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

Prima phacie

Guignardia citricarpa

Pest Risk Assessment: Test Method 4 (Revised)

June 2011

With the EU legislation (EU Council Directive 2000/29/EC) in place

Preface

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

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

Note

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

[1] ISPM No. 11 available at https://www.ippc.int/id/13399

[2] EFSA Journal 2010, **8**(2),1495-1561, Available at http://www.efsa.europa.eu/en/scdocs/doc/1495.pdf

CONTENTS

Table / list of contents

Executive Summary

The purpose of this pest risk assessment was to evaluate the plant health risk associated with *Guignardia citricarpa* (citrus black spot) within the framework of EFSA project CFP/EFSA/PLH/2009/01.

Keywords: Citrus black spot (CBS), citrus fruit, *Citrus* spp., European Community, European Union, *Guignardia citricarpa,* mancha negra, pest risk assessment, pinta preta

Pest biology

- Identity of the pest (see updated datasheet for details)

  Preferred scientific name (teleomorph): *Guignardia citricarpa* Kiely (1948) (citrus black spot, CBS)

  Anamorph: *Phytllosticta citricarpa* (McAlpine) Aa (1973) (macroconidial state)

  Leptodothiorella sp. (microconidial state)

- Life history (see updated datasheet for details):

Two types of infective propagules are involved in CBS epidemiology: ascospores produced within pseudothecia on leaf litter and dead twigs on the orchard floor (Kotzé, 1981; McOnie, 1964b; McOnie, 1965) and conidia (pycnidiospores) produced within pycnidia on fruit, leaves, twigs and abundantly on leaf litter, prior to pseudothecia development (Kiely, 1948; Kotzé, 1963; 1981; 2000). Pseudothecia with ascospores have never been observed to develop on leaves or fruit attached to the tree (Kiely, 1948; Kotzé, 1963; 1981). Both ascospores and conidia have a role in establishing an epidemic, but once the disease reaches epidemic proportions, wind-borne ascospores are considered to be the main inoculum source (Huang and Chang, 1972; Lee and Huang, 1973; Kotzé, 1981; McOnie, 1964b). However, Whiteside (1967) found that conidia were the most important inoculum source at the initial stages of the disease in Rhodesia. Recent studies in Brazil and Argentina (Ortiz *et al*., 2004; Reis, 2002; Spósito *et al*., 2008) have suggested that, due to irregular flowering of the cultivated citrus species/cultivars and the subsequent overlapping of mature and young fruit on the same tree, the role of conidia in the epidemiology of the disease is different than that in other countries, such as South Africa and Australia, where CBS epidemics have been exclusively attributed to ascospores (Kiely, 1948; McOnie, 1964c; 1965). In the latter countries, the uniform flowering and fruiting of the cultivated citrus species/cultivars due to irrigation and cooler temperatures lead to few off-season fruit (Kotzé, 1981).

Pseudothecia develop within 40-180 days after leaf drop (Fig. 1), depending on the frequency of wetting and drying (rain, irrigation, dew, etc) of leaves as well as on the prevailing temperatures (Kotzé, 1981; Kiely, 1950). Ascospores may be produced over several months on leaf litter, even when the leaves are in an advanced stage of decomposition (Kiely, 1948). Maturation of ascospores occurs practically simultaneously in early summer on infected leaves abscised during late autumn, winter and early spring (Kotzé, 1963; Lee and Huang, 1973; McOnie, 1964c). However, severe epidemics have been reported also in areas where ascospores were produced on leaves shed in spring and early summer, as leaves abscised in winter were completely decomposed by other organisms (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 (Lee and Huang, 1973). When mature asci within pseudothecia are moistened with water (rain, irrigation, heavy dew, etc.), ascospores are ejected up to a height of 1.2 cm from pseudothecia and are carried by air currents throughout the canopy and over longer distances (Kiely, 1948 and 1949; Kotzé, 1988; McOnie, 1964b; Huang and Chang, 1972; Wager, 1949). In areas characterized by high rainfall and sandy soils, the effect of irrigation on pseudothecia maturation is of minor importance (McOnie, 1964c). Environmental conditions required for ascospore germination vary from 15-29.5°C and 15-38 h of leaf wetness (Kotzé, 1963).

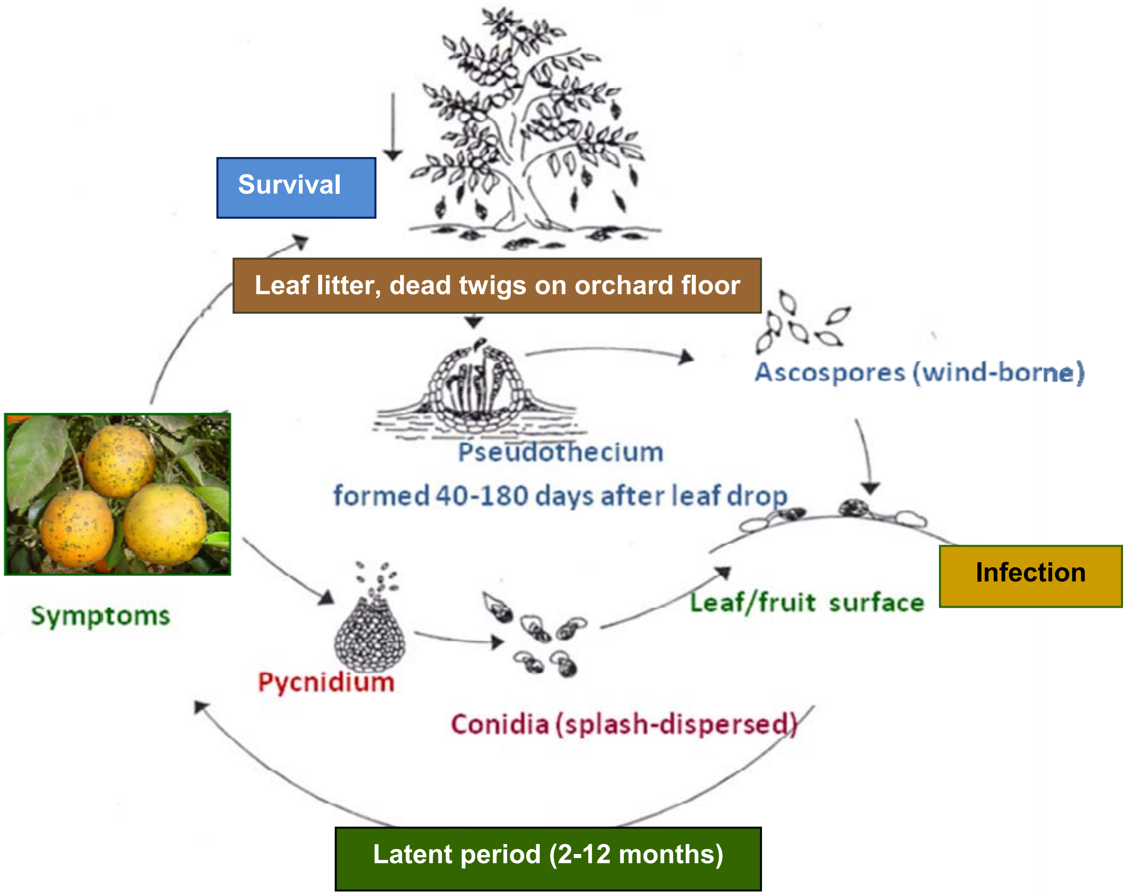

Figure 1. Life cycle of *Guignardia citricarpa* (adapted from Timmer, 1999).

Under wet conditions, mature conidia emerge from the pycnidia embedded in a mucilaginous mass. In contact with water, the mucilage is dissolved and the conidia are splash-dispersed or washed-off by rain or overhead irrigation to nearby susceptible tissues, where new infections may occur (Kiely, 1948; Kotzé, 1981; Spósito *et al*., 2008; Wager, 1952; Whiteside, 1967) (Fig. 1). The sporogenous layers of the pycnidia are regenerative and thus, numerous crops of viable conidia can be produced for several months following regular wetting of the plant tissues (Kiely, 1948; Truter *et al*., 2007; Wager, 1952). There is limited information on the environmental conditions that favour conidial germination. According to Noronha (2002), germination and appressoria formation occurs over a wide range of temperatures (10-40°C) with a minimum of 12 h of wetness.

The microconidial state of *G. citricarpa*, *Leptodothiorella* sp. usually appears on fallen leaves before the formation of pseudothecia (Kiely, 1948), but its role in the biology of *G. citricarpa* is still unclear.

Typically, citrus leaf and fruit infections remain quiescent with no symptom development until after leaves die and fruit mature (Kiely, 1948; Whiteside, 1965). An important characteristic of CBS is the long (2-12 months) quiescent period (Kellerman and Kotzé, 1977; McOnie, 1967).

