</i>     | <p>The likelihood of spread would be very low because the pest:</p> <ul style="list-style-type: none"> <li>• has only one specific way to spread (e.g. a specific vector) which is not present in the risk assessment area;</li> </ul> <p>and/or</p> <ul style="list-style-type: none"> <li>• highly effective barriers to spread exist;</li> </ul> <p>and/or</p> <ul style="list-style-type: none"> <li>• the host is not or is only occasionally present in the area of possible spread;</li> </ul> <p>and/or</p> <ul style="list-style-type: none"> <li>• the environmental conditions for infestation are unsuitable in the area of possible spread.</li> </ul>    |
| <i>Unlikely</i>          | <p>The likelihood of spread would be low because the pest:</p> <ul style="list-style-type: none"> <li>• has one or only a few specific ways to spread (e.g. specific vectors) and its occurrence in the risk assessment area is occasional;</li> </ul> <p>and/or</p> <ul style="list-style-type: none"> <li>• effective barriers to spread exist;</li> </ul> <p>and/or</p> <ul style="list-style-type: none"> <li>• the host is not frequently present in the area of possible spread;</li> </ul> <p>and/or</p> <ul style="list-style-type: none"> <li>• the environmental conditions for infestation are mostly unsuitable in the area of possible spread.</li> </ul> |
| <i>Moderately likely</i> | <p>The likelihood of spread would be moderate because the pest:</p> <ul style="list-style-type: none"> <li>• has few specific ways to spread (e.g. specific vectors) and its occurrence in the risk assessment area is limited,</li> </ul> <p>and/or</p> <ul style="list-style-type: none"> <li>• effective barriers to spread exist;</li> </ul> <p>and/or</p> <ul style="list-style-type: none"> <li>• the host is moderately present in the area of possible spread;</li> </ul> <p>and/or</p> <ul style="list-style-type: none"> <li>• the environmental conditions for infestation are frequently unsuitable in the area of possible spread.</li> </ul>             |
| <i>Likely</i>            | <p>The likelihood of spread would be high because the pest:</p> <ul style="list-style-type: none"> <li>• has some unspecific ways to spread, which occur in the risk assessment area;</li> </ul> <p>and/or</p> <ul style="list-style-type: none"> <li>• no effective barriers to spread exist;</li> </ul> <p>and/or</p> <ul style="list-style-type: none"> <li>• the host is usually present in the area of possible spread;</li> </ul> <p>and/or</p> <ul style="list-style-type: none"> <li>• the environmental conditions for infestation are frequently suitable in the area of possible spread.</li> </ul>   |
| <i>Very likely</i>       | <p>The likelihood of spread would be very high because the pest:</p>   |

|  |   |
|--|---|
|  | <ul style="list-style-type: none"> <li>• has multiple unspecific ways to spread, all of which occur in the risk assessment area;</li> </ul> and/or <ul style="list-style-type: none"> <li>• no effective barriers to spread exist;</li> </ul> and/or <ul style="list-style-type: none"> <li>• the host is widely present in the area of possible spread;</li> </ul> and/or <ul style="list-style-type: none"> <li>• the environmental conditions for infestation are mostly suitable in the area of possible spread.</li> </ul> |
|--|---|

### Rating of magnitude of the potential consequences

| Rating of potential consequences | Descriptors for <i>P. citricarpa</i>  |
|----------------------------------|---|
| <i>Minimal</i>                   | Differences in crop production are within normal day-to-day variation; no additional control measures are required.   |
| <i>Minor</i>                     | Crop production is rarely reduced or at a limited level; additional control measures are rarely necessary.  |
| <i>Moderate</i>                  | Crop production is occasionally reduced to a limited extent; additional control measures are occasionally necessary.  |
| <i>Major</i>                     | Crop production is frequently reduced to a significant extent; additional control measures are frequently necessary.  |
| <i>Massive</i>                   | Crop production is always or almost always reduced to a very significant extent (severe crop losses that compromise the harvest); additional control measures are always necessary. |

### Ratings used for the evaluation of the risk reduction options

The Panel developed the following ratings with their corresponding descriptors for evaluating the effectiveness of the RROs to reduce the level of risk.

### Rating of the effectiveness of risk reduction options

| Rating            | Descriptors for <i>P. citricarpa</i>  |
|-------------------|---|
| <i>Negligible</i> | The risk reduction option has no practical effect in reducing the probability of entry or establishment or spread, or the potential consequences. |
| <i>Low</i>        | The risk reduction option reduces, to a limited extent, the probability of entry or establishment or spread, or the potential consequences.       |
| <i>Moderate</i>   | The risk reduction option reduces, to a substantial extent, the probability of entry or establishment or spread, or the potential consequences.   |
| <i>High</i>       | The risk reduction option reduces the probability of entry or establishment or spread, or the potential consequences, by a major extent.          |
| <i>Very high</i>  | The risk reduction option essentially eliminates the probability of entry or establishment or spread, or any potential consequences.              |

**Rating of the technical feasibility of risk reduction options**

| <b>Rating</b>     | <b>Descriptors for <i>P. citricarpa</i></b>  |
|-------------------|--|
| <i>Negligible</i> | The risk reduction option is not in use in the risk assessment area, and the many technical difficulties involved (e.g. changing or abandoning the current practices, implement new practices and or measures) make their implementation in practice impossible.                           |
| <i>Low</i>        | The risk reduction option is not in use in the risk assessment area, but the many technical difficulties involved (e.g. changing or abandoning the current practices, implementing new practices and/or measures) make its implementation in practice very difficult or nearly impossible. |
| <i>Moderate</i>   | The risk reduction option is not in use in the risk assessment area, but it can be implemented (e.g. changing or abandoning the current practices, implementing new practices and/or measures) with some technical difficulties.   |
| <i>High</i>       | The risk reduction option is not in use in the risk assessment area, but it can be implemented in practice (e.g. changing or abandoning the current practices, implement new practices and or measures) with limited technical difficulties.   |
| <i>Very high</i>  | The risk reduction option is already in use in the risk assessment area or can be easily implemented with no technical difficulties.   |

**Ratings used for describing the level of uncertainty**

For the risk assessment chapter—entry, establishment, spread and impact—as well as for the evaluation of the effectiveness of the risk reduction options, the level of uncertainty has been rated separately in coherence with the descriptors that have been defined specifically by the Panel in this opinion.

| <b>Rating</b> | <b>Descriptors for <i>P. citricarpa</i></b>  |
|---------------|--|
| <i>Low</i>    | No or little information or no or few data missing, incomplete, inconsistent or conflicting. No subjective judgement is introduced. No unpublished data are used.  |
| <i>Medium</i> | Some information is missing or some data are missing, incomplete, inconsistent or conflicting. Subjective judgement is introduced with supporting evidence. Unpublished data are sometimes used.         |
| <i>High</i>   | Most information is missing or most data are missing, incomplete, inconsistent or conflicting. Subjective judgement may be introduced without supporting evidence. Unpublished data are frequently used. |

**Appendix B. Additional trade and interceptions figures**
**Table 31:** Import of citrus fruit (tons) from third countries where *P. citricarpa* is reported into the EU. Product = 0805 citrus. Yearly data (from January to December) from 2002 to 2011. Extracted from Eurostat (online) on 22/02/2013

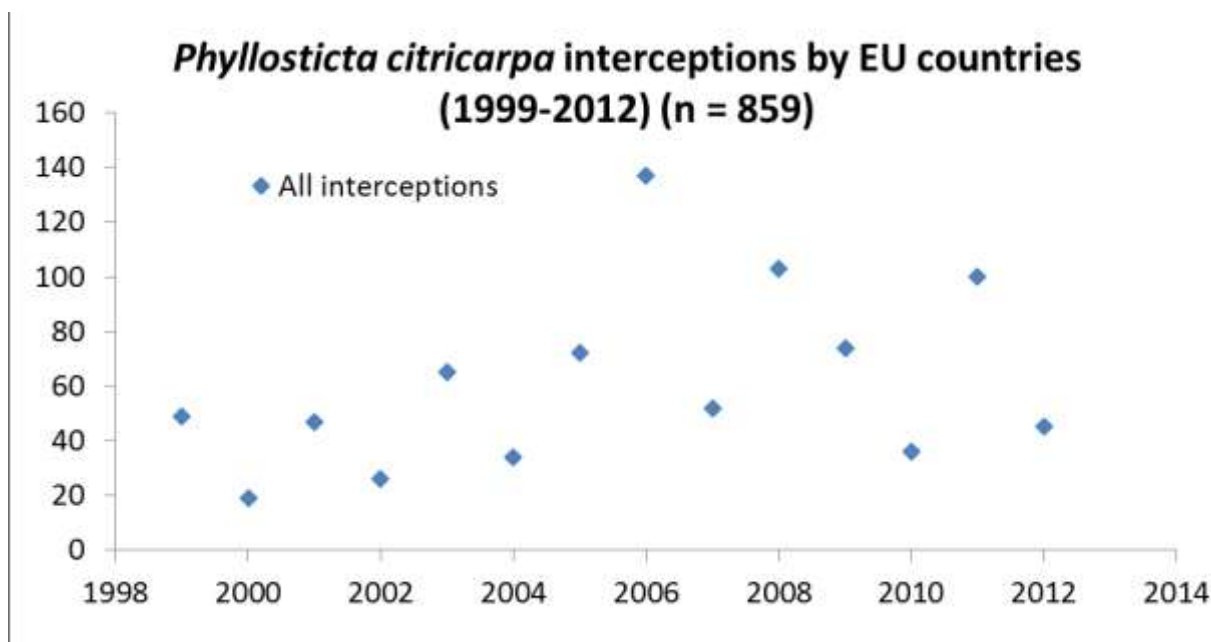
| PERIOD/<br>PARTNER | 2002    | 2003    | 2004    | 2005    | 2006    | 2007    | 2008    | 2009    | 2010    | 2011    |
|--------------------|---------|---------|---------|---------|---------|---------|---------|---------|---------|---------|
| ARGENTINA          | 285 842 | 358 827 | 315 900 | 385 569 | 331 398 | 391 136 | 424 803 | 307 875 | 315 528 | 280 264 |
| AUSTRALIA          | 1 271   | 2 743   | 1 875   | 4 808   | 1 775   | 5 950   | 2 697   | 3 983   | 16 22   | 465     |
| BRAZIL             | 47 200  | 88 630  | 86 358  | 65 004  | 96 139  | 85 222  | 78 142  | 73 094  | 89 206  | 83 795  |
| BHUTAN             |         |         |         |         |         |         |         |         |         |         |
| CHINA              | 41      | 361     | 1 524   | 6 166   | 17 110  | 43 715  | 68 235  | 75 452  | 72 478  | 47 803  |
| CUBA               | 22 217  | 31 598  | 18 964  | 15 989  | 10 363  | 7 281   | 2 979   | 2 197   | 1 374   | 1 375   |
| GHANA              | 7       | 33      |         |         | 20      | 0.7     | 1232    | 2064    | 672     | 312     |
| INDONESIA          | 18      |         |         |         | 0.1     |         |         |         |         |         |
| KENYA              |         | 9       | 0       |         |         |         |         |         |         | 0.1     |
| MOZAMBIQUE         |         | 94      | 910     | 1082    | 121     |         |         | 285     | 989     | 1587    |
| PHILIPPINES        |         |         |         | 0       |         |         | 3       |         |         | 0.3     |
| TAIWAN             | 16      | 1.2     |         |         | 0.3     | 0.8     |         | 0.5     | 0.1     |         |
| UGANDA             | 22      | 13      | 0       |         |         | 0       | 0       | 0.3     | 0       | 0       |
| UNITED STATES      | 122 324 | 107 838 | 100 255 | 54 926  | 55 532  | 65 665  | 8 9823  | 62 964  | 58 743  | 63 673  |
| URUGUAY            | 68 167  | 95 219  | 85 302  | 122 878 | 115 349 | 116 738 | 99 045  | 103 616 | 118 951 | 90 050  |
| VANUATU            |         |         |         |         |         |         |         |         |         |         |
| SOUTH AFRICA       | 463 832 | 473 011 | 407 357 | 539 363 | 468 412 | 641 822 | 676 519 | 527 017 | 604 160 | 535 988 |
| ZAMBIA             | 0       | 1.8     |         | 12      |         |         |         |         |         |         |
| ZIMBABWE           | 37 626  | 37 023  | 18 882  | 36 046  | 15 666  | 29 409  | 18 104  | 15 519  | 25 816  | 13 873  |

EU = EU-27 (AT, BE, BG, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LT, LU, LV, MT, NL, PL, PT, RO, SE, SI, SK)

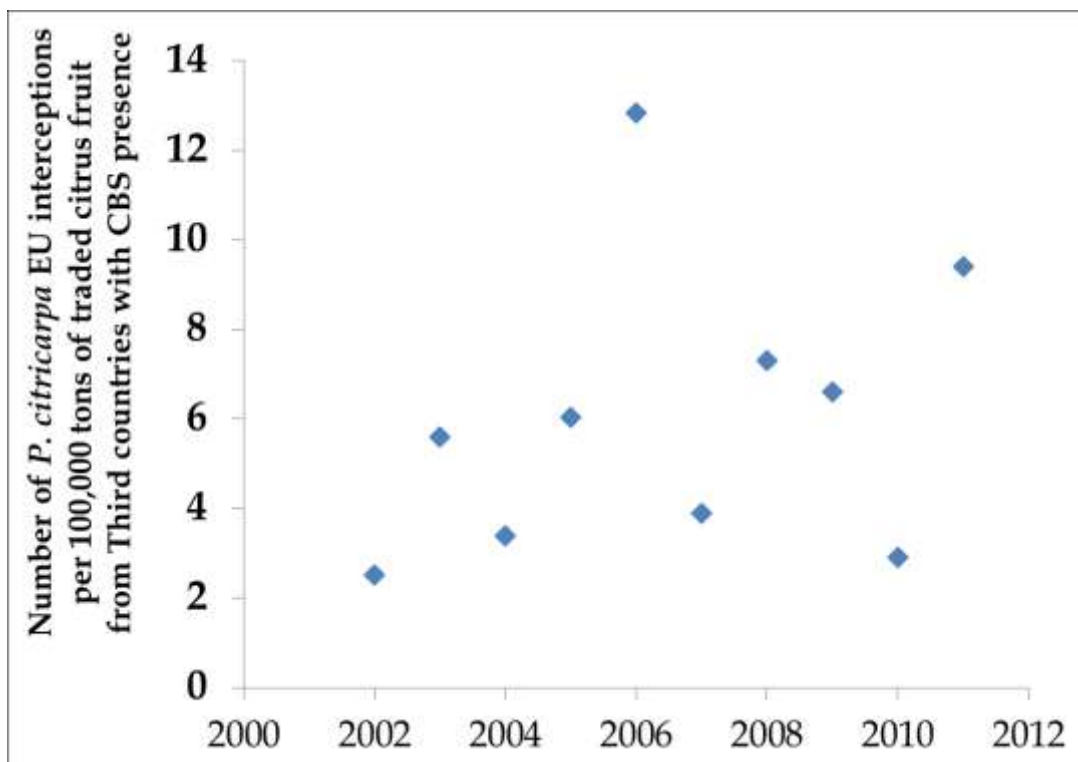
Interception of living stages of *P. citricarpa* on citrus fruit consignments imported into the EU-27 MSs

According to the Europhyt database (Europhyt, online), during the period 1999-2012, there have been 859 notifications from EU-MSs of interceptions of *P. citricarpa* on imported citrus fruit consignments originating in CBS-infested countries, excluding interceptions on pomelo.

Figure 54 shows the trend over time of the number of yearly interceptions (all EU countries, 1999–2012) of citrus fruit, excluded pomelo. There is an increase over time until the onset of the financial crisis (2008), after which the number of interceptions returns to levels comparable to those seen at the beginning of the 2000s (with the exception of 2011).



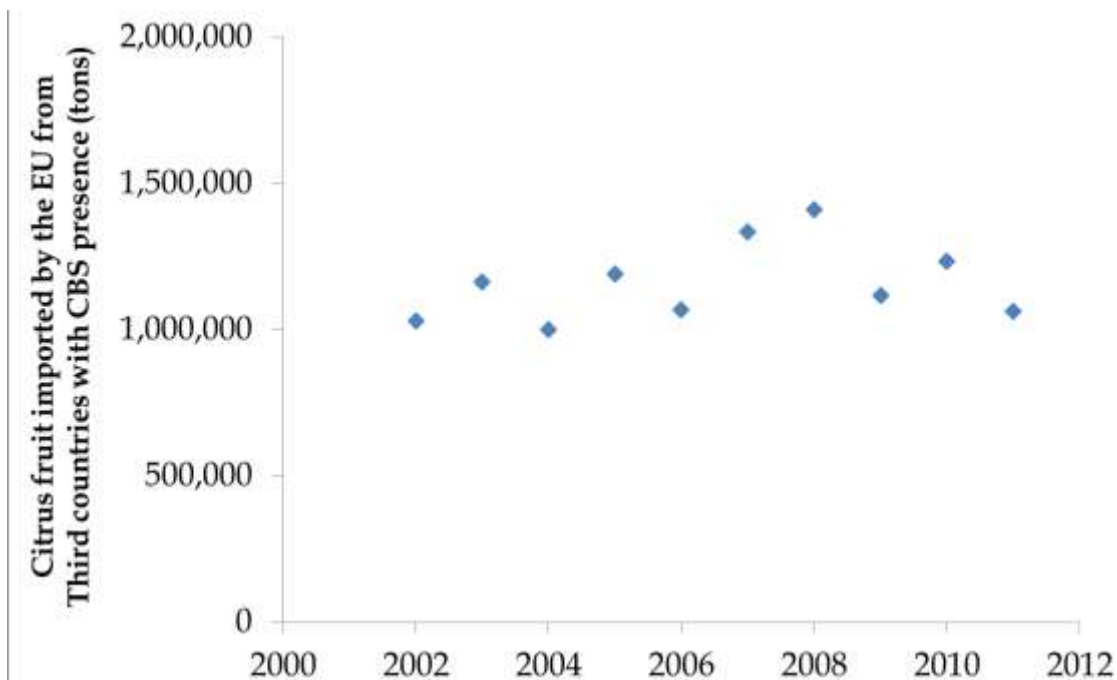
**Figure 54:** Temporal trend in the number of *P. citricarpa* EU interceptions on citrus fruit consignments from third countries, excluded interceptions on pomelo (1999–2012)



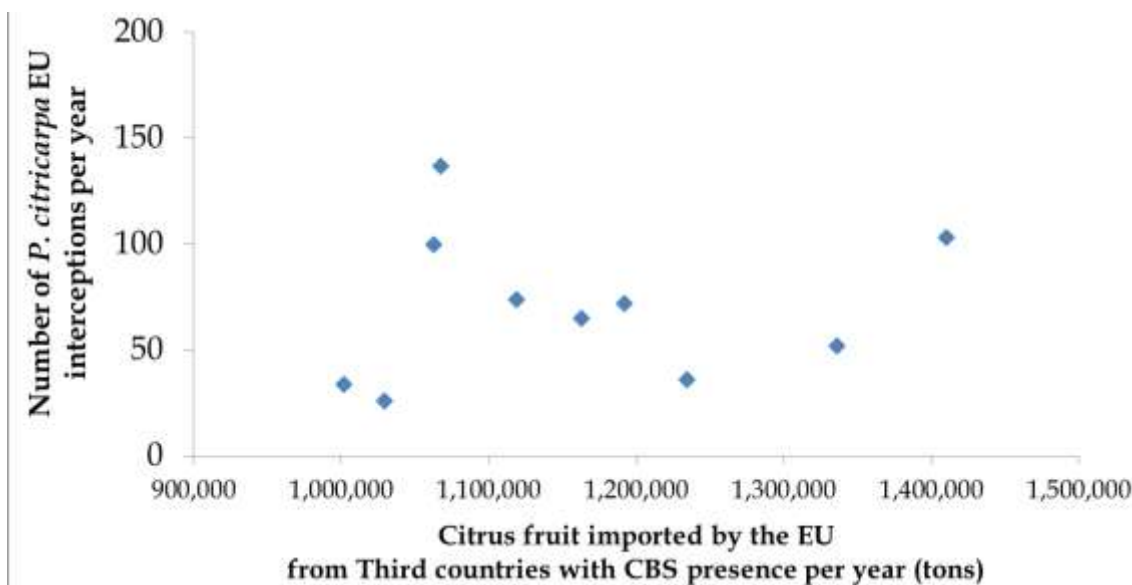
**Figure 55:** Temporal trend in the number of *P. citricarpa* EU interceptions (per unit of trade) on citrus fruit consignments from third countries, excluded limes, (2002–2011). Interceptions on pomelo (*C. maxima*) were not considered

Figure 55 shows the temporal trend in the number of *P. citricarpa* interceptions per unit of trade (100 000 tons of citrus fruit, to all EU countries, from all origins, for the period 2002–2011 for the trade data analysed. The trend is towards a weak increase ( $R^2 = 0.09$ ) in the number of interceptions per unit of trade, with two outliers in 2006 and 2010.

The trend in citrus fruit imported by EU countries from third countries (2002–2011) may also suggest an influence of the economic crisis since 2008 (unless other factors explain the reduced trade volumes for 2009–2010–2011) (Figure 56). Please note that this overall trend for all exporting countries may not be matched by the trends for single countries. For example, over the studied period (2002–2011), citrus fruit imported by the EU from Cuba and the USA fell considerably, whereas imports from China, which were insignificant in 2002, exceeded imports from the USA in 2010 (Table 31).



**Figure 56:** Temporal trend in the amount of citrus fruit imported by the EU from third countries, excluded limes (2002–2011)

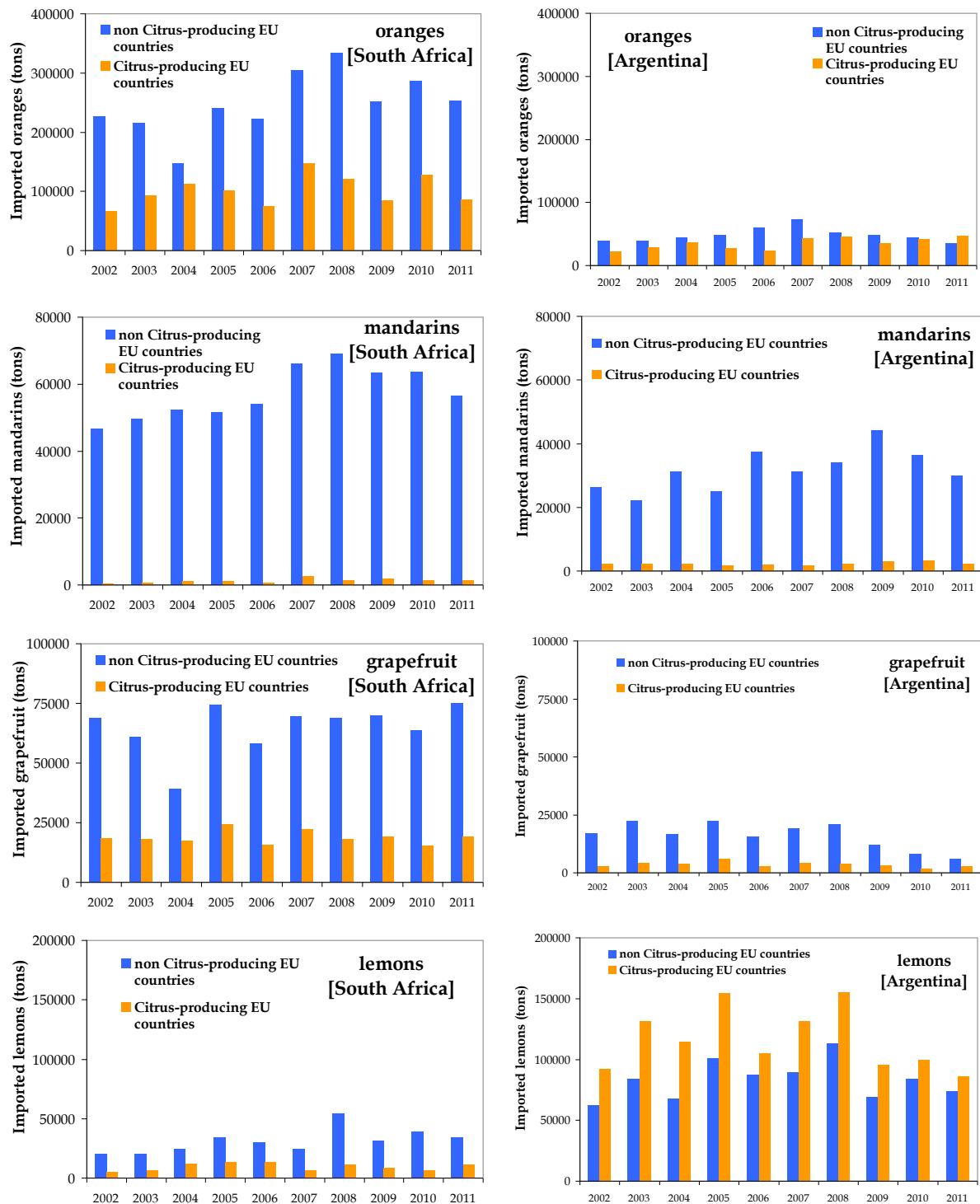


**Figure 57:** Correlation between number of interceptions of *P. citricarpa* in the EU per year (all countries of origin, 2002–2011) and amount of citrus fruit imported by the EU from third countries (all countries of origin, 2002–2011). Trade in limes was not included. Interceptions on pomelo (*C. maxima*) were not included



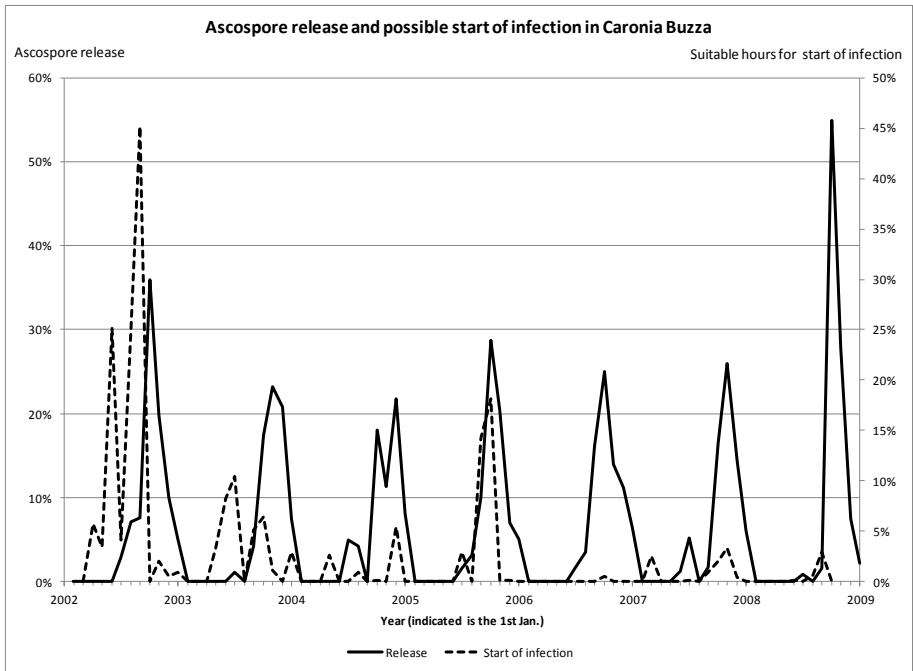
There is no correlation between the number of yearly *P. citricarpa* EU interceptions and the amount of citrus fruit imported by the EU from third countries in the same year. The absence of a correlation is due to the high number of interceptions in 2006 (and, to some extent, in 2011) despite low trade volumes in the same year (Figure 57). The absence of such a correlation does not imply that there is not an increased risk of entry of the pathogen with increasing volume of trade, as other factors also play a role in determining the number of interceptions over a whole year (e.g. climate, management practices and epidemic level during that particular year in the various countries of origin of consignments).

Most of the oranges imported by the EU from South Africa and Argentina (two of the major citrus fruit exporters with reported presence of CBS) go to non-citrus-producing countries, but the quantities of oranges imported by citrus-producing EU countries from these two countries are not negligible (Figure 58), and the pattern was reversed for Argentina in 2011. Mandarins from South Africa and Argentina go nearly exclusively to non-citrus-producing EU countries. In addition, grapefruit from South Africa and Argentina is mostly imported by non-citrus-producing EU countries. Most lemons exported by South Africa to the EU go to non-citrus-producing countries, but this is not the case for Argentina, which mainly exports lemons to citrus-producing EU countries (Figure 58).

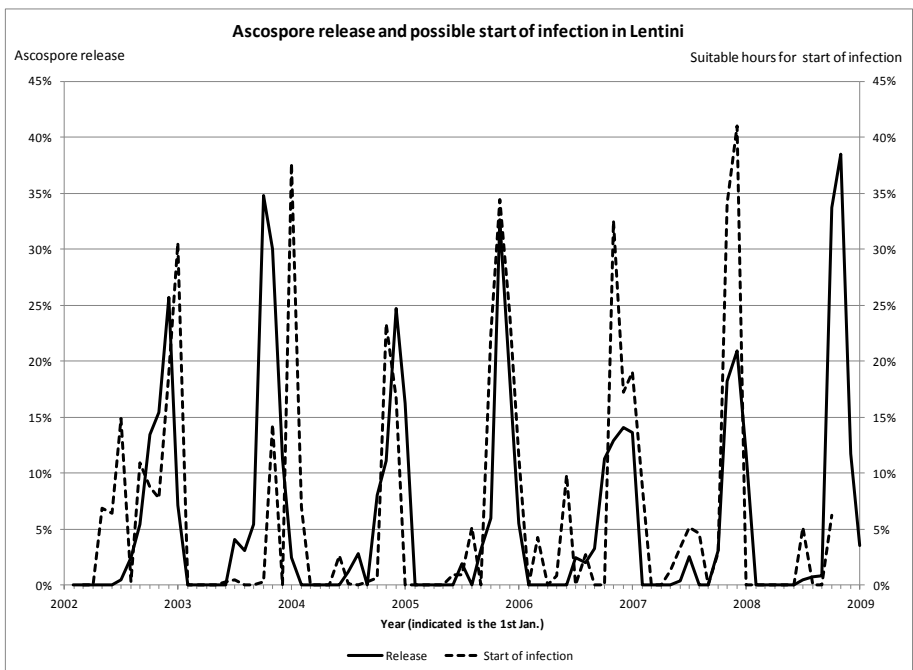


**Figure 58:** Imports of oranges, mandarins, grapefruit and lemons from (left) South Africa and (right) Argentina into citrus-producing and non-citrus-producing EU countries (2002–2011). Note that the y-axis scales are consistent between South Africa and Argentina, but not among the different types of citrus fruit

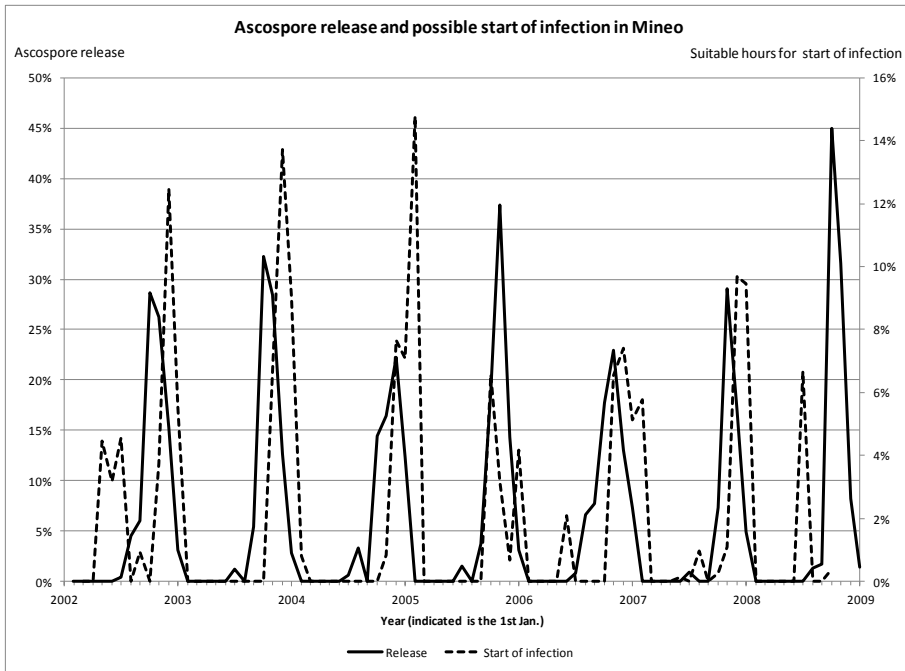
**Appendix C. Comparison of ascospore release with time of possible start of infections for eight Italian stations in citrus-growing areas**



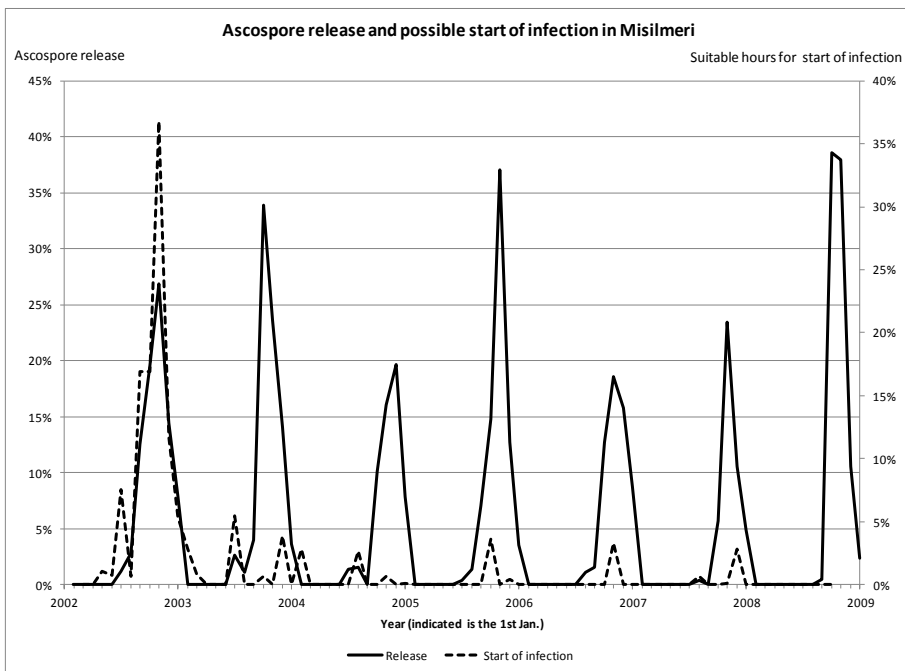
**Figure 59:** Comparison of ascospore release with time of possible start of infections



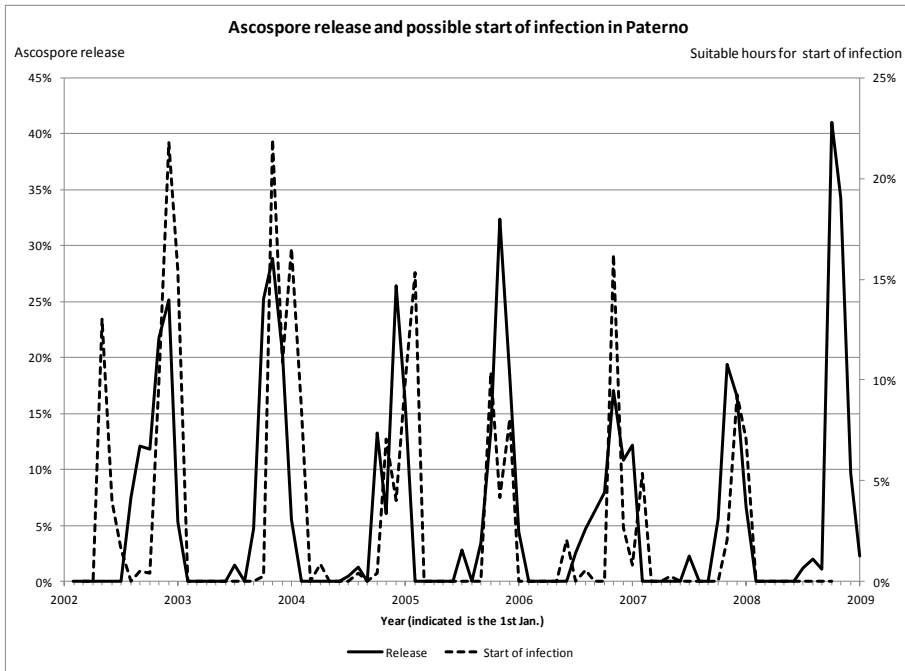
**Figure 60:** Comparison of ascospore release with time of possible start of infections