The epidemiology of CBS is dependent upon the availability of inoculum during the period of host susceptibility, the climate (warm, wet or humid conditions favour infection) and the age of the host tissue in relation to its susceptibility to infection (Kotzé, 2000). Moreover, the flowering and fruiting patterns (e.g. overlapping of mature and new fruit, number of leaf flushes, etc.) of the cultivated citrus species/varieties also influence the epidemiology of the disease (Ortiz *et al.,* 2004; Reis, 2002; Spósito *et al*. 2008).

Symptom development on mature fruit is enhanced by rising temperatures, high light intensity, drought and poor tree vigor. The disease is usually more severe on old trees than on healthy young trees (Kotzé, 1981; 2000). In certain citrus species (e.g. lemons) and/or cultivars (e.g. 'Valencia' oranges), the co-existence of mature symptomatic fruit from the previous year's crop and young fruit from the new fruit set on the same tree extends the period during which susceptible tissues are exposed to *G. citricarpa* inoculum and increases the disease incidence and severity in an orchard (Kiely, 1948; Kotzé, 1981; Spósito *et al.,* 2008).

One of the interesting features of CBS is that the causal organism may be present for many years in a particular area before symptoms appear (Kotzé, 1981). It may take 5-30 years from the time the first symptoms are noticed until the disease reaches epidemic proportions, depending on the presence of lemons (*C. limon* L.) and climatic conditions (Kotzé, 1981). It has been reported that in Western Transvall (South Africa), CBS symptoms had been observed for over 30 years before control measures became necessary (Kotzé, 1981). In South Africa, there is not a single example of CBS declining or disappearing after the epidemic stage had been reached (Kotzé, 1981).

- Host range / habitat:

Guignardia citricarpa affects *Citrus, Poncirus, Fortunella* and their hybrids. Except for sour orange (*Citrus aurantium* L.), its hybrids and Tahiti limes (*C. latifolia* Tan.), all commercially grown *Citrus* species are susceptible (Kotzé, 2000; Aguilar-Vildoso *et al.,* 2002). Lemon (*C. limon* L.) is particularly susceptible and, thus, in an unaffected area, CBS usually appears first on lemons (Kotzé, 2000). Although in the past, late-maturing sweet orange cultivars (e.g. 'Valencia') were considered to be more susceptible than early maturing (Kotzé, 1981), it has been recently shown that some early-, mid- and late-maturing sweet orange cultivars are equally susceptible to infection by *G. citricarpa* (Schinor *et al.*, 2002; Baldassari *et al.*, 2006).

- Means of dispersal / spread (see also revised datasheet):

G. citricarpa infective propagules (conidia, ascospores) are spread by wind and/or water (rain or irrigation). As a rule, pathogens disseminated by wind spread over long distances within a short period of time (Aylor, 1990), whereas splash-dispersed pathogens reach mostly short distances from the primary source of inoculum (Huber *et al*., 1998). Ascospores of *G. citricarpa,* produced abundantly within pseudothecia on leaf litter, dead twigs and petioles on the orchard floor are released during rainfall events and are disseminated by air currents throughout the canopy and over longer distances (Kiely, 1948). *G. citricarpa* conidia, produced within pycnidia on symptomatic mature fruit, dead twigs, fruit pedicels, attached leaves and in abundance on leaf litter, may be either splash-dispersed onto the tree canopy by rain, overhead or sprinkler irrigation water or washed-off by rain, dew or overhead irrigation from the upper infected fruit, leaves, etc to the lower ones that are still at the susceptible stage (Agostini *et al*., 2006; Spósito *et al*., 2008). By this mechanism, conidia of *G. citricarpa* can spread over relatively short distances (Kotzé, 1981; 1996; Kiely, 1948). This is further supported by studies conducted by Spósito *et al*. (2007) in citrus orchards in Brazil with 14-29 year-old trees of 3 m minimum height. According to those studies, CBS-infected trees were aggregated in small foci, with a minimum radius of 24.7 m. Based on these results, the authors assumed that, among the biological, physical and environmental factors that could be involved in the aggregation of infected trees, the most important seemed to be the limitation of the pathogen inoculum dispersal over long distances (Spósito *et al*., 2007).

No other studies have been carried out on the distance or height above inoculum sources over which *G. citricarpa* conidia can be dispersed. However, studies carried out with other splash-dispersed fungi (Fitt *et al.,* 1989) have shown that in still air conidia covered with mucilage, like those of *G. citricarpa,* are splash-dispersed up to a height of not more than 50 cm or up to a distance of 1 m from the inoculum source with their numbers decreasing steeply with increasing height or distance. However, the dispersal pattern of conidia depends on the size of conidia and the velocity of the incident raindrop as well as on the presence of air currents. Rain tower experiments have shown that in still air large raindrops spread the conidia over shorter distances compared with the smaller drops (Fitt *et al.,* 1989). Generally, the size of raindrops that reach the ground are of 0.2-5 mm in diameter, since smaller drops evaporate rapidly, unless relative humidity is near to 100%, and larger drops break up when they fall at speeds approaching their terminal velocities. Nevertheless, rain with many large drops will effectively disperse these pathogens (Fitt *et al*., 1989).

In the presence of wind, conidia carried in small splash drops may also become wind-borne as an aerosol of fine spray (Fitt *et al.*, 1989). The significance of wind in the dispersal of pathogens removed from the inoculum source in splash droplets becomes greater as the size of the inoculum particles becomes smaller. According to Fitt *et al*. (1989), small-sized conidia like those of *Phoma exigua* var. *foveata* (Foister) Boerema (7 × 3 μm), are more likely to be carried in small splash droplets, which become wind-borne. Therefore, under windy conditions, the small-sized *G. citricarpa* conidia (9.4-12.7 x 5-8.5 μm) (Baayen *et al.*, 2002) carried in small splash droplets could also become wind-borne and disperse over longer distances. Moreover, water drops formed on the leaves due to fog, dew, mist, overhead or sprinkler irrigation may cause drip-splash of *G. citricarpa* inoculum under canopies, which may be as important as direct rain-splash. These drip drops may have sufficient impact force for the dispersal of conidia in splash drops because they fall only short distances and thus, they are usually larger than 5 mm in diameter and less likely to break up compared to raindrops (Fitt *et al.*, 1989).

Long distance spread of *G. citricarpa* may also occur with the movement of infested plant material (seedlings, scions, budwood, leaves, fruit, etc). The role of insects, birds, etc. in the spread of *G. citricarpa* has not been documented. However, Kiely (1948) considered that insects, birds etc. may be able to deposit *G. citricarpa* conidia on the wounds they cause on the host.

Geographic Distribution (see updated datasheet for details)

G. citricarpa is present in the following citrus-growing areas (Fig. 2):
<u>Asia</u>: Bhutan, China, Indonesia, Taiwan
<u>Ocaenia</u>: Australia, Vanuatu
<u>Africa</u>: South Africa, Kenya, Mozambique, Uganda, Zambia, Zimbabwe

South America: Argentina, Brazil, Uruguay
North America: Florida (Collier and Hendry counties)

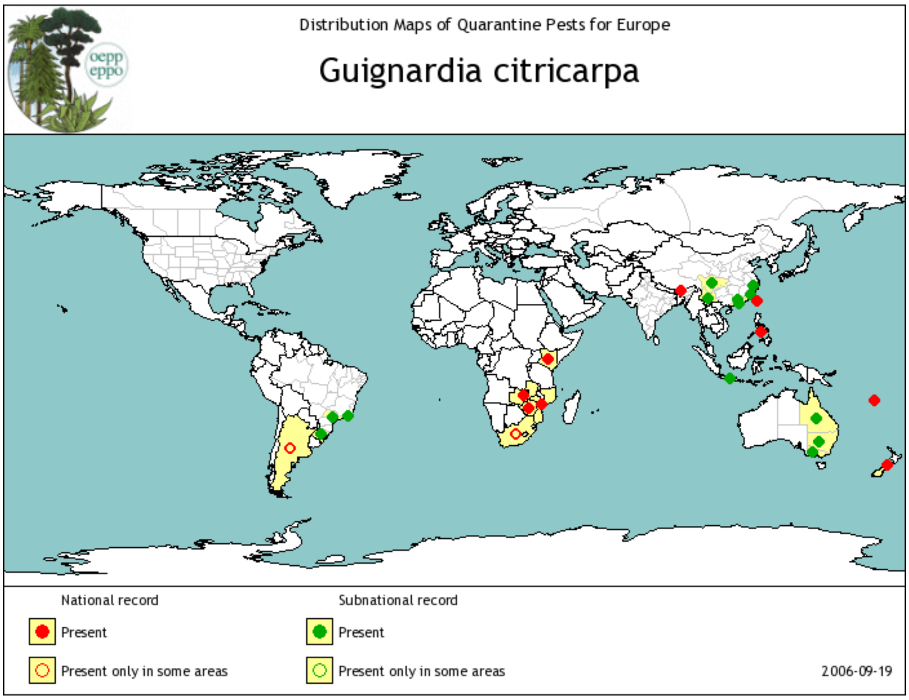

Figure 2. Distribution map of *Guignardia citricarpa* (EPPO, 2006)