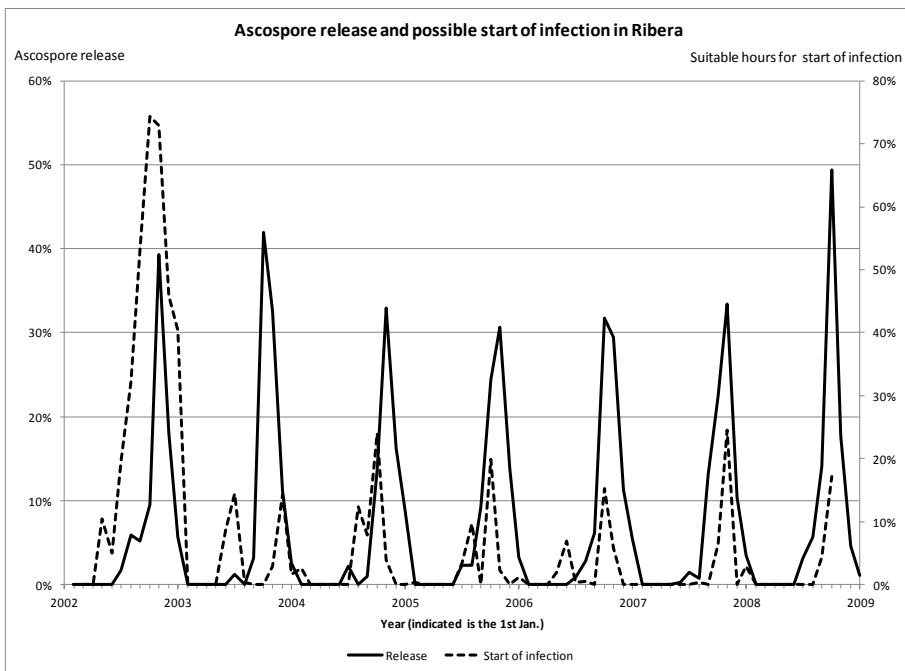
**Figure 61:** Comparison of ascospore release with time of possible start of infections



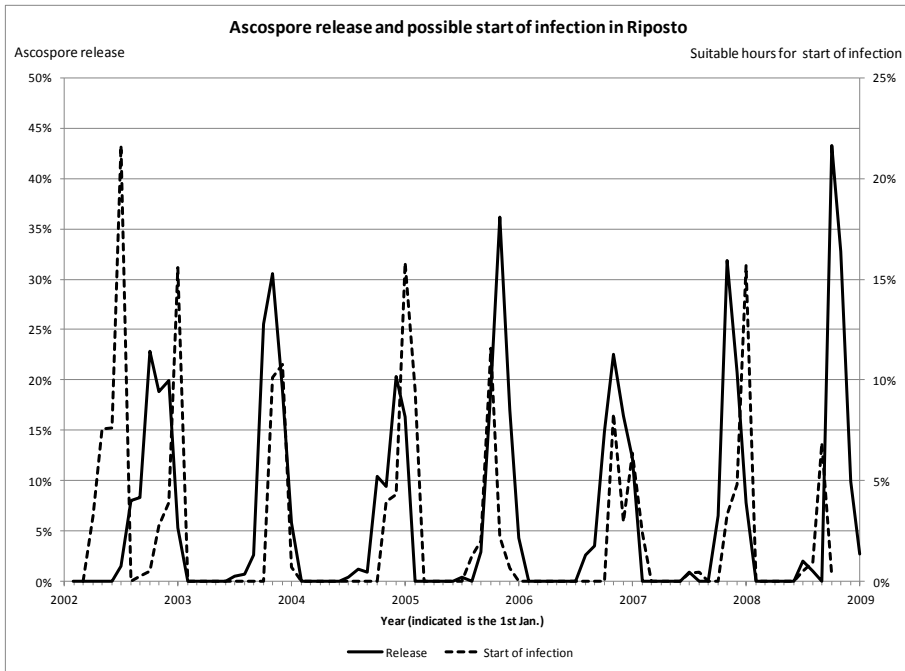
**Figure 62:** Comparison of ascospore release with time of possible start of infections



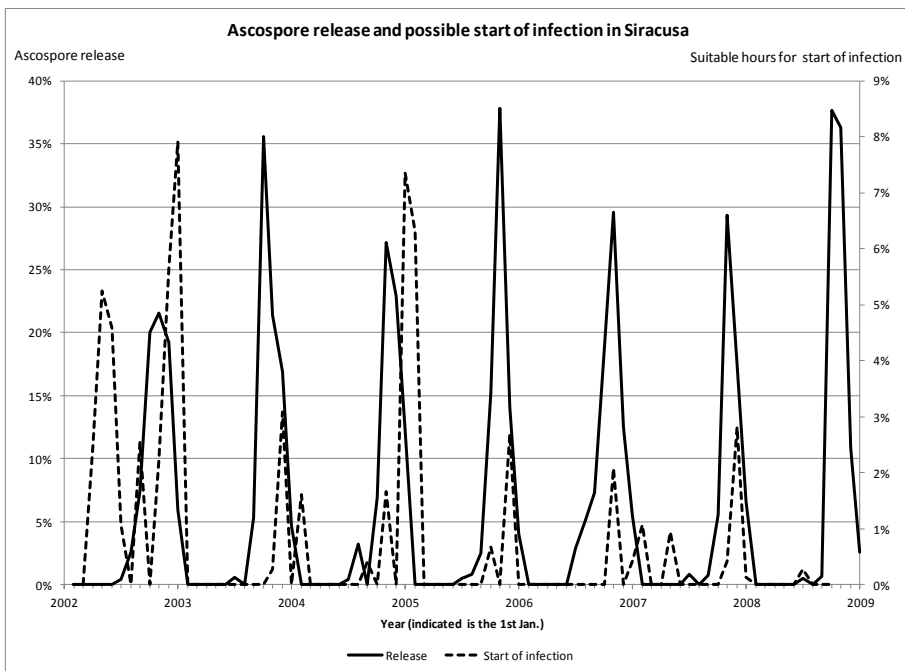
**Figure 63:** Comparison of ascospore release with time of possible start of infections



**Figure 64:** Comparison of ascospore release with time of possible start of infections



**Figure 65:** Comparison of ascospore release with time of possible start of infections



**Figure 66:** Comparison of ascospore release with time of possible start of infections

#### **Appendix D. Analysis of the sensitivity of the Yonow et al., 2013 CLIMEX model results to European climate data with different spatial resolution and time periods coverage**

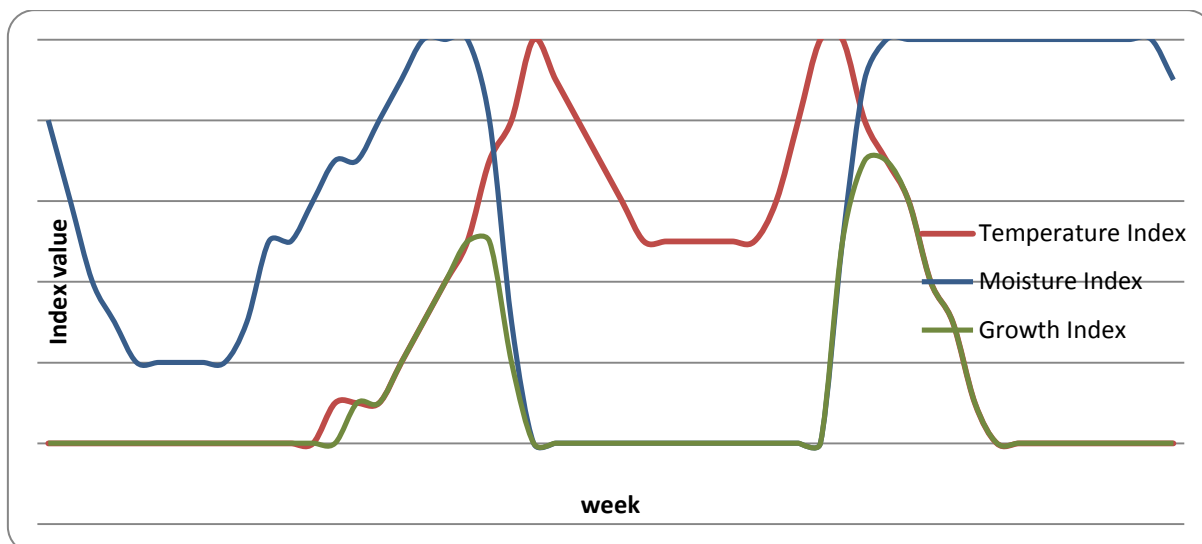
Yonow et al. (2013) recently parameterised a CLIMEX model for *P. citricarpa* to predict the potential global distribution of citrus black spot disease. Yonow et al. (2013) paid particular attention to the risk of citrus black spot disease to Europe and found that “*Within European citrus producing regions, suitable areas are highly constrained, never more than marginally suitable, and all have lower levels of suitability than any area in South Africa and Australia where G. citricarpa is known to occur.*” With regard to marginally suitable areas for citrus black spot disease within its known current distribution range, Yonow et al. (2013) mention Addo in South Africa as an example of an area with marginal suitability for *P. citricarpa*. In Addo, *P. citricarpa* is present in the citrus-growing areas and according to Yonow et al. (2013) the pathogen persists there but “*does not flourish*” because of the marginal climatic suitability of the area for disease development.

The Panel notes that both of the above-mentioned findings are of particular interest for the assessment of the risk posed by *P. citricarpa* to the EU territory. The Panel therefore explored the effect on the output of the Yonow et al. (2013) model when (1) increasing the spatial resolution in the input climate data and (2) using more recent climate data (1961–90 vs. 1998–2007 EU climate averages).

Based on this analysis the Panel concludes that:

- The model results show sensitivity to variation in the spatial resolution and the time period of the input climate data.
- For some of the EU citrus-growing areas the climatic suitability classification varies from “marginally suitable”, through “suitable” and even to “highly suitable” when changing either the spatial resolution or the temporal period covered by the input climate data (using the interpretation of the “Ecoclimatic index” used by Yonow et al. 2013).
- The predicted potential for establishment in some EU citrus-growing areas varies from EI = 3 (1961–90, 0.5° resolution) to EI = 4 (1961–90 0.1° resolution) and EI = 11 (1998–2007, 25 km resolution).

The Panel also undertook a limited investigation of the underlying CLIMEX calculations in order to reveal the climatic factors affecting the output. The CLIMEX model parameterised for *P. citricarpa* by Yonow et al. (2013) indicated that the northernmost citrus-growing areas of Spain, located in the Ebro region, have a climate that is most suitable for citrus black spot. The grid cells with the highest “Ecoclimatic Index” as shown in Figure 67 indicate that there are two periods of the year, one in spring (week 15–22) and one in autumn (week 37–43), when temperature and moisture conditions occur at the same time to provide suitable climatic requirements according to the CLIMEX parameter set published by Yonow et al. (2013).

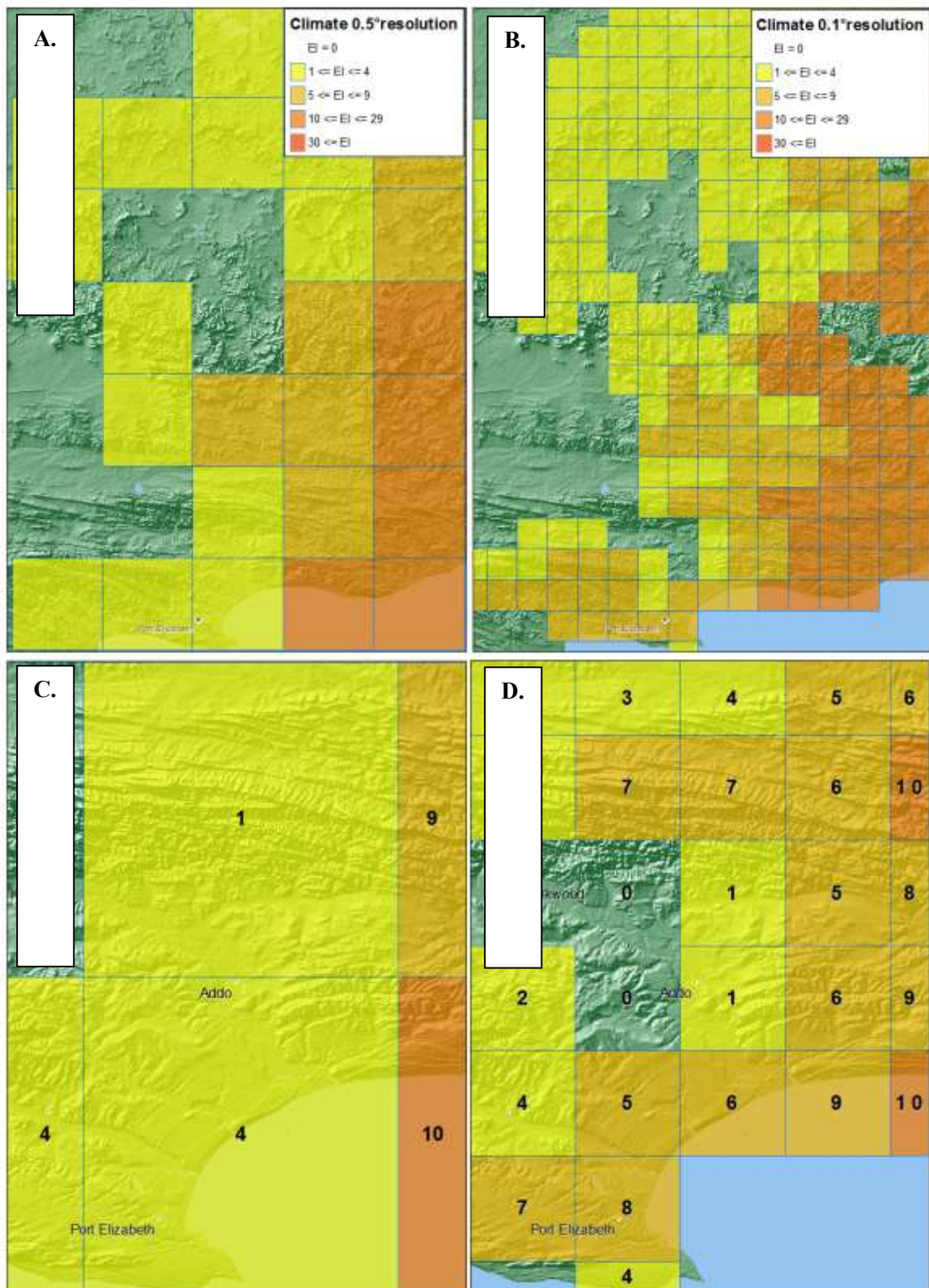


**Figure 67:** Weekly CLIMEX Temperature, Moisture and Growth Index values from the Ebro delta region in eastern Spain for 1961–90 at 0.1° latitude × longitude spatial resolution

The Panel notes that understanding the climatic limitations of *P. citricarpa* is key to determining in which areas the organism can establish (i.e. persist) and it is important therefore to study the marginal areas to obtain these insights. Therefore, the Panel looked in detail at the underlying outputs from the model of Yonow et al. (2013) for a limited number of locations of particular interest in order to understand how the model reacts to climate conditions indicated as marginal both in the EU citrus-growing areas and in the areas indicated as marginal within the known area of occurrence (e.g. Addo in Eastern Cape, South Africa) (Figure 68). The results show that the area around Addo is marginal for CBS, as stated by Yonow et al. (2013). However, when the spatial resolution is increased from 0.5° to 0.1°, the Yonow et al. (2013) model predicts the eastern part of Addo as unsuitable (EI = 0).

As stated in section 3.3.2.2, CLIMEX cannot readily be used to analyse specific periods of the year when the host is at a susceptible stage and inoculum is potentially available. Moreover, it cannot directly take into account the effect of leaf wetness, a critical environmental variable for the successful infection of most fruit and foliage fungal pathogens including *P. citricarpa* (Kotzé, 1963, 1981).





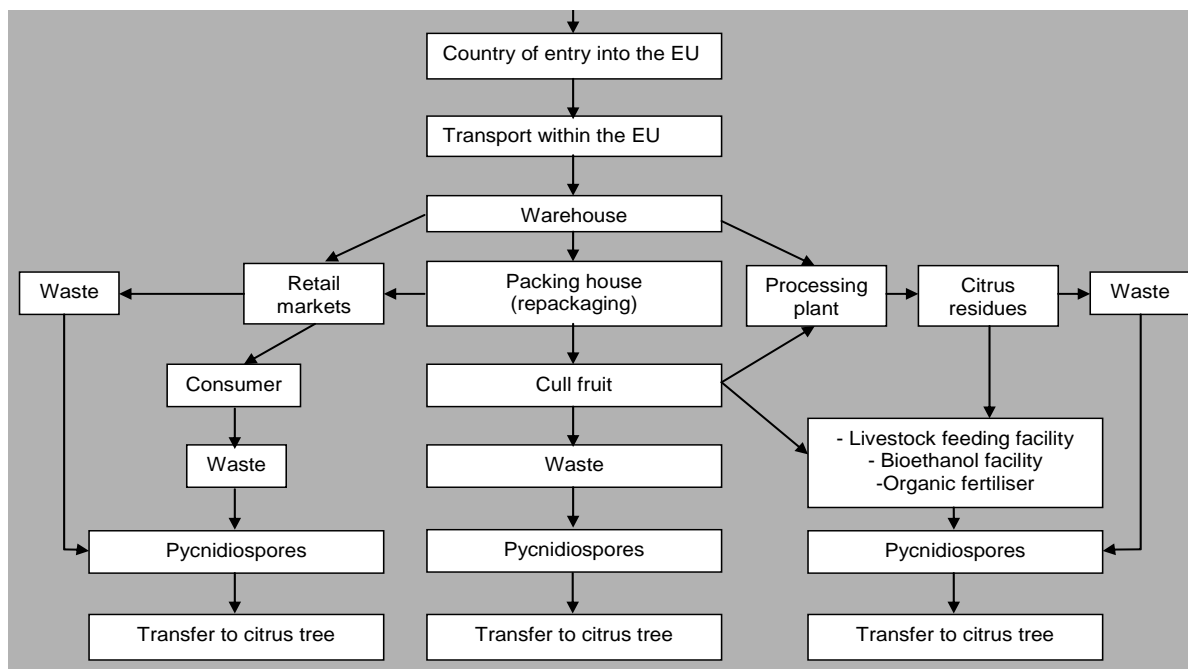
**Figure 68:** Detailed comparison of CLIMEX model output north of Port Elizabeth in South Africa, with two different spatial resolutions in the underlying interpolated climate data (A. and C: 0.5° × 0.5° latitude by longitude; B. and D: 0.1° × 0.1° latitude by longitude)

**Appendix E. Quantitative pathway model for entry of *P. citricarpa* from CBS-affected countries to Spain: pathway citrus fruit without leaves in commercial trade**

*Introduction*

The Panel undertook a simplified quantitative analysis for one of the entry pathways of the pathogen *P. citricarpa* into one country in the risk assessment area, the citrus production regions of Spain, in order to assess the likelihood of entry in more detail for the specific pathway of citrus fruit without leaves in commercial trade. The objective was to quantify the amount of citrus fruit material infected with *P. citricarpa* that is likely to arrive in citrus-growing regions of the EU under different import regulation scenarios and to determine in further detail the steps that are necessary for successful entry to occur. The outputs from this quantitative pathway analysis were compared graphically with the results from models that simulate the conditions conducive to pycnidiospore infection following a rain splash from infected fruit, based on weather conditions. Although the focus of this analysis is mainly on the entry component of the PRA, owing to the inclusion of various scenarios of risk reduction and to the comparison of the pathway model results with infection conditions, this appendix is also relevant to the assessment of establishment and the analysis of risk reduction options. The model was applied to Spain as a case study because this country is the largest citrus producer in the EU and therefore the most relevant country from a risk assessment perspective.

The pathway model follows the conceptual structure of the entry pathway depicted in Figure 8 (repeated here as Figure 69), but only the pathway within the EU has been analysed. The pathway model starts with the entry into the EU of citrus fruit imported from countries where *P. citricarpa* is known to be present. The pathway ends with the final use of the fruit and its disposal as waste, culled fruit or any other citrus by-products within citrus-growing areas where the transfer to susceptible trees can occur if the fruit is disposed of within sufficient proximity to citrus trees.



**Figure 69:** Intra-EU part of the entry pathway model for citrus fruit without leaves in commercial trade. The arrows indicate material flows of citrus fruit. The flow is expressed as the number of fruit on a yearly and a monthly basis

The pathway model addresses the following issues:

1. the amount of imported citrus fruit material infected by *P. citricarpa* entering Spain under different scenarios of phytosanitary regulation
2. the distribution of the imported citrus fruit material infected by *P. citricarpa* to the various trade flows for the different end uses (packing houses, juice industries and retail markets) that can result in the disposal of fruit in a manner that allows the rain-assisted dispersal (ballistic or by wind) of *P. citricarpa* pycnidiospores from the infected fruit to susceptible citrus trees
3. the distribution of the imported citrus fruit infected by *P. citricarpa* into different provinces of Spain.
4. the amount of imported citrus fruit infected by *P. citricarpa* that is disposed of in each province by the various trade flows in a manner that allows the production and dispersal of *P. citricarpa* pycnidiospores
5. the contribution of different trade flows within the citrus fruit entry pathways to the exposure of the citrus-growing areas to infection
6. the temporal association between the disposal of infected fruit waste and the occurrence of weather conditions conducive to the production, dispersal and infection of *P. citricarpa* pycnidiospores in different provinces of Spain throughout the year visualised graphically.

#### *Exposure assessment of the host*

The risk of entry of *P. citricarpa* is mediated by pycnidiospores released from pycnidia on imported infected citrus fruit (Table 5) but also potentially in its waste, e.g. peel (Schutte et al., 2013) and other citrus by-products. Data from phytosanitary inspections at EU borders indicate that pycnidia are observed in most intercepted consignments (J. Meffert, NFCPSA, The Netherlands, personal communication, January 2014; R. McIntosh, FERA, UK, personal communication, January 2014; see also Table 5 and section 3.2.2 of this opinion). If pycnidiospores are dispersed from CBS-affected fruit to a susceptible citrus tree in splashed rain droplets that can be dispersed at longer distances if assisted by wind (Perryman and West, 2014; section 3.2.2.4 of this opinion), infection may occur provided that weather conditions are favourable for pycnidiospore infection. As targets for these pycnidiospores, we consider not only citrus trees in commercial orchards but also trees in parks and private gardens.

There is a potential for transfer of the pathogen to the host if CBS-affected fruit are present at a distance from susceptible citrus trees that is less than the maximum dispersal distance by rain splash or wind dispersal of aerosolised water droplets containing pycnidiospores that have become airborne by rain splash (Perryman and West, 2014). Actual transfer may take place if the presence of CBS-affected fruit within the maximum dispersal distance from the host is combined with conditions that allow spore release and dispersal by rain splash.

The question whether a successful transfer of pathogen propagules to the host (the end point of the entry phase) will result in an infection of the host is discussed in detail in the establishment section of the opinion (section 3.3) and involves the assessment of host susceptibility and separate model calculations based on weather conditions to determine the occurrence/absence of periods conducive to infection by *P. citricarpa* pycnidiospores. *P. citricarpa* ascospores are not known to be formed on CBS-affected fruit and are therefore not relevant for the step from transfer in the entry phase to the first step of the establishment phase.

After completion of the pathway model, the exposure of citrus trees to infection by *P. citricarpa* has been assessed by combining two types of evidence:

1. the quantity of imported citrus fruit infected by *P. citricarpa* disposed of (yearly and monthly) in each province in a manner that can allow the dispersal by rain (ballistic or wind) of *P. citricarpa* pycnidiospores
2. a graphical comparison of the exposure, in terms of the number of infected fruit, and the frequency (monthly and yearly) of the weather conditions enabling rain-assisted splash dispersal of *P. citricarpa* pycnidiospores (i.e. the onset of ballistic or wind dispersal) followed by conditions, simulated by a model, conducive for infection based on weather data.

The results are interpreted by taking into account the area of citrus production in each province, and the potential distance between the disposal site and the susceptible citrus trees in relation to the splash dispersal mechanisms in still air and with wind.

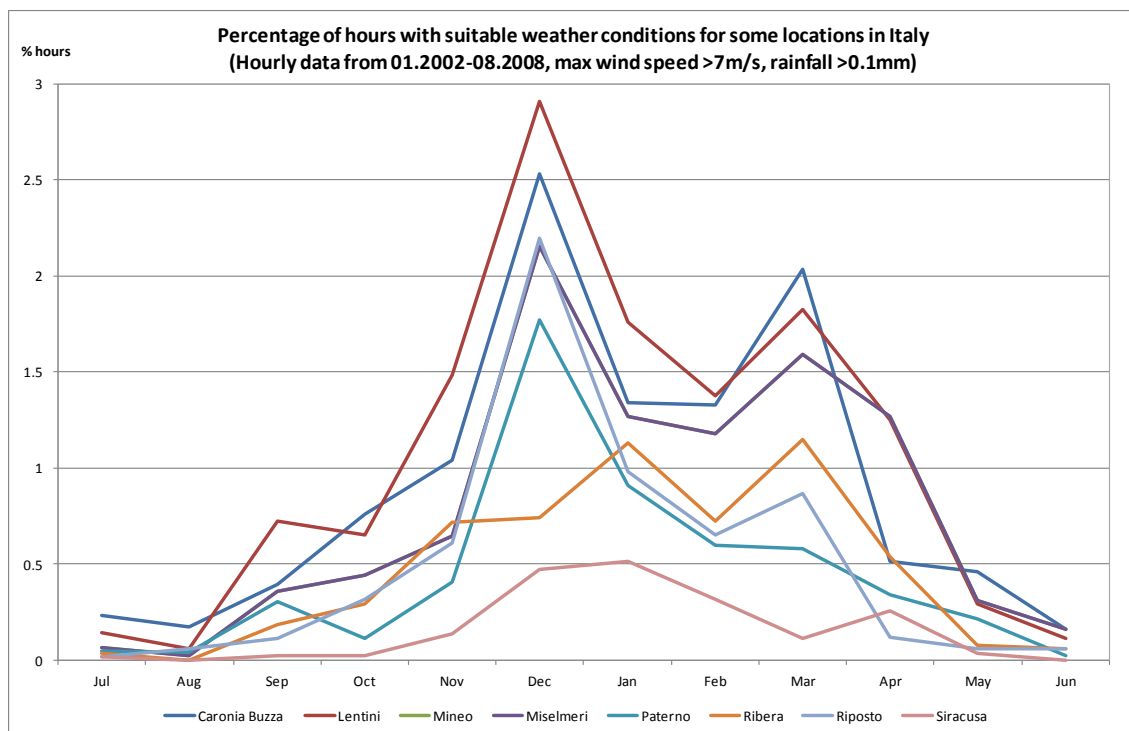
#### *Background and principles of the pathway model*

The pathway model was based on the available data in combination with expert judgement and further justification where appropriate. Assumptions and uncertainties in the model calculations are discussed in the text and taken into account in the model by using minimum and maximum estimates for each parameter in addition to a central estimate. An uncertainty table is presented at the end of this appendix (Table 36), in which the main uncertainties related to the quantitative model and their effects on the model outputs are described and summarised.

The starting point of the pathway model is the volume of citrus fruit directly imported into Spain from CBS-affected countries. The end point of the pathway model is the disposal of the imported fruit or its waste in a manner that can allow the production and dispersal by rain (ballistic in still air or by wind) of *P. citricarpa* pycnidiospores resulting in the exposure of susceptible citrus trees. As indicated in section 3.3.1.1, in the citrus-growing regions of the EU susceptible leaves are present all year round and susceptible fruit are found from May to December. In the case of lemons, susceptible fruit are present all year round.

The Panel considers that, to pose a significant risk, the imported citrus fruit infected by *P. citricarpa* needs to be exposed outdoors to rain or irrigation within the pycnidiospore dispersal distance from susceptible trees. After the spores have been released from the infected fruit by rain splash, the dispersal distance depends on the ballistic mechanism of spore dispersal in still air and on the dispersal in droplet aerosols in windy conditions (see section 3.2.2.4; Perryman and West, 2014). Perryman and West (2014) studied the splash dispersal potential of *P. citricarpa* pycnidiospores from infected sweet orange fruit under controlled conditions. Laboratory experiments showed that fruit misted to simulate light rainfall continue to exude *P. citricarpa* pycnidiospores from pycnidia for at least one hour. In the splash dispersal experiments conducted in still air conditions, 99.4 % of the splashes produced by single incident rain drop on the fruit were of less than 2 mm diameter, with an average of 1–21 pycnidiospores. Larger but less frequent splashes of 4–5.5 mm diameter contained an average of 308 pycnidiospores. In these experiments, the maximum horizontal distance of splash was 70 cm and the maximum height was 47.4 cm. However, when multiple incident rain drops were combined also in still air, splashes were forced higher than occurred in single-drop experiments to over 60 cm. In another experiment combining single incident rain drops and wind, splashes from infected fruit were disseminated up to two metres downwind from the target fruit with a 4 m/s wind speed and up to eight metres at a wind speed of 7 m/s, the highest wind speed evaluated, reaching heights up to 75 cm and even higher as a result of fine droplets becoming aerosolised. These experiments demonstrate that pycnidiospores of *P. citricarpa* can be dispersed from an infected citrus fruit in rain splashes and that, when the rain is combined with

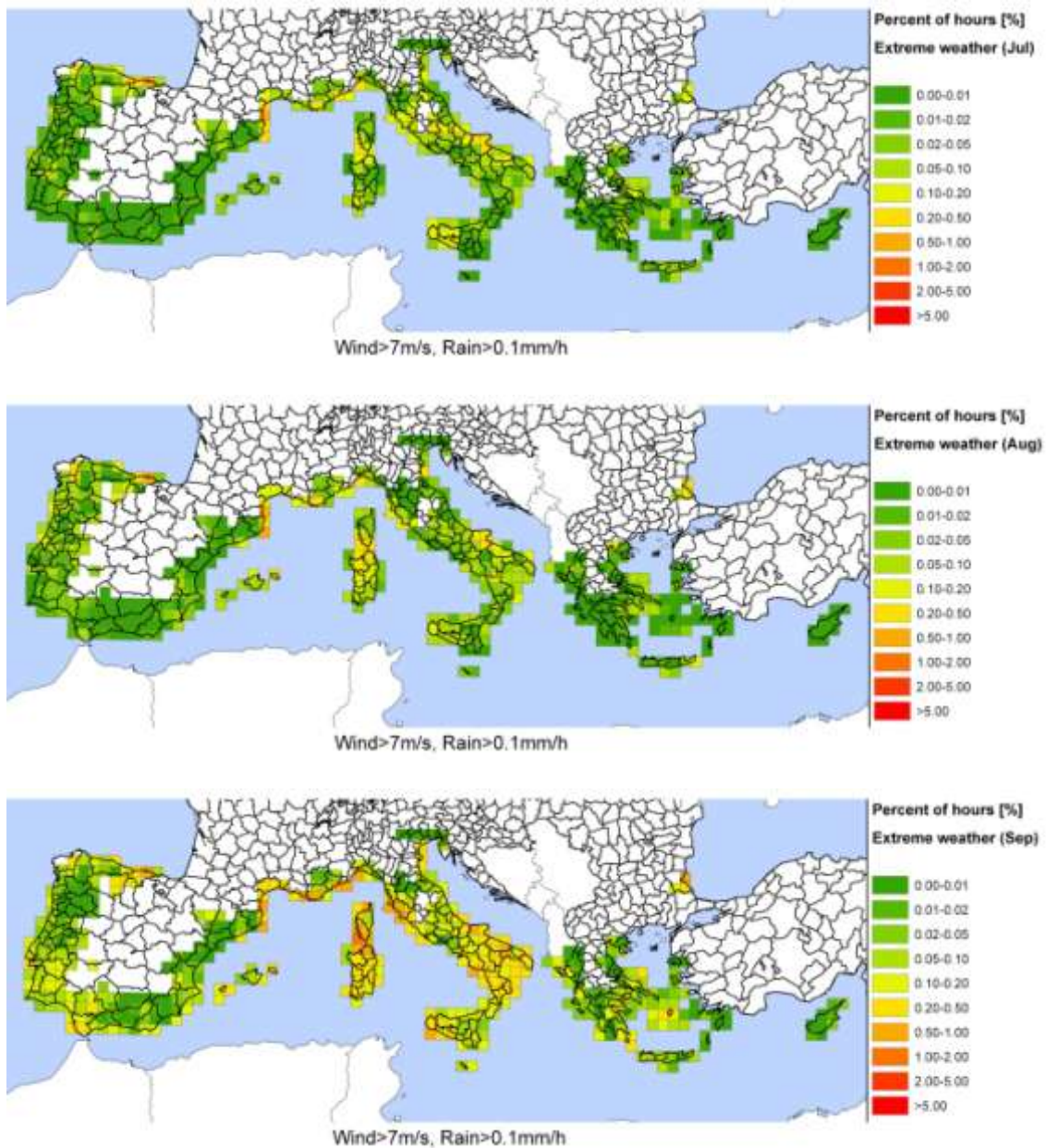
a moderate wind (7 m/s), which is not an unusual meteorological event in southern Europe (Figures 70 and 71; Appendix F), the pathogen can be dispersed at least eight metres downwind from the infected fruit to heights of at least 75 cm. If rain is combined with stronger wind, small aerosolised droplets formed by a rain splash are expected to be dispersed much further (Fitt et al., 1989). Gottwald et al. (2002), studying the dispersal of citrus canker in Florida, found that rain-splashed pathogens can travel several kilometres. Therefore, CBS-affected citrus fruit can provide a source of *P. citricarpa* pycnidiospores with the potential to be splash dispersed to the lower parts of the tree canopy by light rain and sprinkle or micro-sprinkle irrigation and particularly by wind-driven rainfall events.



**Figure 70:** Monthly percentage of hours with wind speed > 7 m/s and rainfall > 0.1 mm in some locations in citrus growing area in Italy based on an average from May 2002 to August 2008

If infected citrus fruit or its waste is disposed of or processed outdoors in regions that have significant citrus cultivation in the presence of weather conditions enabling dispersal of spores (section 3.3.2.5 of this opinion and Figures 70 and 71), the Panel considers that exposure of citrus trees to spores of *P. citricarpa* will depend upon the number of infected citrus fruit or citrus waste placed outdoors, the frequency of suitable weather events and the distance between the infected fruit and the host trees. The likelihood of transfer is greatest if infected citrus fruit are left in the immediate proximity of citrus trees (distance in order of metres), but it is not negligible even at greater distances.

To support the qualitative assessment of this entry pathway, the model described in this appendix aims to quantitatively assess the risk components involved in the pathway processes leading imported infected citrus fruit and waste to outdoor locations in citrus-growing provinces in the EU. The results of the pathway model will be presented together with the results from climate suitability modelling of pycnidiospore infection following a rain event also assists with the qualitative assessment of establishment.



**Figure 71:** Frequencies of rain events with wind > 7 m/s in July to September (monthly average percentage of hours 1998-2007) in citrus growing areas of Europe (grid of 50 x 50 km) (JRC-MARS, online).

*Description of the pathway model*

*Step 1: Importation of citrus fruit from CBS-affected countries into Spain*

The import of citrus fruit from the following CBS-affected countries was considered: Argentina, Australia, Bhutan, Brazil, China, Cuba, Ghana, Indonesia, Kenya, Mozambique, Philippines, South Africa Taiwan, Uganda, the United States, Uruguay, Vanuatu, Zambia and Zimbabwe. Yearly and monthly import data for Spain from these trade partners were extracted from the Eurostat Comext database for the categories 0805 (Citrus fruit, fresh or dried) and 08055090

(Fresh or dried limes “*Citrus aurantifolia*, *Citrus latifolia*”). The total imports were calculated by taking the total citrus imports of all species (category 0805) and subtracting the imports of limes (category 08055090), as all citrus species are considered susceptible to *P. citricarpa* except for Tahiti lime (*C. latifolia*) and sour orange (*C. aurantium*). Since there are no separate import statistical data for *C. latifolia*, the limes category (08055090) as a whole was excluded. Since no statistical data are available for the import into Spain of sour orange, this species could not be distinguished from total citrus imports but is considered to be a species of minor trade relevance.

Although considerable in quantity (see section 3.4.2), intra-EU trade is not considered in this model. *P. citricarpa* could enter Spain via this trade because some EU MSs, particularly the Netherlands and the United Kingdom, import citrus fruit from CBS-affected third countries and then re-export the fruit within the EU. Owing to the free EU internal market, there are no statistical data on the country of production of such fruit that can be used to distinguish production in a CBS-free third country from another EU country. Because of the lack of relevant data, intra-EU trade data are not included in the model calculations. However, it should be kept in mind that there is an extra inflow of potentially infected fruit that is not included in the pathway calculations as presented here. This is further considered in the uncertainty table (Table 36).

Based on the Eurostat data, the Panel took a range of 111 000 to 192 000 tonnes with a central estimate of 142 000 tonnes (Table 37: parameters for the pathway model). These numbers are the minimum, maximum and median of the annual data. Data for the last three years were all close to the minimum as a result of the economic recession, but the fact that they are close together suggests that further reduction is unlikely and that imports may return to normal variation as the economy recovers.