Summary of risk elements:

1. Climate – host interaction

Hosts of *G. citricarpa* are exclusively species of the genera *Citrus, Poncirus* and *Fortunella*. All citrus species and cultivars are susceptible to *G. citricarpa* with the exception of sour orange (*C. aurantium* L.) and Tahiti lime (*C. latifolia* Tan.), which are considered to be tolerant. Citrus species are extensively grown in commercial orchards, nurseries, private gardens for family consumption, and as ornamentals in public gardens and along the roadsides both in rural and urban regions of the PRA area (EU-27 MSs). Citrus orchards are mainly located in coastal areas or next to rivers where the environmental conditions are favourable for the pathogen to complete its life cycle. In the absence of rain, dew or mist, irrigation applied to citrus in these areas may also favour the survival of the pathogen and the disease development. Citrus fruit are susceptible to infection by *G. citricarpa* from petal fall (April to May) up to 4-6 months later and lemon leaves are susceptible from emergence up to 10 months later. In addition, lemon trees, widely grown in the Southern EU-MSs, have 2-3 additional leaf flushing and flowering periods till September extending in this way their susceptibility period. So far, *G. citricarpa* has established in areas with various climatic conditions and particularly in coastal areas that belong to the warm temperate climatic zone, as most of the citrus-growing regions in the PRA area. In the citrus-growing regions of the PRA area, the low winter temperatures may delay the production and maturation of pseudothecia and ascospores in the leaf litter until mid-summer. In this case, there might be asynchrony between the availability of the pathogen's inoculum and the susceptibility stages of the host. However, the environmental conditions during autumn (September and October) would be favourable for infection and susceptible host tissues (young leaves and fruit), especially in the case of lemons and late-maturing citrus cultivars, would be also present. Climatic comparisons and modelling (EFSA, 2008) suggest that, although the climatic conditions in the citrus-growing regions of the PRA area are marginally suitable for the establishment of the pathogen, the microclimatic conditions occurring in spring (April-May) and autumn (September-October) in parts of these regions would greatly favour the establishment of *G. citricarpa*.

Due to the availability of both susceptible citrus species and suitable climatic conditions, *G. citricarpa* has the potential to establish in the citrus-producing regions of the PRA area (i.e. citrus-growing EU-MSs).

2. <u>Host range</u>

Guignardia citricarpa affects *Citrus, Poncirus, Fortunella* and their hybrids. Except for sour orange (*Citrus aurantium* L.), its hybrids and Tahiti limes (*C. latifolia* Tan.), all commercially grown citrus species are susceptible with lemon (*C. limon* L.) being particularly susceptible.

3. <u>Dispersal</u>

The pathogen has the ability to spread by natural means (wind, rain or irrigation water, etc) and by human activities (grafting of citrus trees using infected rootstocks, scions or budwood, movement of infected plant material, disposal of infected plant or fruit waste, etc). Rain and/or irrigation water are the means by which *G. citricarpa* can spread over relatively short distances (within the tree or between neighboring trees). Spread of the pathogen over longer distances may occur by wind and human activities. However, uncertainties exist due to a lack of information on the distance over which *G. citricarpa* spores could be disseminated by wind or wind-driven rain and the role of insects, birds, etc in the dispersal of *G. citricarpa* spores.

4. <u>Potential consequences</u>

CBS is a leaf-spotting and fruit-blemishing disease that has negligible or no effect on citrus tree health. In the areas of its present distribution the pathogen causes pre- and post-harvest fruit losses. Pre-harvest losses arise when severely affected fruit drop prematurely in the orchard and go to waste, particularly in years favourable for disease development and when fruit is held on the trees past peak maturity. Post-harvest losses are not always apparent, as latent, asymptomatic fruit at harvest may develop symptoms while in transit to the export market or in storage. CBS also affects the external quality of the citrus fruit by causing blemishes that make citrus fruit unsuitable for the fresh fruit market. There is only one report on *G. citricarpa* affecting the internal quality of citrus fruit by lowering the acid content. In the areas of its present distribution, *G. citricarpa* can cause considerable losses where protective chemical sprays are not applied.

Nevertheless, it is expected that in case *G. citricarpa* was introduced in the PRA area, the copper-based fungicides, currently applied in commercial citrus orchards for the control of other pathogens, would most probably reduce the disease incidence and severity and subsequently the consequences of CBS on crop yield and quality. However, there are uncertainties with respect to the level of yield/quality reduction as a result of the establishment and spread of the pathogen in the citrus-producing regions of the PRA area (Southern EU-MSs), as there are only a few experimental data from the CBS-infested areas on the yield/quality losses that the pathogen causes to citrus production.

5. Environmental impact

As *G. citricarpa* does not kill its host, it is expected that the introduction of the pathogen in the citrus-growing regions of the PRA area will have no consequences on the biodiversity with respect to the presence of citrus. However, the introduction of the organism into the citrus-growing regions of the PRA area may require specific chemical control programmes and additional copper-based fungicide sprays, which could negatively impact non-target pests and cause accumulation of copper in soil (copper toxicity), respectively.

6. Introduction potential

Pathways: *G. citricarpa* is not reported to be present in the PRA area (EU-27 MSs) and is considered a harmful organism under the Council Directive 2000/29/EC. Hosts of *G. citricarpa* are exclusively species of the genera *Citrus, Poncirus* and *Fortunella*. All citrus species and cultivars are susceptible to *G. citricarpa* with the exception of sour orange (*C. aurantium* L.) and Tahiti lime (*C. latifolia* Tan.), which are considered to be tolerant. Susceptible citrus species/varieties are extensively grown in the Southern EU-MSs in commercial orchards, nurseries, private and public gardens, roadsides, etc. both in rural and urban areas.

Three pathways of entry of *G. citricarpa* into the PRA area exist:
- Citrus plant material for propagation purposes (rootstocks, scions, budwood, etc), excluding seeds.
- Fresh citrus fruit, and
- Natural means (wind, rain, insects, birds, etc)

Of the above mentioned pathways, the citrus plant material for propagation purposes pathway is blocked because of the current EU legislation (EU Council Directive 2000/29/EC, Annex III, Part A, point 16) that 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 Member States. In addition, the natural means pathway (wind, rain, insects, birds, etc) is of minor importance, due to the great distance between the areas of the current distribution of the organism and the PRA area.

Therefore, under the current EU legislation, the fresh citrus fruit pathway is considered as the major pathway for the entry of *G. citricarpa* into the PRA area.

- *Potential for introduction on each pathway*

  Pathway: Fresh citrus fruit. In Annex IV, Part A, Section I, point 16.4 of the Directive, special requirements regarding "*Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*)" are laid down for the importation and movement of fruits of *Citrus* L., *Poncirus* Raf. and *Fortunella* Swingle as well as their hybrids other than fruits of *Citrus aurantium* L., originating in Third countries. However, there have been numerous notifications (560 during the period 2005-2010) from the EU-27 MSs on interceptions of *G. citricarpa* on fresh citrus fruit consignments imported into the PRA from Third countries. The numerous interceptions indicate that the fresh citrus fruit pathway exists. The likelihood of introduction of *G. citricarpa* into the PRA area on fresh citrus fruit, especially latently infected (asymptomatic fruit) and fruit with conspicuous CBS symptoms, has been estimated by the Bayesian Belief Network (BBN) model as **medium** (66% likelihood) to **high** (33% likelihood) (Fig. 10).

- *Overall Risk for individual pathways*

  Pathway: Fresh citrus fruit
  The BBN model suggests that the risk presented to the PRA area by *G. citricarpa* being present in fresh citrus fruit, particularly latently infected and/or with conspicuous symptoms fruit, is **medium** (62% likelihood) to **high** (30% likelihood) (Fig. 10).

Conclusion

G. citricarpa is not reported to be present in the PRA area (EU-27 MSs) and is considered a harmful organism under the Council Directive 2000/29/EC. Hosts of *G. citricarpa* are exclusively species of the genera *Citrus, Poncirus* and *Fortunella*. All citrus species and cultivars are susceptible to *G. citricarpa* with the exception of sour orange (*C. aurantium* L.) and Tahiti lime (*C. latifolia* Tan.), which are considered to be tolerant. Susceptible citrus species/varieties are extensively grown in the Southern EU-MSs in commercial orchards, nurseries, private and public gardens, roadsides, etc. both in rural and urban areas.