#### *Step 2: Incidence of CBS on imported citrus fruit and the effects of border inspection*

Different scenarios are defined to investigate the effect of the incidence of CBS in the imported fruit on the amount of infected fruit disposed of in citrus-growing regions and of the effect of control measures and phytosanitary inspections. Five scenarios have been considered:

1. Current regulations are effective and imported citrus is free from symptoms of CBS. This scenario is trivial as it constitutes no risk as no CBS-infected fruit enters. The Panel considers that a minimal proportion of fruit may carry asymptomatic latent infections that were unnoticed during inspections at the country of origin. These latent infections may develop symptoms during overseas transport (Er et al., 2013) that should be detected during phytosanitary inspections at the point of entry in the EU. However, the duration of the intra-EU transport is probably too short for a significant development of new lesions, so the exposure of citrus trees to incoming inoculum under this scenario is considered to be nil. There is, however, uncertainty as symptoms may develop after cold storage (Er et al., 2013; see section 3.2.2.2) and therefore also after import.
2. Current regulations are partially effective and imported citrus has a low incidence of CBS. Two lines of reasoning were used to derive the likely level of infection with CBS of citrus fruit under current regulations. In the first line of reasoning, the level of infection of fruit admitted for import was calculated by accounting for the effects of culling diseased fruit in the country of origin and of rejecting bad lots by phytosanitary inspection at the point of entry in the EU, using information from the Panel’s meta-analysis (section 3.6.1.1) and data from commercial citrus production provided by Fisher et al. (2008) to estimate the proportion of diseased fruit after harvest under the most effective fungicide programmes. In the second line of reasoning, an estimate of the proportion of infected fruit was made using sampling theory in combination with results

of phytosanitary inspections by the Netherlands Food and Ware Authority. The two methods yielded similar estimates of the proportion of infected fruit under current regulations. Details of the derivation of parameters are given below.

3. There are no regulations and imported citrus has a low incidence of CBS. This may occur in CBS-affected areas with the most effective fungicide spray programmes. For this scenario, the Panel again estimates 2 % of fruit to be infected after effective treatment based on the meta-analysis conducted by the Panel (section 3.6.1 of the opinion) and the data from Fisher et al. (2008), with a range of 0.6–7 % (95 % confidence interval from meta-analysis). However, in contrast to scenario 2, it is assumed that the fruit will not be subjected to CBS-specific inspections, as they are not compulsory in the absence of current phytosanitary regulations and represent an added cost.
4. There are no regulations and imported citrus has a medium incidence of CBS. This may occur in CBS-affected areas with the most affordable, but less effective, fungicide spray programmes. In this scenario, the Panel estimates that 16 % of fruit will be infected after less effective treatment, based on the meta-analysis and the data from Fisher et al. (2008), with a range of 7–32 % (95 % confidence interval from the meta-analysis). As in scenario 3, it is assumed that the fruit will not be subjected to phytosanitary inspections for CBS, as it is unlikely that these will continue in the absence of current regulations owing to the costs involved.
5. No regulation and imported citrus has a high incidence of CBS. This may occur in CBS-affected growing areas in the absence of fungicide sprays. Estimates of the percentage of CBS-affected fruit when there is no treatment range between 46 % and 98 % for different countries based on the meta-analysis and the data from Fisher et al. (2008). The Panel took the midpoint of this range, 72 %, as a simple central estimate for modelling.

#### *Estimation of the proportion of infected fruit at import in scenario 2*

The proportion of infected fruit in imported consignments in scenario 2 was estimated in two ways enabling the results of the two different methods to be used for corroboration and to reduce uncertainty. In the first line of reasoning, the level of infection of fruit admitted for import was calculated by accounting for the effects of culling diseased fruit in the country of origin and of rejecting bad lots at import inspection. The Panel estimates that 2 % of fruit will still be affected by CBS even after the most effective fungicide programmes based on the results of the meta-analysis of fungicide control trials (section 3.6.1) and data from commercial citrus production indicated by Fisher et al. (2008), with a range of 0.6–7 % (95 % confidence interval from meta-analysis). The fruit are then subjected to two stages of phytosanitary inspection, first in the country of origin and, secondly, at the EU border, both following ISPM standards. The commercial incentive for the efficient culling of infected fruit in the country of origin due to the cost of rejected consignments was also taken into account.

A first rough calculation would suggest that culling in the country of origin could attain an efficiency of 99 %, reducing the proportion of infected fruit by a factor of 100, i.e. bringing it down from a medium estimate of 2 % infection in the harvested fruit to 0.02 % in the fruit that is exported. Furthermore, phytosanitary inspection in the country of destination has a nominal likelihood of identifying infected consignments of 95 % under the assumption of random sampling, a sample size of 500 fruit, and a proportion of infestation of 0.6 %. The efficiency of import inspection was used to derive a first tentative estimate of the effect of import inspection on the level of infection in fruit imports. It was assumed that inspection reduces the level of infection in fruit imports by a factor of 20. The assumption that import inspection lowers the proportion of infected fruit in consignments allowed into the EU territory as compared with the



level of infection in consignments arriving at the point of entry in the EU, implies the unverifiable assumption (for lack of data) that there is considerable variability in the infection level between consignments. The resulting medium estimate of the proportion of infected fruit in the citrus flow that enters the territory is 0.001 %, i.e. one infected fruit in 100 000. The associated range, based on the meta-analysis, is 0.0003– 0.0035 %.

Further work was done to corroborate these estimates on the basis of inspection records from the Netherlands Food and Ware Authority. No data are collected on the proportion of infected fruit at import. This proportion needs to be estimated from theoretical considerations of the sampling methods used for inspection and from data on the results of import inspections. The Panel considered that the analysis of results of actual inspections would provide useful information and inform the assessment of the proportion of infected fruit in imported consignments.

Regulation requires pest freedom. It is therefore expected that the proportion of infected fruit, if present at all in a consignment, is very low. Under the assumption of random sampling, the probability of rejection of a consignment is related to the sample size and the proportion of infected fruit via the binomial distribution:

$$P(0)$$

(EFSA, 2012), where  $P(0)$  is the probability of finding zero infected fruit in a sample,  $p$  is the proportion of infected fruit in the consignment and  $N$  is the sample size. When many consignments with an identical proportion of infected fruit are inspected, the proportion of rejected consignments  $f$  is expected to be equal to  $f = 1 - P(0)$ . The proportion of infected fruit can then be estimated from:

$$p$$

It is useful to derive an intuitive formula by using the Poisson approximation to the binomial distribution:

$$f = 1 - \left(1 - p\right)^N = 1 - e^{-\lambda} \approx \lambda = pN \quad (1)$$

where  $\lambda = pN$  is the expected number of infected fruit in the sample. Equation 1 shows that the proportion of rejected consignments is a good approximation and equal to the expected (fractional) number of infected fruit in the sample,  $\lambda = pN$ , provided  $\lambda \ll 1$ . Thus, the proportion of infected fruit  $p$  can be estimated by dividing the proportion of rejected consignments  $f$  by the sample size  $N$ .

An important but untested assumption underlying this calculation is that the proportion of infested fruit in imported consignments is constant, i.e. equal across all of the imported consignments. While an equal proportion of infected fruit across consignments is highly unlikely, the assumption of similar proportion of infected fruit across consignments may be reasonable, considering that quality control in producing countries should be at a high level, and consistently executed, to render the fruit suitable for importation into the EU territory and avoid the risk of rejection. The Panel has considered modelling the variability of the proportion of infected fruit across consignments, e.g. using the beta-binomial distribution (Venette et al., 2002; Anonymous, 2011), but has not taken this further in the present opinion because there are

no data to parameterise this distribution, and the binomial distribution (assuming a fixed proportion of infection,  $p$ ) has wide acceptance in analysing the efficiency of inspection sampling (ISPM 31) despite its limitations.

The sample size is 100 kg for large citrus consignments, approximately equivalent to 500 fruit (but, depending on their weight, this can range from 300 to 1 000 fruit), under current import inspection practices of the Netherlands Food and Ware Authority. Table 32 lists the minimal weight that must be inspected according to the weight of the consignment, while Table 33 provides provisional numbers of fruit calculated from the minimal weight to be inspected, assuming an individual fruit weight of 200 g. (Scenario studies with the pathway model used different fruit weights to assess their effect on the number of imported infected fruit.) In addition, Table 33 provides an upper confidence limit for the true proportion of infected fruit in the consignment if no infected fruit were found in the sample. The upper limit is calculated as the value of  $p$  giving a 5 % chance of zero infected fruit in the sample when using the binomial distribution (EFSA, 2012).

**Table 32:** Relationship between the weight of the citrus consignment and the sample size required to check for the absence of citrus black spot, caused by *Phyllosticta citricarpa* <sup>(a)</sup>

| Weight of consignment (kg) | Minimal weight inspected (kg) |
|----------------------------|-------------------------------|
| ≤ 200                      | 10                            |
| 201–500                    | 20                            |
| 501–1 000                  | 30                            |
| 1 001–5 000                | 60                            |
| > 5 000                    | 100                           |

(a): Personal communication from the Netherlands Food and Ware Authority.

**Table 33:** Approximate number of fruit inspected for different consignment sizes and calculated upper confidence limits of the true probability of infestation if no infected fruit were found in the sample

| Weight of consignment (kg) | No of fruit inspected | $p_{\text{upper}}$ (%) |
|----------------------------|-----------------------|------------------------|
| < 200                      | 50                    | 5.82                   |
| 201–500                    | 100                   | 2.95                   |
| 501–1 000                  | 150                   | 1.98                   |
| 1 001–5 000                | 300                   | 0.99                   |
| > 5 000                    | 500                   | 0.60                   |

The estimate of the proportion of infected fruit in imported consignments is based on the inspection records of the Netherlands, as the Netherlands keeps detailed records of inspections. Moreover, the Netherlands contributed substantially to the total citrus import into the EU 2002–2011 from CBS-affected countries (37.1 %) and the inspections are carried out according to a well-defined protocol, as reported in Tables 32 and 32. The recent Netherlands database on citrus imports (all citrus) shows that 57 128 lots were imported from 2 January 2012 until 8 November 2013, and 40 460 lots were from CBS-affected countries. These lots came from the following CBS-affected exporting countries: South Africa (21 035), Argentina (6 579), China (4 360), Brazil (4 042), Uruguay (2 236), the USA (1 578), Zimbabwe (526), Australia (87), Mozambique (16) and Indonesia (1). The 40 460 imported lots from CBS-affected countries consisted of the categories listed in Table 34.

The majority of consignments consisted of a single lot. Considering that, according to current knowledge, fruit of Tahiti lime (*C. latifolia*) do not produce pycnidiospores and that the Eurostat category for limes for the import trade data used in this model includes both *C. latifolia*

and *C. aurantifolia*, in the estimation of the proportion of infected fruit at import in scenario 2, the Panel excluded from consideration the number of lots of *C. latifolia* (3 499 lots) and *C. aurantifolia* (232 lots), bringing the number of lots included down to 36 729.

**Table 34:** Number of imported lots by citrus species into the Netherlands from 2 January 2012 until 8 November 2013 (Dirk Jan van der Gaag, Netherlands Food and Ware Authority, personal communication, 12 January 2014)

| Citrus species         | No of lots |
|------------------------|------------|
| <i>Citrus</i> sp.      | 39         |
| <i>C. amblycarpa</i>   | 1          |
| <i>C. aurantifolia</i> | 232        |
| <i>C. aurantium</i>    | 0          |
| <i>C. australasica</i> | 78         |
| <i>C. deliciosa</i>    | 0          |
| <i>C. hystrix</i>      | 2          |
| <i>C. latifolia</i>    | 3 499      |
| <i>C. limettioides</i> | 6          |
| <i>C. limon</i>        | 6 106      |
| <i>C. madurensis</i>   | 2          |
| <i>C. maxima</i>       | 4 369      |
| <i>C. medica</i>       | 0          |
| <i>C. reticulata</i>   | 4334       |
| <i>C. sinensis</i>     | 16 210     |
| <i>C. unshiu</i>       | 372        |
| <i>C. paradisi</i>     | 5 210      |

The 36 729 lots of all citrus except limes (*C. latifolia* and *C. aurantifolia*) imported from CBS-affected countries were aggregated into 32 518 consignments with an average consignment size of 31.4 tonnes and a sample size of 500 fruit (Table 1). The total volume of citrus except limes imported from CBS-affected countries was 1 021 679 tonnes. There were 100 confirmed interceptions of *P. citricarpa* in the period 29 May 2012 to 21 November 2013: 80 in *C. sinensis*, 16 in *C. limon*, two in *C. reticulata* and two in *C. paradisi*. Overall, there were 32 518 consignments of all citrus except lime imported in 675 days and 100 rejections due to the presence of *P. citricarpa* in 541 days, corresponding to an import of  $32\,518/675 = 48.2$  consignments per day and a rejection rate of  $100/541 = 0.185$  rejections per day. Under the assumption that the level of infection of consignments with *P. citricarpa* does not vary over time, the proportion of consignments infested by *P. citricarpa* is estimated at  $(100/541)/(32\,518/675) = 0.185/48.2 = 0.003837$ .

According to equation 1, 0.003837 is the expected number of infected fruit in a sample of 500. The estimated proportion of infected fruit is thus  $0.003837/500 = 7.67$  infected fruit per million fruit.

To account for uncertainties in the data and the calculation method, the Panel scaled this calculated proportion by a factor 3.5 up or down, giving three values of the proportion of fruit infected by *P. citricarpa* in imported consignments in scenario two:  $2.19 \times 10^{-6}$  (lower bound),  $7.67 \times 10^{-6}$  (most likely value), and  $26.9 \times 10^{-6}$  (upper bound).

These estimates are very similar to those based on the meta-analysis and assumptions on the efficiency of culling infected fruit in the country of origin and removing badly infected consignments at the EU border. The proportions of infestation resulting from the data analysis are based on observations from actual inspection practice and are therefore considered to be more trustworthy. These are therefore used in the pathway calculations.

*Step 3: Allocation of fruit to three main pathways: packing houses, retail and food processing industries*

Fruit is imported mostly by boat, and transported by road in trucks to packing houses, distribution centres for retail and food processing industries. The fruit is transported under protected and refrigerated conditions, and the risk of dissemination of pycnidiospores by rain splash is considered to be nil. Furthermore, no significant waste is produced during this step unless occasional traffic accidents are considered. Based on a personal communication from a citrus expert (M.A. Forner, IVIA), the proportion going to packing houses is estimated at 40 % (plausible range 30–50 %), to retail is 40 % (plausible range 30–50 %), and to food processing (predominantly juice making) 20 % (plausible range 15–25 %).

*Step 4: Packing house pathway*

Packing houses receive fruit and repack it before forwarding it to distribution centres for retail. Packing houses will process fruit mainly to ensure that the fruit that reaches local markets fulfils current European quality regulations and even the more stringent quality criteria established by retail companies. All packing houses in Spain are located in the citrus-growing areas because they are associated with local fruit production (Table 38). Consequently, CBS-affected fruit going to packing houses is in close proximity to the citrus orchards, with distances between the waste and the nearest citrus trees often in the order of metres.

The Panel assumed that fruit sent to packing houses is distributed between regions in proportion to the number of packing houses per region, based on regional and central government records for 2013 (M. Pastor Pichardo, Ministerio de Agricultura, Alimentación y Medio Ambiente, Spain, personal communication, January 2014). This distribution reflects the situation in which the average turnover of packing houses is the same across all regions. The data on the number of packing houses are considered to be accurate because registration is required by law (Directive 93/50/EEC) and illegal non-registered packing houses will be detected and regulated or otherwise closed. Although not all packing houses are currently registered with the authorities for the direct import of citrus fruit from third countries, all packing houses are able to work with fruit imported by another authorised Spanish importer or with fruit imported via another EU country, which may be originally imported from a third country. Ideally, account would be taken of the size of the packing houses (i.e. volume of fruit packed), which might vary between packing houses by a factor of 10, but this information was not available and therefore needs to be considered as an unquantified source of uncertainty affecting the model.

Packing houses purchase fruit, at the quality standard they require, during the season and then apply further checks during the packing process. Packing houses produce waste, but they select not specifically for spots on the peel, such as those produced by *P. citricarpa*, but for major blemishes and bruises. Data on the proportion of fruit going to waste are available from FAO and WRAP (Gustavson et al., 2011; WRAP, 2011, 2012) and indicate a waste fraction of 3 % in the grading process followed by a further 0.1–0.5 % in the packing process, with the total loss being quoted as up to 4 %. If the upper bound of 4 % is assumed to include upper bound loss in the packing process, this leaves 3.5 % as an upper bound for the grading process (assuming that deviations in the grading and packing wastes are positively correlated). Arbitrarily assuming an equivalent lower bound gives a range of 2.5–3.5 % for waste in grading, which in turn implies a range of 2.6 % (2.5 + 0.1 %) to 4 % (WRAP, 2011 and 2012; Gustavsson et al., 2011) for the overall waste. This range is used for modelling, with the midpoint of 3.3 % as a central estimate.

However, these data have been produced based on a UK study; thus, there is uncertainty over their extrapolation to other countries, leading to a possible underestimation of the waste volumes.

The waste from packing houses is usually mixed with rotten fruit so it cannot be used for juicing. Instead, it is stored in open containers, generally under cover, until full and then spread outdoors in open-air facilities for solar drying (Kimball, 1991; Caparra et al., 2007). Subsequently, it may be used to feed livestock or to produce compost. Resting structures of some ascomycetes are able to survive in the digestive tract of animals, and ascomycetes are common in animal dung (Melo et al., 2011) but it is not known whether *P. citricarpa* can survive passage through the digestive tract of farm animals. There is no information on the survival of *P. citricarpa* during composting. Consequently, the Panel has not assigned risk values to the potential exposure pathways through manure and compost, but takes these into consideration when drawing overall conclusions as it is not possible to exclude such risks altogether. However, there is positive evidence for an opportunity for exposure through the packing house pathway when the fruit waste is left outdoors for solar drying. During exposure of fruit waste in the open air, pycnidiospores may be dispersed by rain splash and further transported in aerosols with wind (Perryman and West, 2014), and spread to nearby citrus trees. Therefore, the volume of CBS-affected fruit exposed outdoors for solar drying is estimated by multiplying the number of units assigned to packing houses in each region by the fraction of waste they produce.

#### *Step 5: Retail pathway*

The Panel assumed that fruit sent directly to retail (not via packing houses) is distributed between regions in proportion to their populations, based on official population statistics for Spain (INE, 2014). Data on the proportion of fruit going to waste in retail is available from FAO and WRAP and indicate a waste fraction of 2.25 % for three suppliers accounting for 60 % of market share in the United Kingdom.