Three pathways of entry of *G. citricarpa* into the PRA area exist:

- Citrus plant material for propagation purposes (rootstocks, scions, budwood, etc), excluding seeds.
- Fresh citrus fruit, and
- Natural means (wind, rain, insects, birds, etc)

Of the above mentioned pathways the citrus plant material for propagation purposes pathway is blocked because of the current EU legislation (EU Council Directive 2000/29/EC, Annex III, Part A, point 16) that 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 Member States. In addition, the natural means pathway (wind, rain, insects, birds, etc) is of minor importance, due to the great distance between the areas of the current distribution of the organism and the PRA area.

Therefore, under the current EU legislation, the fresh citrus fruit pathway is considered as the major pathway for the entry of *G. citricarpa* into the PRA area.

The current EU legislation (EU Council Directive 2000/29/EC, Annex IV, Part A. Section I, paragraph 16.4; Annex IV, Part A, Section I, point 16.1.; Annex V, Part B, point 3) requires that fruit of *Citrus* L., *Poncirus* Raf. and *Fortunella* Swingle as well as their hybrids originating in Third countries:

- shall be subjected to a plant health inspection in the country of origin or the consignor country, before being permitted to enter the Community
- shall be free from peduncles and leaves and the packaging shall bear an appropriate origin mark, and
- will originate in a country recognised as being free from *Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*), in accordance with the procedure laid down in Article 18, or in an area recognised as being free from *Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*), in accordance with the procedure laid down in Article 18, and mentioned on the certificates referred to in Articles 7 or 8 of this Directive, or no symptoms of *Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*), will 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 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.

Citrus fruit imported into the PRA area from Third countries are mainly destined for human consumption. Citrus fruit originating in citrus black spot (CBS)-infested areas may carry the pathogen in the form of (i) conidia within pycnidia in lesions (symptomatic fruit) and/or (ii) latent mycelium (asymptomatic fruit). However, the pathogen has a long latent period on citrus fruit, whereas fruit symptoms are variable in appearance and similar to those caused by other pathogens, mechanical or insect damage. Moreover, *G. citricarpa* has the ability to survive cultural practices, pre- and post-harvest treatments as well as transport and storage conditions and remain undetected on latently infected fruit (asymptomatic) and fruit with low disease incidence and severity.

Susceptible citrus species and varieties are extensively grown in the Southern EU-MSs in commercial orchards and nurseries, private gardens for family consumption, and as amenity trees in public gardens, roadsides, etc. both in rural and urban areas.

So far *G. citricarpa* has established in areas with various climatic conditions and particularly in coastal areas that belong to the warm temperate climatic zone, as do most of the citrus-growing regions in the PRA area. Climatic comparisons and modelling (EFSA, 2008) suggest that, although the climatic conditions in the citrus-growing regions of the PRA area are marginally suitable for the establishment of the pathogen, the microclimatic conditions occurring in spring (April-May) and autumn (September-October) in these regions would greatly favour the establishment of *G. citricarpa*. In addition, it is unlikely that the existing management practices applied in commercial orchards and nurseries in the PRA area would prevent establishment. The long latent period of the pathogen, the widespread occurrence of lemon, which is the most susceptible species and thus, the first to be infected in a new area, the presence of citrus in semi- and/or unmanaged environments, where cultural practices and chemical control measures are not usually undertaken (e.g. private and public gardens, roadsides, etc) and the absence of enemies/antagonists could greatly facilitate the establishment of the pathogen in the PRA area. Irrigation (drip or sprinkler) applied to citrus orchards during the dry periods would promote the maturation of *G. citricarpa* ascocarps produced on leaf litter and provide favourable conditions for the infection of susceptible plant tissues. Moreover, the existence of citrus species/varieties with overlapping crop periods (i.e. co-existence of mature and young fruit on the same tree) and more than one leaf flushing and flowering periods in the same year (e.g. lemon, 'Valencia' sweet orange, etc) are likely to extend the period during which citrus species/varieties grown in the PRA area will be susceptible to the pathogen.

The risk of introduction of *G. citricarpa* on the citrus fruit pathway is mainly associated with the fruit waste (whole symptomatic fruit or peels) derived from packinghouses, households and fresh fruit markets, and its management. In case this waste is not properly managed, the pathogen has the ability to spread by natural means (wind, rain or irrigation water, etc) to susceptible hosts grown in close proximity.

Given the end-use of the fresh citrus fruit (consumption) and the numerous interceptions (560 during the period 2005-2010) of living stages of the pathogen on citrus fruit consignments imported into the PRA area from CBS-infested areas, it is considered that, under the current EU legislation, the likelihood of *G. citricarpa* introduction into the PRA area, as estimated by the BBN model, is **medium** (66% likelihood) to **high** (33% likelihood). However, there are uncertainties with respect to (i) the concentration of *G. citricarpa* on the fresh citrus fruit imported into the PRA area, particularly on the latently infected (asymptomatic) fruit, (ii) the volume and frequency of infected fruit being disposed in the vicinity of susceptible hosts in the citrus-growing regions of the PRA area (i.e. Southern EU-

MSs), and (iii) the time taken for discarded asymptomatic whole fruit or peel to produce pycnidia and conidia before being decomposed by other organisms.

Spread of *G. citricarpa* within the citrus-growing regions of the PRA area is very likely. The pathogen has the ability to spread by natural means (wind, rain or irrigation water, etc) and by human activities (grafting of citrus trees using infected rootstocks, scions or budwood, movement of infected plant material, disposal of infected plant or fruit waste, etc). Rain and/or irrigation water are the means by which *G. citricarpa* can spread over relatively short distances (within the tree or between neighbouring trees). Spread of the pathogen over longer distances may occur by wind and human activities. However, uncertainties exist due to a lack of information on the distance over which *G. citricarpa* spores could be disseminated by wind or wind-driven rain and the role of insects, birds in the dispersal of *G. citricarpa* spores.

G. citricarpa has negligible or no effect on citrus tree health. In the areas of its present distribution it causes pre- and post-harvest fruit losses. Pre-harvest losses arise when severely affected fruit drop prematurely in the orchard and go to waste, particularly in years favourable for disease development and when fruit is held on the trees past peak maturity. CBS also affects the external quality of the citrus fruit by causing blemishes that make citrus fruit unsuitable for the fresh fruit market. Nevertheless, it is expected that in case *G. citricarpa* was introduced in the PRA area, the copper-based fungicides, currently applied in commercial citrus orchards for the control of other pathogens, would most probably reduce the disease incidence and severity and subsequently the consequences of CBS on crop yield and quality. The introduction of the pathogen in the citrus-growing regions of the PRA area will have no consequences on the biodiversity with respect to the presence of citrus. However, the introduction of the organism may require specific chemical control programmes and additional copper-based fungicide sprays, which could negatively affect non-target pests and soil (i.e. copper toxicity), respectively.

In the absence of any control measures, the overall consequences on citrus production as a result of the introduction of *G. citricarpa* into the citrus-growing regions of the PRA area (i.e. Southern EU-MSs) is estimated by the BBN model as **medium** (62% likelihood) to **high** (30% likelihood). However, uncertainties exist around the level of yield/quality reduction as a result of the introduction and spread of the pathogen in the citrus-growing regions of the PRA area, as there are only a few experimental data from the CBS-infested areas on the yield/quality losses that the pathogen causes to citrus production.

Based on the above, it can be concluded that *G. citricarpa* satisfies the IPPC definition of a harmful organism for the EU.

Stage 1 – Initiation

1.1 Background and Initiation

The purpose of this assessment is to evaluate the plant health risk of *Guignardia citricarpa* (citrus black spot) within the framework of EFSA project “Pest risk assessment for the European Community: plant health: a comparative approach with case studies (Prima phacie)”.

The terms of reference are described in EFSA call CFP/EFSA/PLH/2009/01, Pest risk assessment for the European Community plant health: A comparative approach with case studies (EFSA, 2009). The text in Section 1.4 of the call, “Structure and essential requirements of the proposal”, pages 7-9, provide the terms of reference e.g. that a systematic review of risk assessment methodologies, with emphasis on quantitative and semi-quantitative approaches, used in pest risk assessment to analyse and predict the likelihood of entry, establishment and spread, the potential negative consequences, the overall risk characterisation and the associated level of uncertainties be assessed, together with a systematic review of the methods used to assess the effectiveness of management options in reducing the risk of introduction and/or spread. The quantification of economic losses in monetary values and the assessment of potential effects on export markets, employment and tourism were not to be included.