The waste will be processed as organic residue either brought to landfill or collected separately and processed together with other waste for production of compost in the open air, but the Panel has no quantitative information on the relative proportion of waste going to landfill or used for composting. During landfill, citrus may be exposed to open air, allowing rain splash dispersal by air, resulting in exposure of susceptible trees. Furthermore, scavenging animals at landfills, notably birds such as seagulls, consume waste at landfill sites (Belant et al., 1995) and could theoretically contribute to dispersal of pathogens in the waste. However, the Panel has no information on the dispersal of *P. citricarpa* inoculum by birds and has not considered it further in the calculations.

Considering the waste used for composting, not all of the facilities have a rain cover and, without such cover, exposed fruit would be exposed to rain, thus providing an opportunity for pycnidiospore dispersal by rain splash and transfer to susceptible trees. The Panel has no data on the fraction of retail waste that is exposed to rain. To explore the impact of this source of uncertainty, the Panel uses 5 % as the central estimate and 0.5–50 % as the range. The volume of CBS-affected fruit reaching this end point is estimated by applying these fractions to the number of units assigned to retail in each region.

#### *Step 6: Consumer pathway*

Consumers buying citrus fruit from retail will produce organic waste by disposing of the fruit that has gone bad before consumption and the peel. Although much of the pulp is consumed, only a very small proportion of peel will be consumed (e.g. in homemade marmalade or after grating) and consumers are likely to avoid using visibly affected peel. Therefore, to focus the model on the relevant part of the fruit (the peel), it is assumed that 100 % of the units purchased will eventually be discarded as waste.

Citrus waste from the consumer will be processed as organic residue and disposed in a similar way to the citrus waste from retail, i.e. to landfill or for composting. Similarly to the retail pathway, the Panel has no data on the fraction of consumer waste that is exposed to rain. To explore the impact of this source of uncertainty, the Panel used 5 % as the central estimate and 0.5–50 % as the range.

#### *Step 7: Food industry pathway*

The Panel assumed that fruit used by the food industry is distributed in proportion to the number of citrus processing plants in each region. The food industry obtains imported fruit directly from warehouses, mainly to make juice but also to make other products, such as marmalade. Fruit is covered during transport, and there is no significant risk of spore release during storage or transport before processing. After pressing the fruit for production of juice, all of the remainder is sent to the open-air facilities for solar drying and is later used for livestock feeding or bioethanol production. The Panel assumes in its model that peel from 100 % of the units entering the food industry is disposed of in this way.

At the open-air drying facilities, the fruit from the food industry is usually mixed with the culled fruit and waste from the packing houses in order to standardise the residue for animal feed purposes. The drying facilities are in the citrus-producing areas, with susceptible trees in close proximity. The waste is placed in a thin layer which is ploughed mechanically to dry the material quickly without rotting, so it can be assumed that all units of fruit are exposed to air (although some part of each unit will face downwards). They are not covered, and hence there is opportunity for dispersal of pycnidiospores after rain splash. Waste from the packing houses and from food processing is placed in the same locations at the same proximity to citrus orchards. However, the Panel decided to consider them separately because there are some mitigation options that apply only to the juicing industry. As a result of this, risk managers can understand what part of the risk is mitigated.

#### *Quantifying uncertainty in the pathway model*

Uncertainty was quantified by using ranges, as well as central estimates, for some of the parameters in the pathway model, as specified in the preceding sections. To examine the impact of these uncertainties on the results, the calculations were repeated three times: once to give a central estimate (using the central estimate for all parameters), once to give a worst case estimate (using lower or upper bounds according to their impact on the result) and once to give a best case estimate. Other uncertainties (Table 36) were not quantified and need instead to be taken into account when interpreting the quantitative results.

#### *Per cent of time with conditions suitable for transfer and pycnidiospores infection*

The risk of infection depends not only on the volume of infected fruit coming into proximity with citrus production areas, as assessed with the pathway model, but also on the suitability of the local conditions for transfer of the pathogen to citrus trees and subsequent infection. The generic infection model by Magarey et al. (2005) was used to estimate the percentage of time with conditions suitable for infection by pycnidiospores. This model requires estimates of the three cardinal temperatures ( $T_{max}$ ,  $T_{min}$ ,  $T_{opt}$ ), of two wetness duration thresholds ( $W_{max}$ ,  $W_{min}$ ) and of a parameter describing tolerance to dry interruptions ( $D_{50}$ ). It computes the leaf surface wetness duration requirement for infection. This model does not define a relationship between the predicted number of infection events and the incidence/severity of the disease. The parameter values were estimated by EFSA (2008; Table 3, page 36) based on published experiments on pycnidiospore germination. The requirement for transfer starting with rain splash was integrated in the model by considering only those potential infections starting with a rain event. This model was run by EFSA (2008) with climatic data interpolated to a 50-km grid for the EU citrus growing areas with simulated wetness data (Bregaglio et al., 2010, 2011). The model was also applied to climatic datasets from locations where CBS is present as well as

extra-EU locations where it is not known to occur. Here (EFSA PLH Panel, 2014), the climatic suitability for *P. citricarpa* infection in EU citrus-growing areas was updated using the new 25-km grid of interpolated climatic data.

Four estimates of the percentage of time suitable for transfer and infection by *P. citricarpa* pycnidiospores were generated for each 25-km grid square, representing different combinations of parameter values from the Magarey et al. (2005) model. The average value of proportion of suitable time for transfer and infection of the 25-km squares in each province was considered as the central estimate using the most likely parameter values defined by EFSA (2008). A range for each province was used to take account of the uncertainty regarding the different parameter values, and uncertainties regarding the locations of pathway end points (packing houses, drying facilities, etc.) within each province. These ranges were obtained by taking the minimum and maximum estimates from all combinations of parameter values and grid cells within each province.

Both environmental suitability and the volume of CBS-affected fruit reaching pathway end points vary seasonally, in different ways, and the level of risk will depend on the extent to which they co-occur in time. To examine this, monthly estimates of the proportion of suitable time for transfer and infection were also calculated for those province(s) which showed a high level of risk.

#### *Pathway model results and assessment of scenarios*

The risk of transfer followed by infection is a function of the volume of CBS-affected fruit coming into proximity with citrus trees and the suitability of the local conditions for transfer of the pathogen to citrus trees and subsequent infection. These two factors were quantified by pathway modelling and climate modelling for different Spanish provinces under the five contrasting scenarios for regulation, as described in the preceding sections.

On a yearly basis, the results are presented in Figures 72 to 75. Within each scenario, results are presented in separate figures for each pathway end point (packing house waste, retail waste, consumer waste and food industry waste). In each figure, the horizontal axis represents the percentage of time for which environmental conditions are suitable for transfer and infection, while the vertical axis represents the volume of CBS-affected fruit (plotted as log number of units, i.e. 1 = 10 units, 2 = 100 units, etc.). Each of the plotted points represents a single province and is plotted as a cross. The centre of the cross shows the central estimate on each axis, while the vertical and horizontal lines of the cross show the uncertainty of the estimate on each axis (quantified as explained above). The NUTS3 code identifying each province is plotted at the centre of each cross and a key to these codes is provided in Table 33.

Fewer points (provinces) are plotted in the figures for packing houses and food industry because packing houses and food processing plants are not present in the unplotted provinces, so no waste from the corresponding pathway is present. Within each graph, points towards the top-right corner (high volume of CBS-affected fruit, high percentage time with suitable conditions for transfer and infection) have higher risk than those towards the bottom left. However, it must be borne in mind that risk is additionally influenced by factors that are not plotted on the graphs including, most importantly, the proximity of the waste to citrus trees. This will generally be higher for the packing house waste, because packing houses are generally situated on the same property as citrus orchards, with trees often within a few metres from the packing house. Waste from consumers, retail and the food industry is exposed in facilities that are not necessarily close to citrus orchards; in these cases, the chance of citrus trees being present nearby might be expected to be higher in those provinces with larger densities of citrus orchard such as Valencia, Murcia, Alicante and Castellón.

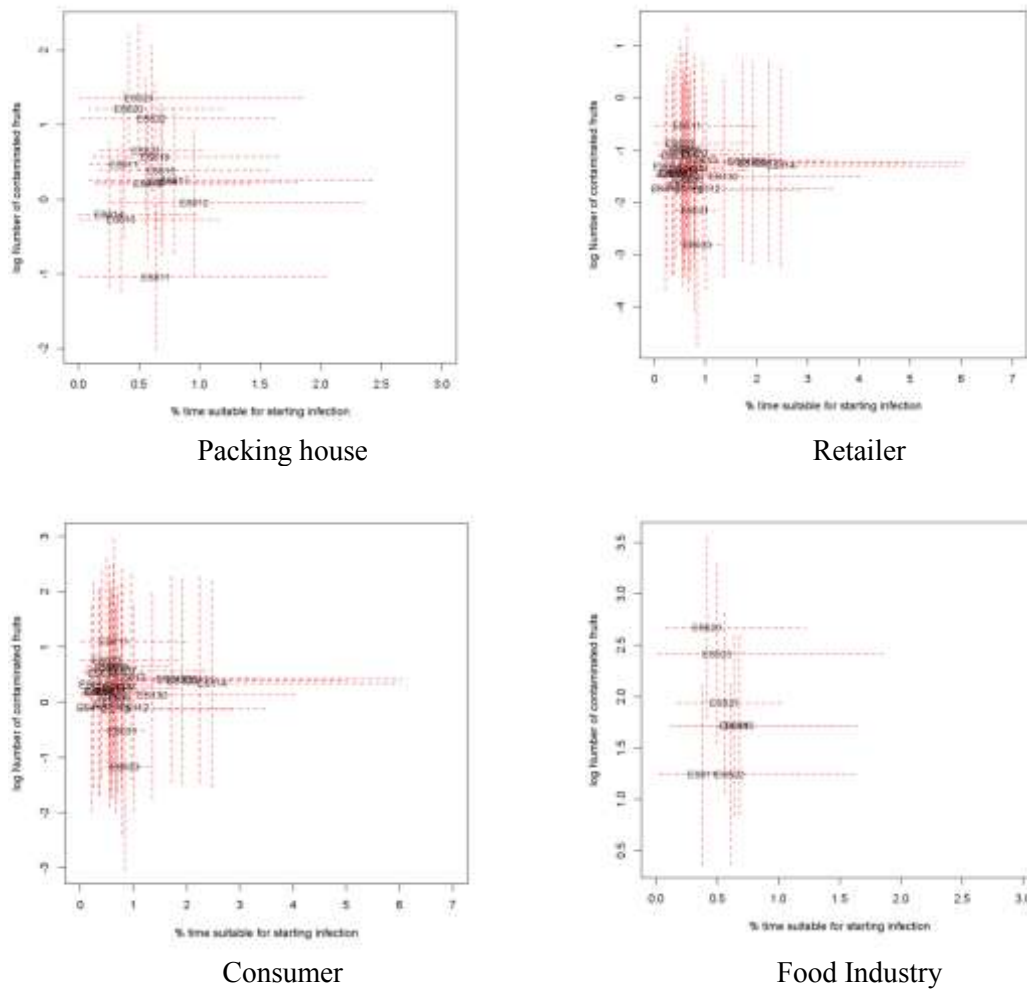
**Table 35:** The list of codes employed for each of the Spanish provinces included in the pathway model

| Code  | Province   | Code  | Province             |
|-------|------------|-------|----------------------|
| ES111 | A Coruña   | ES523 | Valencia             |
| ES112 | Lugo       | ES531 | Eivissa Y Formentera |
| ES113 | Ourense    | ES532 | Mallorca             |
| ES114 | Pontevedra | ES533 | Menorca              |
| ES120 | Asturias   | ES611 | Almería              |
| ES130 | Cantabria  | ES612 | Cádiz                |
| ES213 | Vizcaya    | ES613 | Córdoba              |
| ES415 | Salamanca  | ES614 | Granada              |
| ES431 | Badajoz    | ES615 | Huelva               |
| ES432 | Cáceres    | ES616 | Jaén                 |
| ES511 | Barcelona  | ES617 | Málaga               |
| ES514 | Tarragona  | ES618 | Sevilla              |
| ES521 | Alicante   | ES620 | Murcia               |
| ES522 | Castellón  |       |                      |

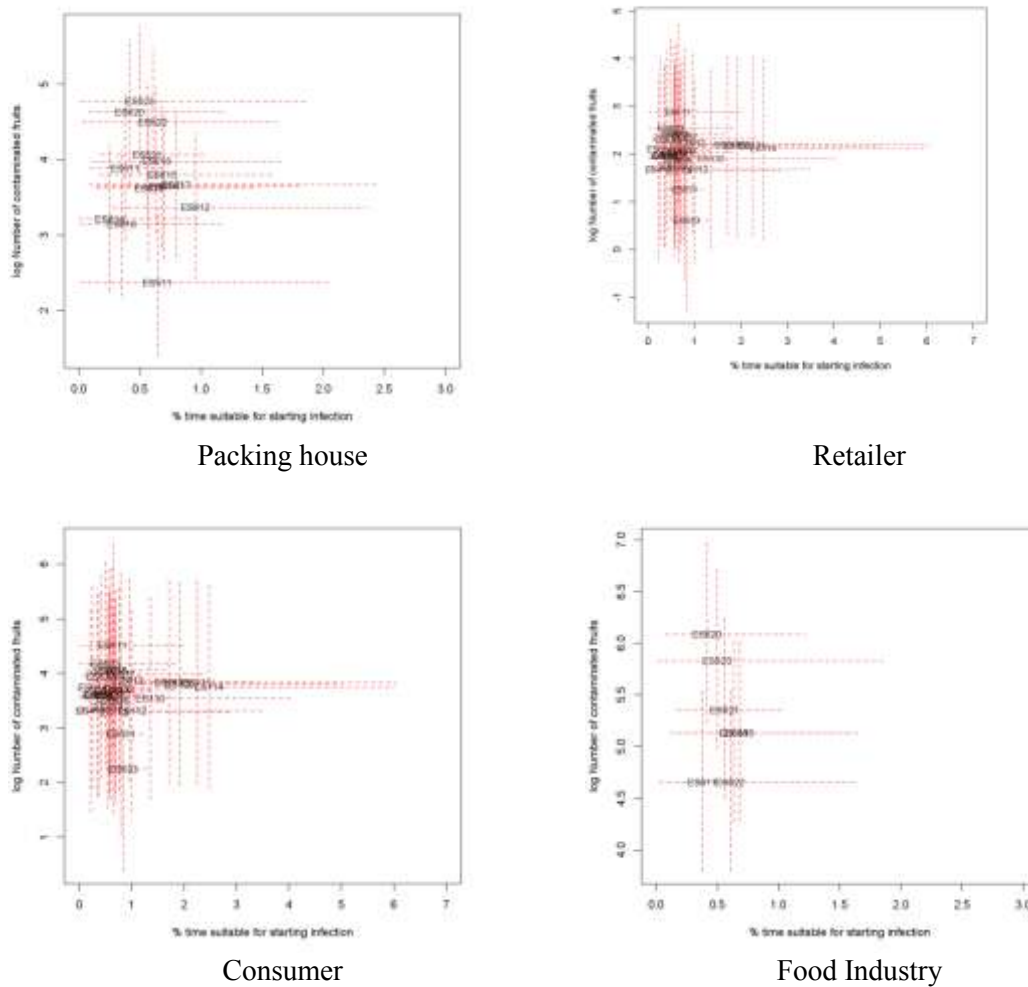
In all scenarios, the largest amount of fruit waste exposed to the air belongs to the food industry pathway, with values of 10–400 fruit for scenario 2 up to 1 000 000 to 50 000 000 under scenario 5 (Figures 72 and 75). Packing house fruit waste is more important than fruit waste from consumers and retailers. The amount of fruit waste from the packing house varied from 0 to 10 fruit under scenario 2 and from up to 100 000 to 1 000 000 fruit under scenario 5.

It is also important to note that the results of the pathway model show that under scenario 3 there is a dramatic increase in the number of fruit exposed to the air when compared with scenario 2. The time suitable for starting infection varies depending on the citrus waste pathway, being higher with fruit from retailers and consumers and lower for packing houses and food industry waste (Figures 72 to 75).