Initiation Point
This Pest Risk Assessment was initiated as a case study pest to be examined within EFSA project CFP/EFSA/PLH/2009/01 (Prima phacie). *Guignardia citricarpa* had been selected as a case study pest because it satisfied a number of criteria needed to provide a range of contrasting pest examples for consideration in the project (see report of kick-off meeting, (MacLeod, 2010).

1.2 Identification of the PRA Area

The PRA area is the 27 Members States of the European Union (Fig. 3) with focus on the continental European area, specifically excluding the ultra-peripheral regions, i.e. the French Overseas Departments, the Spanish Canary Isles and the Portuguese Azores and Madeira

1.3 Available pertinent regulatory information

Previous PRAs

- **Magarey, R.D., Chanelli, S., and Holtz, T. 2009**. Validation study and risk assessment *Guignardia citricarpa* (citrus black spot). United States Department of Agriculture, Animal and Plant Health Inspection Service, Plant Protection and Quarantine, Center for Plant Health Science and Technology, Plant Epidemiology and Risk Analysis Laboratory (PERAL), Raleigh, NC. 19 pp. (http:/www.nappfast.org/pest%20reports/guignardia_citricarpa.pdf).
- **EFSA, 2008**. Pest risk assessment and additional evidence provided by South Africa on *Guignardia citricarpa* Kiely, citrus black spot fungus–CBS. Scientific Opinion of the Panel on Plant Health. *The EFSA Journal* (2008) 925, 1-108
- **Magarey, R.D., and Borchert, D.M. 2007**. Risk Assessment: *Guignardia citricarpa*, (Citrus black spot), CPHST Ad-Hoc Project. USDA-APHIS-PPQ-CPHST-PERAL

Figure 3. The PRA area (i.e. the 27 EU-Member States); Source: http://www.eucountrylist.com

- **USDA/APHIS, 2007.** Risk Assessment for the importation of fresh lemon (*Citrus limon* (L.) Burm. F.) fruit from Northwest Argentina into the Continental United States. 83 pp.
- **USDA/APHIS, 2006.** A Qualitative Pest Risk Analysis for the Importation of Fresh Unshu Orange Fruit (*Citrus reticulata* Blanco var. *unshu* Swingle) from the Republic of Korea into Alaska. 49 pp.
- **DPA and USDA/APHIS, 2004.** Importation of fresh commercial citrus fruit: Clementine (*Citrus reticulata* Blanco var. Clementine) Mandarin (*C. reticulata* Blanco) and Tangerine (*C. reticulata* Blanco) from Chile into the United States. A pathway initiated Plant Pest Risk Assessment. Departamento Protección Agrícola, Servicio Agricola y Ganadero, Santiago, Chile and United States Department of Agriculture, Plant Protection and Quarantine, USA. 74 pp.
- **USDA/APHIS, 2003.** Importation of Fresh Commercial Citrus Fruit: Grapefruit (*Citrus* x *paradisi* Macfad.); Lime (*C. aurantiifolia* [Christm.] Swingle); Mandarin Orange or Tangerine (*C. reticulate* Blanco); Sweet Orange (*C. sinensis* [L.] Osbeck); Tangelo (*C.* x *tangelo* J.W. Ingram & H.E. Moore); from Peru into the United States. A Pathway-Initiated Plant Pest Risk Analysis. 92 pp. (http://www.senasa.gob.pe/servicios/eng/plant_health/phytosanitary_surveillance/PeruCitrusPRA1028.pdf)
- **Hattingh, V., Roux le, H. and Schutte, G.C. 2000**. Citrus Black Spot: Pest Risk Assessment for the review of current phytosanitary regulations pertaining to the export of fresh Citrus fruit from the Republic of South Africa to the EU. South African National Department of Agriculture, Directorate Plant Health and Quality, 25 pp.

Available Pest Fact Sheets/ Pest Alerts, etc.:

- **Chung, K.-R. and Timmer, L.W. 2009.** Citrus diseases exotic to Florida: Black Spot. University of Florida, Institute of Food and Agricultural Sciences, Florida Cooperative Extension Service, Plant Pathology Department. 4 pp. (http://edis.ifas.ufl.edu)
- **COSAVE, (2008).** Hojas de datos sobre organismos cuarentenarios para los paises miembros del COSAVE: *Guignardia citricarpa* Kiely. (http://www.cosave.org/admin/files/bc499d5254121f5_4.pdf)
- **CAB International. 2007.** Crop Protection Compendium. Wallingford, UK (http://www.cabicompendium.org/cpc/home.asp)
- **EPPO/CABI 1997**. *Guignardia citricarpa*. In: Quarantine Pests for Europe, 2nd edition, CAB International, Wallingford, UK. pp. 773–781
- **Schubert, T., Sutton, B. and Jeyaprakash, A. 2010.** Pest Alert: Citrus Black Spot (*Guignardia citricarpa*) discovered in Florida. Florida Department of Agriculture and Consumer Services, Division of Plant Industry. (http://www.doacs.state.fl.us/pi/pest_alerts/pdf/citrus-black-spot-pest-alert.pdf)
- **OEPP/EPPO. 2003**. EPPO Standards PM 7/17 (1). Diagnostic protocols for regulated pests: *Guignardia citricarpa*. *OEPP/EPPO Bulletin* 33: 245–247

A datasheet for *G. citricarpa* was prepared in July 2010, as part of the Prima phacie 1st interim report. As new findings on the organism and the disease it causes have been published or became available in the mean time, an updated datasheet was prepared in June 2011 (available in the EFSA extranet).

Current regulatory status

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

G. citricarpa is considered a harmful organism for many citrus-producing countries, which have implemented regulations to prevent its introduction and spread into their territory. The organism is listed as an A1 pest by EU, EPPO, CPPC and as A2 pest by COSAVE, APPPC and IAPSC.

G. citricarpa is considered a harmful organism for many citrus-producing countries, which have implemented regulations to prevent its introduction and spread into their territory. The organism is listed as an A1 pest by EU, EPPO, CPPC and as A2 pest by COSAVE, APPPC and IAPSC.

---

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

EU Legislation

- <u>Legislation specific to *G. citricarpa*</u>

- *G. citricarpa* is classified as a harmful organism for the European Community and is listed in Annex II, Part A, Section I of the Council Directive 2000/29/EC ("*Harmful organisms not known to occur in the community and relevant for the entire community and whose introduction into, and spread within, all EU Member States shall be banned if they are present on certain plants or plant products*").
- Annex IV, Part A. Section I, paragraph 16.4 describes special requirements which must be laid down by all Member States for the introduction into the Community of fruits of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, other than fruits of *Citrus aurantium* L., originating in third countries. According to these requirements:

  (a) the fruits originate in a country recognised as being free from *Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*), in accordance with the procedure laid down in Article 18,

  or

  (b) the fruits originate in an area recognised as being free from *Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*), in accordance with the procedure laid down in Article 18, and mentioned on the certificates referred to in Articles 7 or 8 of this Directive,

  or

  (c) 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

  (d) the fruits 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.

The organism is mentioned in the Council Directive 2000/29/EC as "*Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*)", as in the past, there was a confusion with respect to the identity of the fungus (i.e. pathogenic and non-pathogenic to *Citrus* strains were reported to exist). However, Baayen *et al.* (2002) showed that the non-pathogenic to citrus *Guignardia* type belonged to a distinct species, *G. mangiferae* (anamorph *Phyllosticta capitalensis*), which is a common endophyte in many plant families.

- <u>Legislation not specific to *G. citricarpa*</u>
  - Annex III, Part A, point 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 Member States.
  - Annex IV, Part A, Section I, point 16.1. of the Directive 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.
  - Annex V, Part B, point 3, states that fruits of *Citrus* L., *Poncirus* Raf. and *Fortunella* Swingle and their hybrids originating outside 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.

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

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

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

- EPPO Reporting Service
- EUROPHYT for notifications of interceptions
- Prima phacie Interim Report No 1: Annex 1g-Datasheet for Prima phacie case study pest: *Guignardia citricarpa*
- Prima phacie Interim Report No 1: Annex 4g-WP2: Systematic literature review to support assessment of risk for *Guignardia citricarpa*
- Prima phacie Interim Report No 1: Annex 5g: WP3: Systematic literature review to evaluate risk management options for *Guignardia citricarpa*
- New information on the organism and the disease it causes, which became available in the literature since the first data sheet was compiled (see Prima phacie Interim Report No 1, Annex 1g)

Stage 2 - Pest Risk Assessment
(Outline approach)

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

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

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

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

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

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

Contact for queries regarding operation of this approach:
Willem Roelofs (w.roelofs@fera.gsi.gov.uk) Tel: +44(0)1904 462495

or

Alan MacLeod (a.macleod@fera.gsi.gov.uk) Tel: +44(0)1904 462350

Stage 2 - Pest Risk Assessment

Consequences of Introduction

2.1 Climate-Host Interaction

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

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

Information / evidence: *Provide reasoning then give judgment.*

The availability, quantity and distribution of hosts in the PRA area

Hosts of *G. citricarpa* are exclusively within the genera *Citrus, Poncirus, Fortunella*. Except for sour orange (*Citrus aurantium* L.), its hybrids and Tahiti limes (*C. latifolia* Tan.), all commercially grown citrus species are susceptible, with lemon (*C. limon* L.) being particularly susceptible (Kotzé, 2000; Aguilar-Vildoso *et al.,* 2002). Citrus species grown in in the risk assessment area include *C. sinensis* L. (sweet orange), *C. limon* (lemon) and *C. reticulata* (mandarin) (FAOSTAT, 2010). *C. paradisi* (grapefruit), *C. bergamia* (bergamot) and *C. japonica* (kumquat, syn. *Fortunella japonica*) are grown to a lesser extent. *Poncirus trifoliata* (trifoliate orange) and its hybrids (e.g. Troyer citrange, Carrizo citrange, etc) are used as rootstocks and thus, they are grown mainly in nurseries.

C. aurantium (sour orange), which is used as a rootstock and an amenity tree, is also widely grown in nurseries, private and public gardens and roadsides in the PRA area. However, it is considered not to be susceptible to *G. citricarpa*.

The European citrus fruit sector is strongly orientated towards growing for the fresh produce market. Citrus species are widely grown in commercial orchards and nurseries, private gardens for family consumption, and as ornamentals in public gardens and along the roadsides both in rural and urban regions of the PRA area (EU-27 MSs). Lemon (*C. limon* L.), which is the most susceptible citrus species and the first to be infected by the pathogen in a new area, is also widely distributed in the citrus-growing EU-MSs in both rural and urban regions.

In 2007, the total area grown commercially with citrus (including small-fruited citrus) in the PRA area was 493,412 ha (Eurostat, 2010), with Spain (314,908 ha), Italy (112,418 ha), Greece (44,252 ha), Portugal (16,145 ha), Cyprus (3985 ha) and France (1705 ha) being the main citrus-producing EU-MS (Tables 1-3; Fig.3). Of the 493,412 ha grown commercially with citrus, 62,854 ha were grown with lemons (Eurostat, 2010; Table 2).

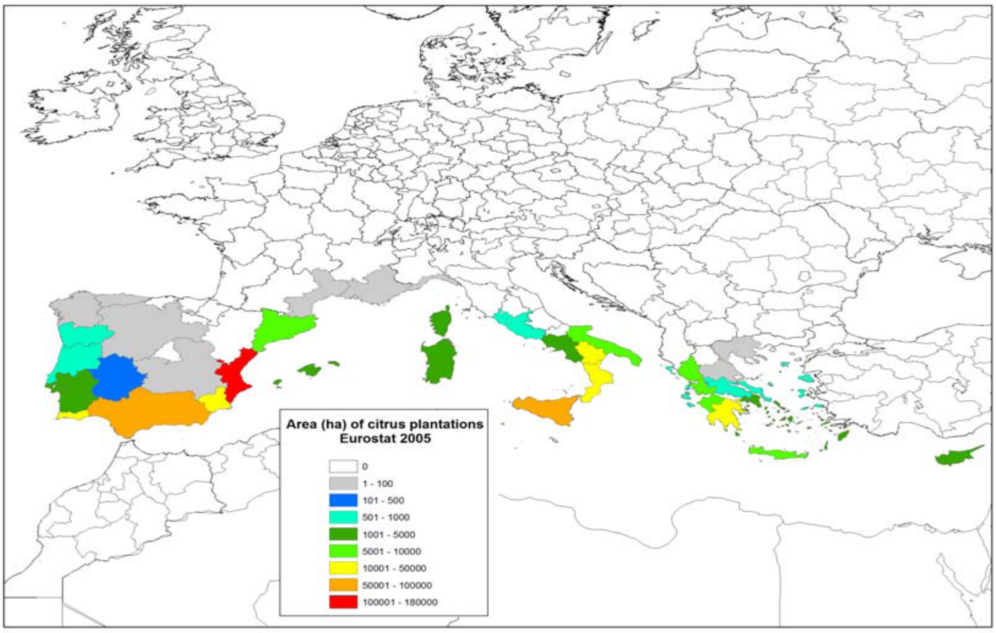

Figure 3. Area (ha) grown with commercial citrus orchards in the PRA area (Eurostat, 2005)

The suitability of the environment in the PRA area

Citrus spp. do not tolerate cold climates, they are grown mainly in areas situated at latitude between 40º North and 40º South. Therefore, they are typically grown in "Mediterranean" type climates.

G. citricarpa has established in areas with various climatic conditions, such as cool, misty, semi-arid, dry, hot, sub-tropical, etc. (Wager, 1952). Kiely (1949) reported that in Australia the most favourable conditions for the development of CBS from its quiescent condition are hot days accompanied by dry winds in spring and summer as citrus fruit approach maturity.

The most rapid development of CBS symptoms on fruit occurs approximately at 30^{0}C. When such temperatures coincide with the peak maturity of the fruit, CBS epidemic in its most severe form occurs (Kiely, 1949; Sutton and Waterston, 1966). In South Africa, the disease was first reported in 1929 in the cool misty belt of Natal, but in 1945 it assumed more serious proportions when it spread to hot, dry sub-tropical East and North Transvaal (Sutton and Waterston, 1966). According to Kotzé (1981), climate influences the rate of CBS spread, but not necessarily the establishment of *G. citricarpa*. He also reported that in the past, the over-reliance on a climatic barrier for the establishment of CBS in a new area was proven unsatisfactory.

Based on the Köppen-Geiger Climate Classification System (Fig. 4), which groups the world into various zones based on the vegetation type, average annual and monthly temperatures and precipitation and the present geographic distribution of CBS (EPPO, 2006; Paul *et al.,* 2005, WTO, 2003, NAPPO, 2010), *G. citricarpa* occurs mainly in coastal areas that belong to the warm temperate zone (C). However, within these areas, the disease is present in regions with various climatic conditions.

Table 1. Area grown commercially with oranges in the each of the citrus-producing EU-Member States in 2007 (Eurostat, 2010)

EU Member State	Area (ha)
European Union (27 countries)	**279,048**
Greece	**32,439,9**
Kentriki Ellada, Evvoia	6,531.02
Ipeiros	3,993.92
Peloponnisos	17,347.7
Nisia Aigaiou, Kriti	883.87
Kriti	3,410.53
Other Greek regions	266.51
Spain	**158,824**
Extremadura	278.56
Cataluña	2,080.09
Comunidad Valenciana	76,593
Illes Balears	660.89
Andalucía	64,158.7
Región de Murcia	14,514
Canarias (ES)	538.36
France	**28.55**
Provence-Alpes-Côte d'Azur	1.23
Corse	27.1
France, non alloué	0.22
Italy	**73,785.9**
Liguria	7.27
Toscana	6.67
Lazio	399.43
Abruzzo	178.14
Molise	9.08
Campania	689.25
Puglia	3,462.78
Basilicata	4,640.92
Calabria	17,273.7
Sicilia	43,731.3
Sardegna	3,387.33
Cyprus	**1,554**
Portugal	**12,416**
Norte	734.26
Centro (PT) (NUTS95)	401.86
Lisboa e Vale do Tejo (NUTS95)	256.47
Alentejo (NUTS95)	1,585.9
Algarve	9,437.46

Table 2. Area grown commercially with lemons in the each of the citrus-producing EU-Member States in 2007 (Eurostat, 2010)	
EU-Member State	**Area (ha)**
European Union (27 countries)	**62,854.8**
Greece	**5,180.49**
Kentriki Ellada, Evvoia	1,969.14
Peloponnisos	1,730.95
Nisia Aigaiou, Kriti	308.3
Kriti	277.4
Other Greek regions	885.8
Spain	**39,859**
Principado de Asturias	1.31
Cataluña	20.38
Comunidad Valenciana	9,127.21
Illes Balears	397.51
Andalucía	5,646.85
Región de Murcia	24,561
Canarias (ES)	104.78
France	**22.7**
Provence-Alpes-Côte d'Azur	5.06
Corse	17.34
France, non alloué	0.3
Italy	**16,633.6**
Piemonte	0.01
Liguria	17.24
Toscana	0.23
Lazio	82.5
Campania	954.63
Puglia	146.5
Basilicata	39.79
Calabria	967.09
Sicilia	14,338.8
Sardegna	86.75
Cyprus	**665**
Portugal	**494.04**
Norte	52.75
Centro (PT) (NUTS95)	27.08
Lisboa e Vale do Tejo (NUTS95)	196.67
Alentejo (NUTS95)	11.01
Algarve	206.53