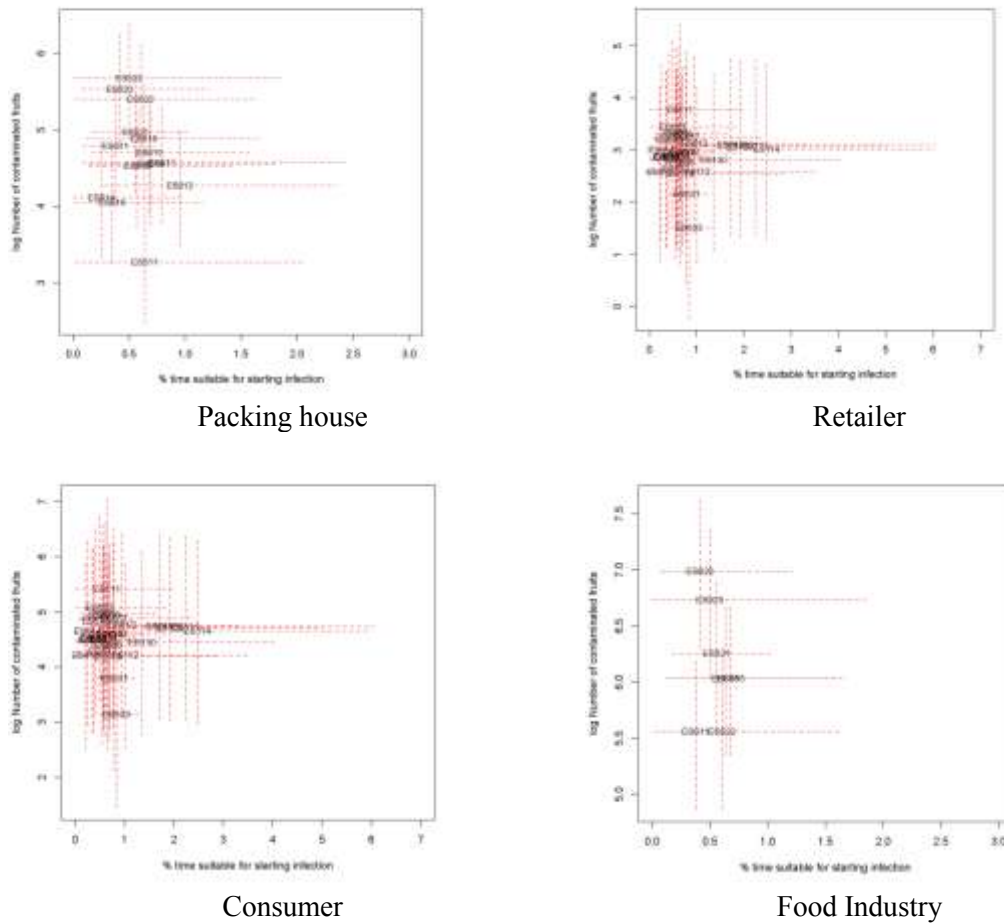




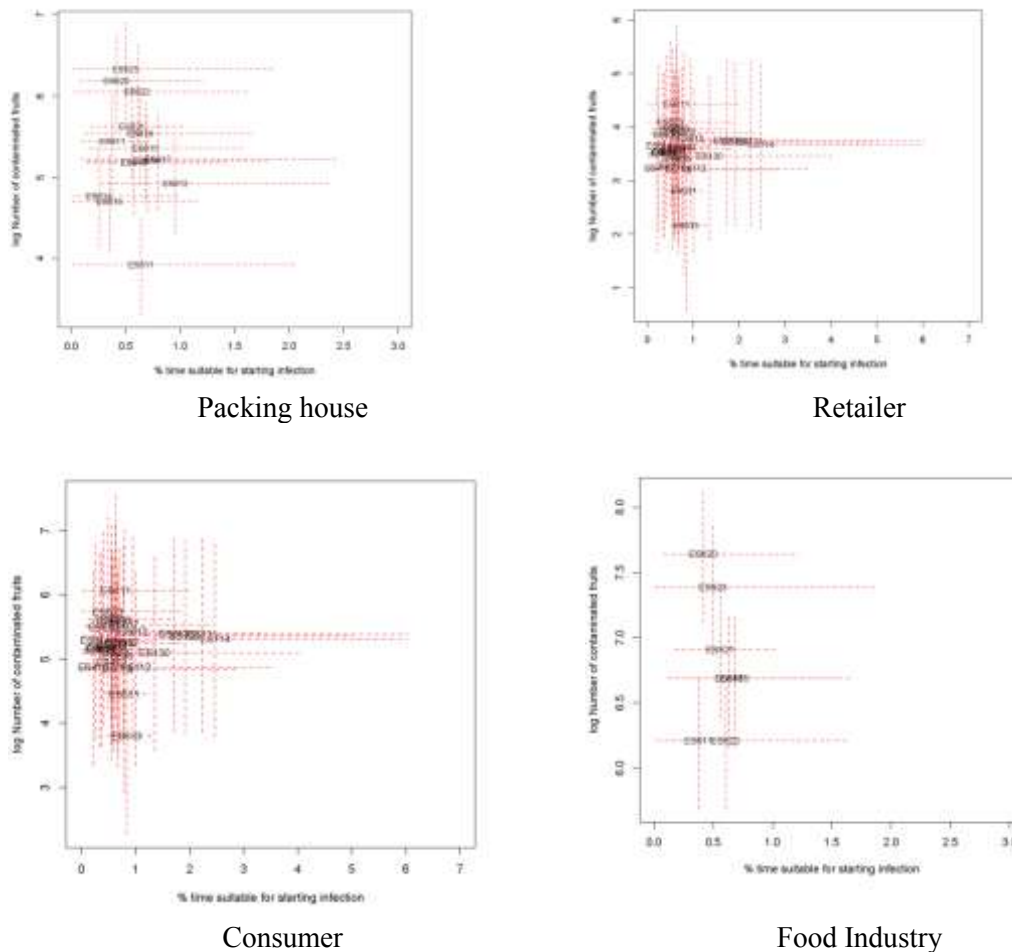
**Figure 72:** Log number of contaminated fruit in the yearly waste of the packing house, retailer, consumer and food industry for scenario 2. Each Spanish province is identified by its NUTS3 code. Vertical bars indicate the range (min–max) of log number of contaminated fruit simulated by the pathway model using the most extreme combinations of parameter values. Horizontal bars indicate the range of percentage of time suitable for starting infection predicted for the considered provinces



**Figure 73:** Log number of contaminated fruit in the yearly waste of the packing house, retailer, consumer and food industry for scenario 3. Each Spanish province is identified by its NUTS3 code. Vertical bars indicate the range (min–max) of log number of contaminated fruit simulated by the pathway model using the most extreme combinations of parameter values. Horizontal bars indicate the range of percentage of time suitable for starting infection predicted for the considered provinces



**Figure 74:** Log number of contaminated fruit in the yearly waste of the packing house, retailer, consumer and food industry for scenario 4. Each Spanish province is identified by its NUTS3 code. Vertical bars indicate the range (min-max) of log number of contaminated fruit simulated by the pathway model using the most extreme combinations of parameter values. Horizontal bars indicate the range of percentage of time suitable for starting infection predicted for the considered provinces

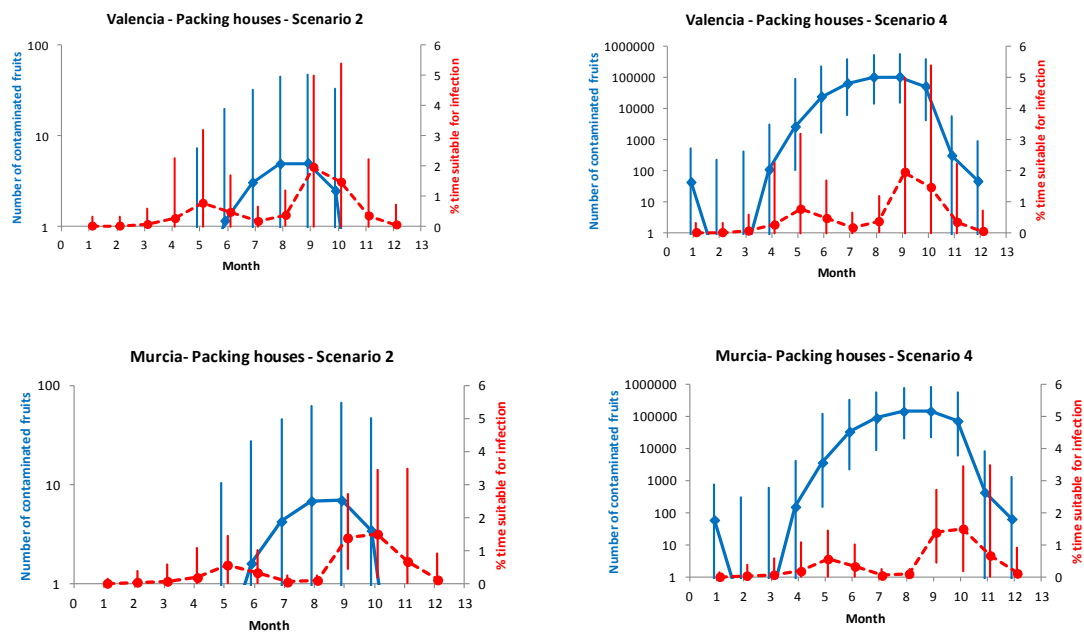


**Figure 75:** Log number of contaminated fruit in the yearly waste of the packing house, retailer, consumer and food industry for scenario 5. Each Spanish province is identified by its NUTS3 code. Vertical bars indicate the range (min–max) of log number of contaminated fruit simulated by the pathway model using the most extreme combinations of parameter values. Horizontal bars indicate the range of percentage time suitable for starting infection predicted for the considered provinces

#### *Seasonal variation in risk*

Both environmental suitability and the volume of CBS-affected fruit reaching pathway end points vary seasonally, in different ways, and the level of risk will depend on the extent to which they co-occur in time. To examine this, the model was run with monthly import data (Table 39) and monthly estimates of percentage of time suitable for pycnidiospore infection after a rain event. The results are shown for Valencia and Murcia, the provinces with the largest total citrus and lemon production. Results are shown for packing houses for scenarios 2 and 4 (Figure 76).

In both provinces, the period of time when imported contaminated fruit could be exposed to the open air coincides with periods of the season when the percentage of time for infection is at the highest levels. For instance, in Valencia, during September (month 9), the percentage of time suitable for infection reaches the highest value when the model simulates that there are around 100 000 contaminated fruit under scenario 4. Similar trends are found for Murcia province (Figure 76).



**Figure 76:** Results from pathway modelling showing the temporal overlap of the abundance of the CBS-contaminated citrus fruit waste with climate suitability for pycnidiospore infection after rain splash. The figures shown consider the packing house citrus waste under scenarios 2 and 4 for two Spanish provinces (Valencia and Murcia). Data are monthly values. The dotted lines indicate the range of variations for each of the variables graphed

### Uncertainties

The uncertainties related to the model outputs are discussed and summarised in Table 36. Uncertainties that do not affect directly the model outputs, but which need to be considered when model outputs are interpreted and discussed, are also presented.

**Table 36:** Uncertainties on model outputs

| Description of uncertainty  | Effect of uncertainty on model outputs  | Direction and magnitude of the effect on model outputs                                  |
|---|---|---|
| <p>The internal EU trade in citrus fruit from the other EU countries to Spain is not included in the model. The EU intra-trade statistics do not report the country of origin of the fruit traded within the common EU market (there are no internal border customs inspections). Therefore, the internal EU trade is excluded from the model.</p>                        | <p>The proportion of citrus imported into Spain from other EU countries (EU intra-trade) is about 50 % of the direct import to Spain from CBS-affected countries. Part of the intra-EU trade may come from CBS-free third countries or from production by other EU countries, but at least some is expected to originate from CBS-affected countries and be re-exported to Spain. Therefore, depending on trade patterns (which may vary with time), EU internal trade could considerably increase the model outputs which are for this reason underestimated. To precisely estimate such an increase, market studies and industry elicitation should be conducted but these activities could not be carried out within the timeframe of this assessment.</p> | <p>(++)<br/>(model outputs for all pathways and scenarios)</p>                          |
| <p>CBS symptoms and pycnidia may also develop after cold storage (Er et al., 2013) and therefore after import.</p>  | <p>This uncertainty may slightly increase the number of imported infected fruit as pycnidia may develop after import, with the effect of a minor increase in model outputs.</p>   | <p>(+)<br/>(model outputs for all pathways and scenarios)</p>                           |
| <p>The WRAP (2011 and 2012) and the FAO (Gustavsson et al., 2011) studies on food waste, which are the references for the citrus waste parameters used in this model, are based on a UK study. No specific data were available for Spain or other Mediterranean EU countries.</p>   | <p>The UK industry and its waste disposal system, used as a reference, is well organised, and therefore when extrapolating these results to other countries there is a risk of underestimating the importance of waste.</p>   | <p>(+)<br/>(model outputs for packing houses and retail pathways for all scenarios)</p> |
| <p>The Spanish import of limes (category 08055090) was not included in the model, as Tahiti lime (<i>C. latifolia</i>) has not been shown to bear fruiting bodies (pycnidia) of <i>P. citricarpa</i> on its fruit. However this excluded Eurostat category also includes data on the lime <i>C. aurantifolia</i>, which is not reported as resistant/tolerant to CBS.</p> | <p>The import data used in the model do not include the import into Spain of <i>C. aurantifolia</i> from CBS-affected countries; however, this trade is expected to be small in volume and related only to the retailer and consumer pathways, with a minor effect on the model outputs.</p>  | <p>(+)<br/>(model outputs for retailer and consumer only)</p>                           |
| <p>In reality, of the imported citrus fruit processed by the packing houses will enter the Spanish retail market, but the Panel has no data on this flow and therefore excluded it from the model.</p>  | <p>The Panel considers that, as most of the imported citrus fruit processed by the packing houses is for re-export (Diego Intrigliolo, IVIA, Valencia, Spain, personal communication, December 2013), only a small proportion of imported citrus fruit processed by the packing houses will enter the Spanish retail market. It is estimated that adding this fraction to</p>   | <p>(+)<br/>(models outputs for retailer only)</p>                                       |

| Description of uncertainty   | Effect of uncertainty on model outputs  | Direction and magnitude of the effect on model outputs          |
|--|---|---|
|  | the model would increase the retail waste flow by up to 10%.  |   |
| Citrus imports to Spain are assumed to have a similar level of infection throughout the year despite different origins.  | The proportion of imported citrus fruit that is infected by <i>P. citricarpa</i> may vary depending on the months and the country of origin. This is not expected to affect the overall trends for the yearly pathway model, whereas it may have more of an effect on the monthly pathway model.  | (+/-)<br>(monthly model outputs for all scenarios and pathways) |
| Interception data from the Netherlands have been used to parameterise the model for scenario 2. However the Netherlands imports citrus from a much larger number of sources than Spain, whose direct citrus imports are primarily from Brazil. | The extrapolation from the Dutch interception data to assess the CBS disease incidence in imported citrus fruit may not be representative of the Spanish imports; however, it is not known whether this would affect positively or negatively model outputs for scenario 2. However, because these estimates are consistent with those based on the meta-analysis and on assumptions on the efficiency of culling infected fruit in the country of origin, the effect of this uncertainty on model outputs is considered minor.   | (+/-)<br>(model outputs for scenario 2)                         |
| The citrus fruit imported for processing is only used in the model for juice production. Citrus fruit for marmalade is not considered.   | The use of peel in marmalade will decrease the amount reaching the waste pathway. However, the Panel considers that quantities of imported citrus fruit into Spain used for marmalade are small and therefore the effect on model output will be minor.   | (–)<br>(models outputs for juice industry only)                 |
| No data are available for the import into Spain of sour orange, which is not considered susceptible to CBS, therefore this species could not be excluded from the total citrus Spanish imports from CBS-affected countries.                    | Sour orange is considered to be of minor trade relevance and is normally used only for marmalade. Therefore, the effect of this uncertainty is minor on model outputs.  | (–)<br>(model outputs for all scenarios and pathways)           |
| Not all CBS symptomatic fruit bear pycnidia (see Table 5) as observed in samples from intercepted consignments in the Netherlands and in United Kingdom.   | The model estimates the number of infected fruit reaching the waste pathways. If some of these fruit do not bear pycnidia and therefore cannot disperse the fungal spores, this means that the amount of fruit capable of transmitting infection will be smaller than the number estimated by the model. From Table 5, the average proportion of fruit without pycnidia in samples taken from intercepted citrus fruit consignments is 18 % under the scenario of current phytosanitary measures. Although this value is derived from data for citrus imported into only two EU | (–)<br>(model outputs for all pathways and scenarios)           |

| Description of uncertainty  | Effect of uncertainty on model outputs  | Direction and magnitude of the effect on model outputs   |
|---|---|--|
|   | countries, these countries are among the highest EU importers of citrus fruit; thus, it is considered that this uncertainty will, on average, cause a reduction of up to 18 % in model outputs.   |  |
| The Eurostat citrus trade data for import from CBS-affected countries that used in the model do not distinguish trade coming from pest-free areas in CBS-affected countries.              | The figures on import of CBS-infected fruit are overestimated for scenario 1 and 2 as part of the current import can come from pest-free areas, however it is more difficult to make an estimation of such uncertainty for scenario 3 to 5 because in absence of CBS-specific phytosanitary measures the proportion of import from non pest-free areas may increase.  | (–)<br>(model outputs for all pathways for scenario 1 and 2)<br><br>(+/–)<br>(model outputs for all pathways for scenario 3, 4 and 5)  |
| Uncertainties remain concerning the distances between drying/landfill/composting facilities and citrus orchards.  | For logistics/transportation reasons, the treatment of citrus fruit waste for feed or for composting is expected to occur generally within short distances from packing houses or juice industries, which are located in citrus-growing areas (see Table 38). In still air, without wind, presumably only spores from the citrus fruit at the very edge of the drying area or at the surface/edge of landfill could reach neighbouring trees. However, with wind and rain, which is not a rare event in southern Europe, the dispersal distances of splashed pycnidiospores can considerably increase. Therefore, it is expected that a fraction of the citrus fruit or waste disposed in drying/landfill/composting facilities will have opportunities for ballistic and wind-assisted dispersal of rain-splashed pycnidiospores. To model such a fraction, more information and data could be obtained in the future via a formal knowledge elicitation process with industry experts, but this was not possible within the timeframe of this assessment. The effect of this uncertainty could lead to a reduction in the amount of citrus waste acting as a source of splashed and ballistically dispersed pycnidiospores. | This uncertainty does not affect results of model outputs, but their interpretation and discussion in the context of assessing the probability of transfer to a suitable host and its uncertainty. |
| Owing to the lack of specific studies, there are uncertainties related to survival of the pathogen (a) during processing, (b) during temporary storage until the container is full before | The effect of processing on the probability of transfer is discussed in section 3.2.2.4 of this opinion. This uncertainty, due to the lack of studies conducted on survival and splash  | This uncertainty does not affect the model outputs, but their interpretation and discussion in the   |



| Description of uncertainty  | Effect of uncertainty on model outputs   | Direction and magnitude of the effect on model outputs  |
|---|--|---|
| being dumped in landfill or spread out for solar drying and (c) during drying in the hot sun for feed processing or composting at high temperature. | dispersal of <i>P. citricarpa</i> in citrus waste and its processing, does not directly affect the outputs of this model but it has a major effect on the interpretation and discussion of the results   | context of assessing the probability of transfer to a suitable host and its uncertainty.  |
| Ranges were used by the Panel to represent uncertainties affecting some model parameters.   | Although necessarily subjective and approximate, the ranges used in the model represent the Panel's judgement of the uncertainties affecting the model parameters, given the information currently available to it. Better estimates could be obtained in the future via a formal knowledge elicitation process with a wider range of experts, but this was not possible within the time permitted for this request. | These uncertainties are already represented within the model (vertical bars in figures indicating the range from minimum to maximum). |
| OVERALL ASSESSMENT OF UNCERTAINTIES ON MODEL OUTPUTS  | Considering all the additional uncertainties above, the Panel estimates that their combined effect would possibly lead to a slight increase in model outputs but this would not significantly affect the differences between scenarios.  | (+) (model outputs for all pathways and scenarios)  |

Key: (-) or (+) = minor effect; (++) or (--) = major effect.

Considering all the additional uncertainties above, the Panel estimates that the combined effect of the uncertainties would possibly lead to a slight increase in model outputs but this would not significantly affect the differences between the scenarios.

Some of the uncertainties listed in Table 36 above do not directly affect the model outputs but need to be carefully considered when they are interpreted. The processing of citrus waste derived from packing houses and juice industries will be located mostly within citrus-growing areas with opportunities for ballistic and wind-assisted splash dispersal of pycnidiospores from infected citrus fruit; however, there are uncertainties concerning the fraction of such waste that will be disposed or placed within the maximum dispersal distances. There is also high uncertainty owing to the lack of studies on the survival of the pathogen during processing for feed and for waste purposes.