Table 3. Area grown commercially with small-fruited citrus (mandarins, tangerines, etc) in each of the citrus-producing EU-Member States in 2007 (Eurostat, 2010)	
EU-Member State	**Area (ha)**
European Union (27 countries)	**151,510**
Greece	**6,631.71**
Peloponnisos	3,379.98
Nisia Aigaiou, Kriti	213.8
Kriti	356.9
Other Greek regions	2,598.09
Spain	**116,225**
Extremadura	38.68
Cataluña	10,777.2
Comunidad Valenciana	90,878.5
Illes Balears	98.5
Andalucía	9,999
Región de Murcia	4,433.55
France	**1,654.21**
Provence-Alpes-Côte d'Azur	1.95
Corse	1,648.42
France, non alloué	3.84
Italy	**21,997.9**
Liguria	3.89
Lazio	178.31
Molise	9.08
Campania	634.9
Puglia	4,059.31
Basilicata	2,093.54
Calabria	10,774.2
Sicilia	3,106.62
Sardegna	1,138.1
Cyprus	**1,766**
Portugal	**3,235.21**
Norte	133.24
Centro (PT) (NUTS95)	54.01
Lisboa e Vale do Tejo (NUTS95)	37.3
Alentejo (NUTS95)	247.52
Algarve	2,763.14

More specifically, CBS occurs in fully humid regions with warm or hot summers (Cfa or Cfb) [e.g. south-eastern parts of South Africa, eastern parts of Australia, southern parts of Brazil, south-eastern parts of China, Florida (USA)] and in regions with dry winters and warm or hot summers (Cwa or Cwb) (e.g. north-eastern parts of South Africa, Zambia, Zimbabwe, south-western parts of China). Moreover, CBS is also present in a few areas that belong either to the equatorial zone (A) and are characterised by dry winters (Aw) (e.g. northern parts of Australia, Mozambique) or to the arid zone (B) with dry and hot summers (Bsh) (e.g. some regions in the north-eastern part of Australia) (Fig. 4).

Based on the map of the European Hardiness Zones (Magarey *et al.,* 2008), most of the citrus-growing regions in the PRA area (EU-27) belong to the warm temperate climatic zone (C) with dry warm or dry hot summers (Csa or Csb) (Fig. 5). Similar climatic conditions also occur in the south-western part of the Western Cape (South Africa) (Fig. 4), but based on the published information, *G. citricarpa* has not been detected in that region.

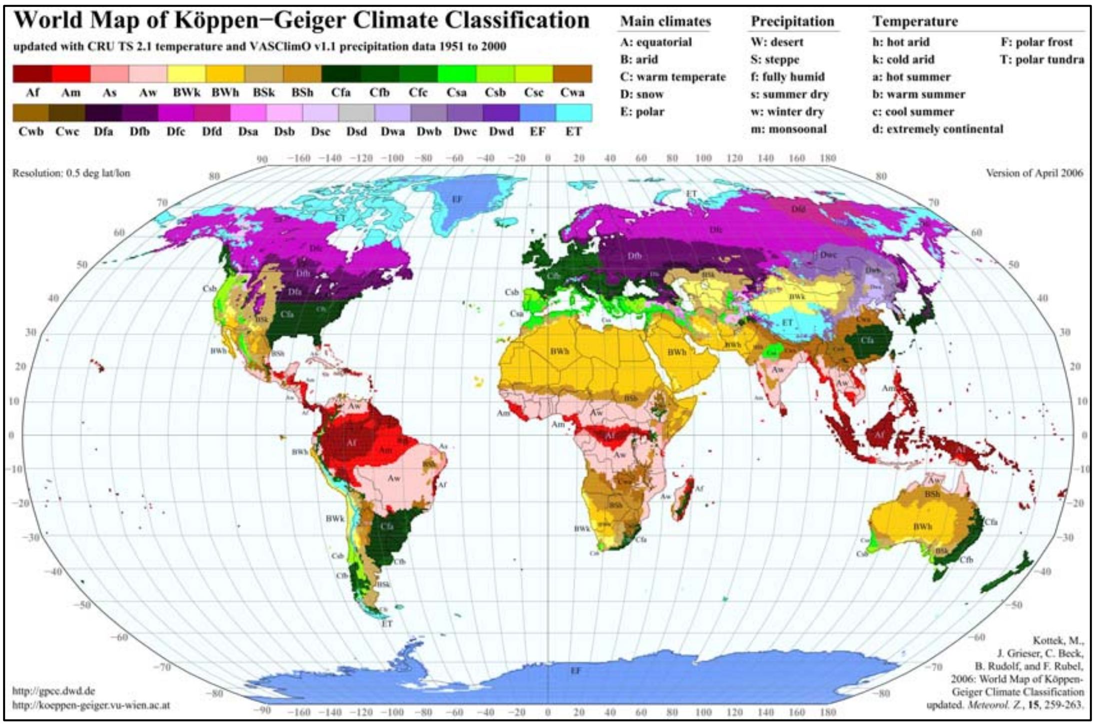

Figure 4. The world Köppen-Geiger climate classification system (Source: http://koeppen-geiger.vu-wien.ac.at/shifts.htm#maps)

When Paul *et al.* (2005) used the CLIMEX model to compare the climate in Europe with that in the CBS-infested areas of South Africa and Australia, they found that the climate in the majority of the citrus-growing EU-MS is unsuitable for the establishment of *G. citricarpa.* Paul *et al.* (2005) assumed that the main factor limiting the establishment of *G. citricarpa* in some citrus-growing areas is the low winter temperatures that do not allow the survival of the pathogen.

EFSA (2008), using the same CLIMEX parameter values selected by Paul *et al.* (2005) and the same thresholds for favourability but recent weather station and gridded data averaged over the last 8-10 years, found that:

- Some parts of the EU citrus-growing regions are marginally suitable for the establishment of *G. citricarpa* and the development of the disease.
- The lower limiting temperature and the cold stress temperature threshold parameters for CLIMEX were set very high by Paul *et al.* (2005) compared to the published information on cold temperature survival for *G. citricarpa* (Agostini *et al*., 2006; Peres *et al*., 2007).
- Citrus orchards in Europe generally have lower maximum winter temperatures (but similar minimum temperatures) compared to those in South Africa. For example, the

minimum winter temperatures in Valencia, Messina and Porto in Spain are similar to those occurring in Addo region in the Eastern Cape Province of South Africa.

In the CBS-infested areas, the maturation of ascospores on infected leaves shed during late autumn, winter and early spring occurs almost simultaneously in early summer (Kotzé, 1963; McOnie, 1964c; Lee and Huang, 1973). However, according to Lee and Huang (1973) severe CBS epidemics have also occurred in areas where ascospores were produced on leaves shed in spring and early summer. It has also been reported that occasionally citrus leaves abscised in winter are decomposed by other organisms before ascospore maturation (Kotzé, 1981). Nevertheless, citrus leaves are shed all year around and, depending on the temperature and wetting regimes, pseudothecia with ascospores can develop in infected leaf litter within 40 days from leaf drop (McOnie, 1964c; Lee and Huang, 1973; Kotzé, 1981).

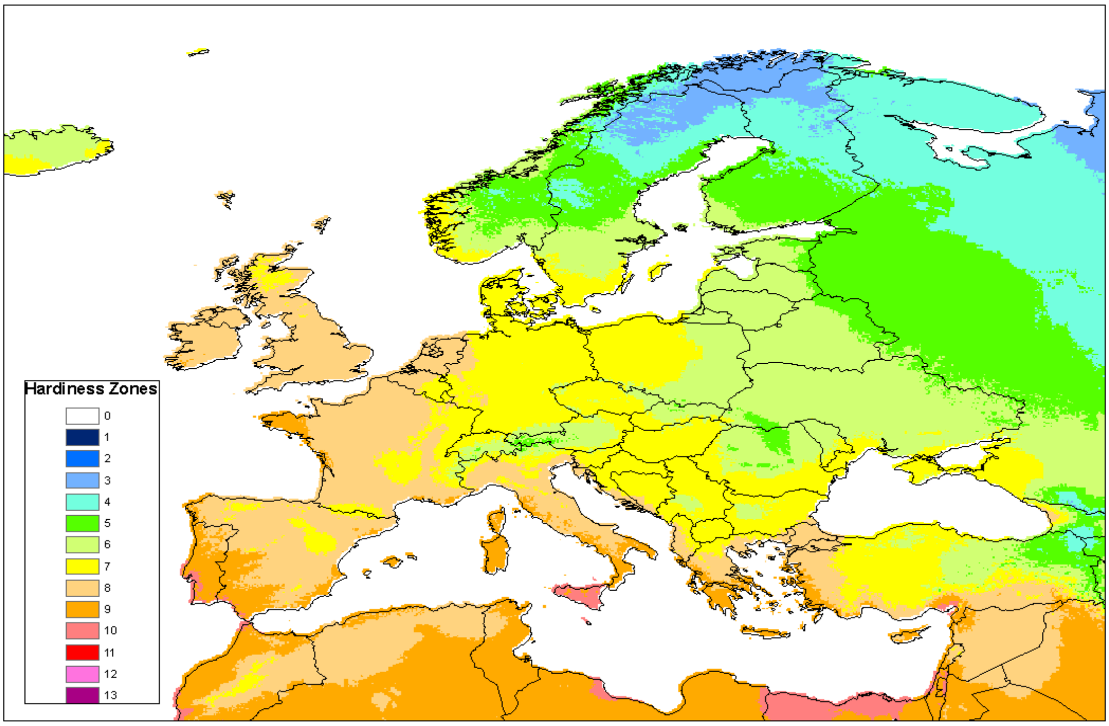

Figure 5: The European Hardiness Zones (updated by Magarey *et al*., 2008)