### Conclusions

The pathway model indicates that, under current regulations (scenarios 1 and 2), the number of fruit infected with *P. citricarpa* entering the citrus-growing regions of Spain is likely to be small, in the order of zero to several dozen fruit per region. Thus, under current regulations, entry via the citrus fruit trade pathways is very unlikely, with low uncertainty, owing to the minor amounts of inoculum that may reach the trees from the small number of infected fruit moving along the pathway. This finding is of key relevance to the analysis of RROs.

Since regulations currently targeted at *P. citricarpa* are not taken into account in the entry section of the pest risk assessment, only the results from scenarios 3–5 can be considered when assessing the likelihood of entry. Compared with scenarios 1 and 2, scenarios 3–5 all show major increases, by a factor of 10 000 or more (four or more orders of magnitude), in the

potential for entry. The uncertainties quantified by the Panel within the model are substantial, but change these estimates by a factor of only 1–2 orders of magnitude and could be either positive or negative (see uncertainty bars in Figures 72–76). Additional uncertainties have been identified in Table 36 above.

The Panel concludes that, without regulation, the number of infected fruit entering citrus-growing regions and arriving within the maximum distance for spores to move to citrus trees is high and there is good temporal overlap between the timing of entry and the weather conditions suitable for rain splash and spore dispersal. Of the four main pathways, the levels of exposure resulting from these pathways is considered to be highest for the juice industry, followed by packing houses, consumers and the retail chain. In relating this conclusion to the qualitative rating according to the EFSA harmonised framework for pest risk assessment (EFSA PLH Panel, 2010), we need to take into account the rating descriptors for entry in Appendix A. For *P. citricarpa*, the likelihood of transfer is the key issue and, even though the volume moving along the pathway is high, this needs to be compared with other pests and other pathways and the Panel concludes that the pest still “has some limitations for transfer to a suitable host in the risk assessment area” and therefore that the pathway should be assessed as moderately likely. Although the uncertainties taken into account in the model can be considered to be low, when the additional uncertainties outlined in Table 36 are taken into account, the Panel considers that the uncertainty score should be medium.

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**Table 37:** Input parameters used for the different scenarios

| Name                 | Definition                        | Scenario 2 |            |           | Scenario 3 |           |           | Scenario 4 |           |           | Scenario 5 |           |           |
|----------------------|-----------------------------------|------------|------------|-----------|------------|-----------|-----------|------------|-----------|-----------|------------|-----------|-----------|
|                      |                                   | Median     | Min        | Max       | Median     | Min       | Max       | Median     | Min       | Max       | Median     | Min       | Max       |
| Import               | Quantity of imports in kg         | 142273400  | 110582500  | 192370800 | 142273400  | 110582500 | 192370800 | 142273400  | 110582500 | 192370800 | 142273400  | 110582500 | 192370800 |
| FruitWeight          | Standard fruit weight in kg       | 0.2        | 0.25       | 0.15      | 0.2        | 0.25      | 0.15      | 0.2        | 0.25      | 0.15      | 0.2        | 0.25      | 0.15      |
| PropInfectedFruit    | Proportion of infected fruit      | 0.00000767 | 0.00000219 | 0.0000269 | 0.02       | 0.006     | 0.07      | 0.16       | 0.07      | 0.32      | 0.72       | 0.46      | 0.98      |
| Allocation_PackHouse | Fraction of fruit allocated to PH | 0.4        | 0.3        | 0.5       | 0.4        | 0.3       | 0.5       | 0.4        | 0.3       | 0.5       | 0.4        | 0.3       | 0.5       |
| Allocation_Retailer  | Fraction of fruit allocated to R  | 0.4        | 0.3        | 0.5       | 0.4        | 0.3       | 0.5       | 0.4        | 0.3       | 0.5       | 0.4        | 0.3       | 0.5       |
| Allocation_Industry  | Fraction of fruit allocated to I  | 0.2        | 0.15       | 0.25      | 0.2        | 0.15      | 0.25      | 0.2        | 0.15      | 0.25      | 0.2        | 0.15      | 0.25      |
| ExpoPropPacking      | Prop of inf fruit from PH exposed | 0.033      | 0.026      | 0.04      | 0.033      | 0.026     | 0.04      | 0.033      | 0.026     | 0.04      | 0.033      | 0.026     | 0.04      |
| ExpoPropRetailer     | Prop of inf fruit from R exposed  | 0.0225     | 0.02       | 0.025     | 0.0225     | 0.02      | 0.025     | 0.0225     | 0.02      | 0.025     | 0.0225     | 0.02      | 0.025     |
| ExpoPropConsum       | Prop of inf fruit from C exposed  | 0.05       | 0.005      | 0.5       | 0.05       | 0.005     | 0.5       | 0.05       | 0.005     | 0.5       | 0.05       | 0.005     | 0.5       |
| ExpoPropIndustry     | Prop of inf fruit from I exposed  | 1          | 1          | 1         | 1          | 1         | 1         | 1          | 1         | 1         | 1          | 1         | 1         |
| FruitToConsum        | Prop of fruit of R sold to C      | 0.9775     | 0.98       | 0.975     | 0.9775     | 0.98      | 0.975     | 0.9775     | 0.98      | 0.975     | 0.9775     | 0.98      | 0.975     |
| ConsumWaste          | Prop of waste produced by C       | 1          | 1          | 1         | 1          | 1         | 1         | 1          | 1         | 1         | 1          | 1         | 1         |

**Table 38:** Input parameters used for the different Spanish provinces

| Code  | Name                 | Numb PackHouses | Prop PackHouses | Numb Industries | Prop Industries | Population | Prop Population |
|-------|----------------------|-----------------|-----------------|-----------------|-----------------|------------|-----------------|
| ES111 | A Coruna             | 0               | 0               | 0               | 0               | 1138494    | 0.024364334     |
| ES112 | Lugo                 | 0               | 0               | 0               | 0               | 344845     | 0.007379853     |
| ES113 | Ourense              | 0               | 0               | 0               | 0               | 325389     | 0.006963485     |
| ES114 | Pontevedra           | 0               | 0               | 0               | 0               | 953241     | 0.020399828     |
| ES120 | Asturias             | 0               | 0               | 0               | 0               | 1067802    | 0.02285149      |
| ES130 | Cantabria            | 0               | 0               | 0               | 0               | 590037     | 0.012627083     |
| ES213 | Vizcaya              | 0               | 0               | 0               | 0               | 1158000    | 0.024781772     |
| ES415 | Salamanca            | 0               | 0               | 0               | 0               | 347249     | 0.0074313       |
| ES431 | Badajoz              | 0               | 0               | 0               | 0               | 690894     | 0.014785473     |
| ES432 | Caceres              | 0               | 0               | 0               | 0               | 410074     | 0.008775786     |
| ES511 | Barcelona            | 1               | 0.001242236     | 0               | 0               | 5493078    | 0.117554584     |
| ES514 | Tarragona            | 19              | 0.023602484     | 0               | 0               | 802806     | 0.017180445     |
| ES521 | Alicante             | 50              | 0.062111801     | 5               | 0.079365079     | 1854244    | 0.039681738     |
| ES522 | Castellón            | 135             | 0.167701863     | 1               | 0.015873016     | 585729     | 0.01253489      |
| ES523 | Valencia             | 255             | 0.316770186     | 15              | 0.238095238     | 2547044    | 0.054508        |
| ES531 | Eivissa Y Formentera | 0               | 0               | 0               | 0               | 132637     | 0.002838497     |
| ES532 | Mallorca             | 0               | 0               | 0               | 0               | 869067     | 0.018598463     |
| ES533 | Menorca              | 0               | 0               | 0               | 0               | 29580      | 0.000633027     |
| ES611 | Almeria              | 33              | 0.040993789     | 1               | 0.015873016     | 691680     | 0.014802294     |
| ES612 | Cadiz                | 10              | 0.01242236      | 0               | 0               | 1247578    | 0.026698786     |
| ES613 | Cordoba              | 18              | 0.022360248     | 0               | 0               | 800414     | 0.017129255     |
| ES614 | Granada              | 7               | 0.008695652     | 0               | 0               | 922138     | 0.019734209     |
| ES615 | Huelva               | 27              | 0.033540373     | 3               | 0.047619048     | 520948     | 0.011148545     |
| ES616 | Jaen                 | 6               | 0.007453416     | 0               | 0               | 661716     | 0.014161049     |
| ES617 | Malaga               | 20              | 0.02484472      | 0               | 0               | 1611983    | 0.034497233     |
| ES618 | Sevilla              | 41              | 0.050931677     | 3               | 0.047619048     | 1936703    | 0.041446401     |
| ES620 | Murcia               | 183             | 0.227329193     | 27              | 0.428571429     | 1461987    | 0.031287244     |

**Table 39:** Monthly import inputs (tons)

| <b>Month</b> | <b>Median</b> | <b>Min</b> | <b>Max</b> |
|--------------|---------------|------------|------------|
| Jan          | 17.75         | 0          | 54.2       |
| Feb          | 0             | 0          | 22.9       |
| Mar          | 0             | 0          | 43.2       |
| Apr          | 45.65         | 0          | 311.3      |
| May          | 1 084.3       | 216        | 9078.7     |
| Jun          | 9 993.3       | 3 287      | 24 358.2   |
| Jul          | 26 437.4      | 12 745.9   | 40 010.4   |
| Aug          | 42 486.8      | 28 831.6   | 55 222.9   |
| Sep          | 43 219.1      | 32468.8    | 58 493.7   |
| Oct          | 21 349.9      | 8486.7     | 40 922.1   |
| Nov          | 127.75        | 0          | 600.6      |
| Dec          | 19.1          | 0          | 92         |

**Annex 1 to Appendix E. The code and input parameters used for running the pathway modelling simulations for the R-statistical software.<sup>13</sup>**

Code used for simulation

```
#####
##Number of infected fruit in Spain##
#####
Infected_Fruit<-function(Import,          FruitWeight,          PropInfectedFruit,
Allocation_PackHouse, Allocation_Retailer, Allocation_Industry) {

NumberFruit<-Import/FruitWeight

#Total number of infected fruit
Total_Infected<-NumberFruit*PropInfectedFruit

#Number of infected fruit in packing houses
Infected_PackHouse<-Total_Infected*Allocation_PackHouse

#Number of infected fruit in retailers
Infected_Retailer<-Total_Infected*Allocation_Retailer

#Number of infected fruit in industries
Infected_Industry<-Total_Infected*Allocation_Industry

InfectedFruitSpain<-c(Total_Infected,          Infected_PackHouse,          Infected_Retailer,
Infected_Industry)

return(InfectedFruitSpain)

}

#####
##Number of infected fruit in Spanish provinces##
#####
Infected_Fruit_Provinces<-function(Inf_fruit_Spain,          PropPacking,          PropRetail,
PropIndustry) {

#Inf_fruit_Spain is c(Total_Infected,Infected_PackHouse,          Infected_Retailer,
Infected_Industry)

#Number of infected fruit in packing houses
Infected_PackHouse_Prov<-Inf_fruit_Spain[2]*PropPacking

#Number of infected fruit in retailers
Infected_Retailer_Prov<-Inf_fruit_Spain[3]*PropRetail

#Number of infected fruit in industries
Infected_Industry_Prov<-Inf_fruit_Spain[4]*PropIndustry

InfectedFruitProvinces<-cbind(Infected_PackHouse_Prov,          Infected_Retailer_Prov,
Infected_Industry_Prov)

return(InfectedFruitProvinces)

}

#####
##Number of exposed fruit in provinces##
#####
```

<sup>13</sup> R Development Core Team (2011), R: A Language and Environment for Statistical Computing. Vienna, Austria : the R Foundation for Statistical Computing. ISBN: 3-900051-07-0. Available online at <http://www.R-project.org/>.

```

Exposed_Fruit_Provinces<-function(Inf_Fruit_Prov,                               ExpoPropPacking,
ExpoPropRetailer,FruitToConsum, ConsumWaste, ExpoPropConsum, ExpoPropIndustry) {

#Inf_fruit_Prov is cbind(Infected_PackHouse_Prov,      Infected_Retailer_Prov,
Infected_Industry_Prov)

#Number of infected fruit in waste from packing houses
Exposed_Waste_PackHouse<-Inf_Fruit_Prov[,1]*ExpoPropPacking

#Number of infected fruit in waste from retailers
Exposed_Waste_Retailer<-Inf_Fruit_Prov[,2]*ExpoPropRetailer*ExpoPropConsum

#Number of infected fruit in waste from consumers
Exposed_Waste_Consumer<-
Inf_Fruit_Prov[,2]*FruitToConsum*ConsumWaste*ExpoPropConsum

#Number of infected fruit in waste from industries
Exposed_Waste_Industry<-Inf_Fruit_Prov[,3]*ExpoPropIndustry

NumberExposedFruit<-cbind(Exposed_Waste_PackHouse,                               Exposed_Waste_Retailer,
Exposed_Waste_Consumer, Exposed_Waste_Industry)

return (NumberExposedFruit)

}

#####
##Main program for simulating the packinghouse pathway##
#####

#####
#Province-specific data#
#####

ProvinceTable<-read.table("SpanishProvinces_2.txt", sep="\t", header=T)

PropPacking<-ProvinceTable$PropPackHouses
PropRetail<-ProvinceTable$PropPopulation
PropIndustry<-ProvinceTable$PropIndustries

#####
#Pathway parameters#
#####

ParameterTable<-read.table("PathwayParametersPH.txt", sep="\t", header=T)

#####
##Loop for running the functions for all provinces and all scenarios##
#####

scenarios<-c(3,6,9,12)

for (k in 1:4) {
#Column for scenarios 2, 3, 4, 5 (3, 6, 9, 12)
  Num<-scenarios[k]
  dev.new()

  FruitMin<-rep(NA, length(ProvinceTable$Code))
  FruitMed<-rep(NA, length(ProvinceTable$Code))
  FruitMax<-rep(NA, length(ProvinceTable$Code))
  RiskPackingHouse<-
data.frame(ProvinceTable$Code,ProvinceTable$Name,FruitMed,FruitMin,FruitMax)

  for (i in 1:3) {

    Import<-ParameterTable[ParameterTable$Name=="Import", (Num+i-1)]
    FruitWeight<-ParameterTable[ParameterTable$Name=="FruitWeight", (Num+i-
1)]

```



```

    PropInfectedFruit<-
ParameterTable[ParameterTable$Name=="PropInfectedFruit", (Num+i-1)]
    Allocation_PackHouse<-
ParameterTable[ParameterTable$Name=="Allocation_PackHouse", (Num+i-1)]
    Allocation_Retailer<-
ParameterTable[ParameterTable$Name=="Allocation_Retailer", (Num+i-1)]
    Allocation_Industry<-
ParameterTable[ParameterTable$Name=="Allocation_Industry", (Num+i-1)]
    ExpoPropPacking<-
ParameterTable[ParameterTable$Name=="ExpoPropPacking", (Num+i-1)]
    ExpoPropRetailer<-
ParameterTable[ParameterTable$Name=="ExpoPropRetailer", (Num+i-1)]
    ExpoPropConsum<-
ParameterTable[ParameterTable$Name=="ExpoPropConsum", (Num+i-1)]
    ExpoPropIndustry<-
ParameterTable[ParameterTable$Name=="ExpoPropIndustry", (Num+i-1)]
    FruitToConsum<-
ParameterTable[ParameterTable$Name=="FruitToConsum", (Num+i-1)]
    ConsumWaste<-ParameterTable[ParameterTable$Name=="ConsumWaste", (Num+i-
1)]

    Inf_fruit_Spain<-Infected_Fruit(Import, FruitWeight,
PropInfectedFruit, Allocation_PackHouse, Allocation_Retailer, Allocation_Industry)

    Inf_Fruit_Prov<-Infected_Fruit_Provinces(Inf_fruit_Spain, PropPacking,
PropRetail, PropIndustry)

    Exposed_Fruit_Prov<-Exposed_Fruit_Provinces(Inf_Fruit_Prov,
ExpoPropPacking, ExpoPropRetailer, FruitToConsum, ConsumWaste,
ExpoPropConsum, ExpoPropIndustry)

    Inf_fruit_Spain

####Number of infected fruit in each province for each pathway####
    data.frame(ProvinceTable$Code, ProvinceTable$Name, Inf_Fruit_Prov)

####Number of exposed fruit in each province for each pathway####
    data.frame(ProvinceTable$Code, ProvinceTable$Name, Exposed_Fruit_Prov)

    RiskPackingHouse[, (3+(i-1))]<-Exposed_Fruit_Prov[,1]

}

####Data for Y axis###
    RiskPackingHouse

####Data for X axis###
    RiskInfection<-read.table("Pycnidiospore.txt", sep="\t", header=T)

    plot(0,0, pch=" ", xlim=c(0,3),
ylim=c(log10(min(RiskPackingHouse$FruitMin[RiskPackingHouse$FruitMin!=0])), log10(max
x(RiskPackingHouse$FruitMax))), xlab="% time suitable for starting infection",
ylab="log Number of contaminated fruit")

###Graph###

    for (j in 1:length(RiskPackingHouse[,1])) {

        Code_j<-RiskPackingHouse[j,1]

        X_j_med<-RiskInfection$AVE[RiskInfection$NUTS_ID==Code_j]
        X_j_min<-RiskInfection$MIN[RiskInfection$NUTS_ID==Code_j]
        X_j_max<-RiskInfection$MAX[RiskInfection$NUTS_ID==Code_j]

        Y_j_med<-
RiskPackingHouse$FruitMed[RiskPackingHouse$ProvinceTable.Code==Code_j]
        Y_j_min<-
RiskPackingHouse$FruitMin[RiskPackingHouse$ProvinceTable.Code==Code_j]

```

```
Y_j_max<-
RiskPackingHouse$FruitMax[RiskPackingHouse$ProvinceTable.Code==Code_j]

      if (Y_j_med!=0) {
#points(X_j_med,log(Y_j_med), pch=19, col="red")
      lines(c(X_j_min,X_j_max),
log10(c(Y_j_med,Y_j_med)),lwd=1,col="red",lty=2)
      lines(c(X_j_med,X_j_med),
log10(c(Y_j_min,Y_j_max)),lwd=1,col="red",lty=2)
      text(X_j_med,log10(Y_j_med),Code_j,cex=0.85)
      }

}

write.table(RiskPackingHouse,
file=paste("PackingHouse_Scenario_",(k+1),".txt",sep=""))
}
```

**Appendix F. Data supplement**

Available online: <http://www.efsa.europa.eu/en/efsajournal/doc/3557ax1.pdf>