However, the time required for ascospore maturation in the CBS-free areas has not been studied and thus, it is difficult to estimate the periods during which ascospores will be potentially released in the PRA area. However, in the citrus-growing regions of the PRA area, the low winter temperatures are unlikely to affect the survival of the organism but may delay the production and maturation of pseudothecia and ascospores in the leaf litter (Kiely, 1948; Kotzé, 1981; Lee and Huang, 1973) until mid-summer. In this case, there might be asynchrony between the availability of the pathogen's inoculum and the susceptibility stages of the host. However, the environmental conditions during autumn (September-October) would be potentially favourable for infection and susceptible host tissues (young leaves and fruit), especially in the case of lemons and late-maturing citrus cultivars, would be also present (EFSA, 2008).

There is no information available in the literature on the environmental conditions, particularly temperature regimes and wetness durations that favour infection of citrus by *G. citricarpa*. Nevertheless, as spore germination is a requisition for infection, it may be assumed that the environmental conditions that favour spore germination would also be conductive for infection. Kiely (1948) reported that *in vitro* no germination of *G. citricarpa* ascospores occurred within the first 24 h of incubation and that the highest percentage of germination was observed after 48 h of incubation. On the contrary, McOnie (1967) obtained peak ascospore germination (approximately 100%) within 24 h of incubation on PDA at 25ºC, with most of the appressoria being formed between 24 and 48 h of incubation. According to Kotzé (1963) studies, 15.7% of *G. citricarpa* ascospores germinated after 15 h of incubation at 29.5 ºC. In artificial inoculations of citrus fruit in the orchard, McOnie (1967) showed that ascospores could infect with at least 15 h of continuous wetness, which is in agreement with the germination data obtained by Kotzé (1963). According to Noronha (2002), at a temperature of 10 ºC, 20% of *G. citricarpa* conidia formed appressoria after 36 h of incubation, with the highest percentage of appressoria being formed between 25 and 30 ºC. Kotzé (1963) reported that the highest germination rate of *G. citricarpa* conidia was observed at 29.5 ºC, which was the highest temperature tested. However, preliminary studies with artificial inoculations of lemon seedlings with *G. citricarpa* conidia showed that at 22.5 ºC high levels of infection occurred with 14 h of leaf wetness duration (IAM-UPV, 2008).

No information is available in the literature on the ability of *G. citricarpa* ascospores and conidia to withstand dry interruptions during the germination and infection process. However, studies in Brazil have shown that 10 h/day of leaf wetness were adequate for infection to occur (Reis *et al.,* 2006), which suggests that either the requirement for leaf wetness duration is lower than that reported in other studies (Kotzé, 1963; McOnie, 1967) or the pathogen can withstand dry periods during the infection process.

Citrus fruit are susceptible to CBS for 4-6 months after petal fall (Baldassari *et al*., 2001; Kellerman and Kotzé, 1977; Kotzé, 1981; Spósito *et al*., 2008), whereas leaves, in particular lemon leaves, are susceptible from emergence up to 10 months later (Truter *et al.,* 2004; 2007). The number of citrus leaf flushes per year may vary depending on the variety, the cultural practices (e.g. pruning, fertilisation, irrigation, etc.), the rootstock, etc. However, the main flowering period for most citrus species/cultivars grown in the PRA area is from early-April to early-May. The main leaf flush period is in spring (April-May) and coincides with the flowering period (Guardiola, 1997; Krezdorn, 1986). In the case of lemon, one or two additional flowering and leaf flushing periods occur until September (Agustí, 2000; CORERAS, 2007; Cutuli *et al*., 1985) extending in this way the period during which lemon trees are susceptible to infection by *G. citricarpa*. The above-mentioned are further supported by the information provided by the Italian National Plant Protection Organisation (NPPO) as a response to the questionnaire distributed to the EFSA Network on Plant Health. According to this information, lemon cultivars grown commercially in orchards and nurseries, in private gardens for family consumption and as ornamentals in public gardens, roadsides, etc both in urban and rural regions in Italy have 2-3 leaf flushes and 1-3 flowering periods per year (Table 4).

The CLIMEX model cannot readily be used to analyse specific periods of the year when citrus are at a susceptible stage and *G. citricarpa* inoculum is potentially available. Moreover, the model cannot directly take into account the effect of leaf wetness, a critical environmental factor for the successful infection of citrus by a fruit and foliage fungal pathogen like *G. citricarpa* (Kotzé, 1963 and 1981). Due to the above limitations of the CLIMEX model, EFSA (2008) applied the Magarey generic infection model for foliar pathogens (Magarey *et al.,* 2005) to predict potential infection events in the PRA area over a

10-year period (1998-2007). The results showed that significant numbers of infection events by *G. citricarpa* conidia and ascospores were predicted to occur at many locations in spring (April-May) and autumn (September-October), but no infection was predicted to occur in summer (June-August). Citrus leaves are susceptible almost throughout the year (i.e. from emergence up to 10 months of age) and fruit for 4-6 months after petal fall. However, as lemon trees have more than one leaf-flushing and flowering period from spring until September, they would be susceptible in autumn too.

Table 4. Number of leaf-flushing and flowering periods of lemon cultivars grown commercially in Italy (Source: NPPO of Italy, 2010)

Region	Cultivar	No of leaf flushes/year	No of flowering periods/year
Sicilia	Femminello Comune	3	3
	Femminello Siracusano	3	3
	Femminello Zagara Bianca	3	3
	Monachello	2	2
	Interdonato	2	2
Campania	Sfusato Amalfitano	2	1
	Ovale di Sorrento	2	1
Puglia/Basilicata/Sardenia	Femminello Comune	2	1

Due to the availability of both suitable citrus species and suitable climate, *G. citricarpa* has potential to establish in two to three plant hardiness zones (see Fig. 5. Plant hardiness zones 8, 9 and 10) in the PRA area.

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

2.2 Host Range

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

Information / evidence

Guignardia citricarpa affects *Citrus, Poncirus, Fortunella* and their hybrids. Except for sour orange (*Citrus aurantium* L.), its hybrids and Tahiti limes (*C. latifolia* Tan.), all commercially grown *Citrus* species are susceptible (Kotzé, 2000; Aguilar-Vildoso *et al.,* 2002). Lemon (*C. limon* L.) is particularly susceptible and, thus, in an unaffected area, CBS usually appears first on lemons (Kotzé, 2000). Although in the past, late-maturing sweet orange varieties (e.g. 'Valencia') were considered to be more susceptible than early maturing (Kotzé, 1981), it has been recently shown that some early-, mid- and late-maturing sweet orange varieties are equally susceptible to infection by *G. citricarpa* (Schinor *et al.*, 2002; Baldassari *et al.*, 2006).

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

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

2.3 Dispersal potential

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

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

Information / evidence:

G. citricarpa infective propagules (conidia, ascospores) are spread by wind and/or water (rain or irrigation). As a rule, pathogens disseminated by wind spread over long distances within a short period of time (Aylor, 1990), whereas splash-dispersed pathogens reach mostly short distances from the primary source of inoculum (Huber *et al*., 1998). Ascospores of *G. citricarpa,* produced abundantly within pseudothecia on leaf litter, dead twigs and petioles on the orchard floor are released during rainfall events and are disseminated by air currents throughout the canopy and over longer distances (Kiely, 1948). *G. citricarpa* conidia, produced within pycnidia on symptomatic mature fruit, dead twigs, fruit pedicels, attached leaves and in abundance on leaf litter, may be either splash-dispersed onto the tree canopy by rain, overhead or sprinkler irrigation water or washed-off by rain, dew or overhead irrigation from the upper infected fruit, leaves, etc to the lower ones that are still at the susceptible stage (Agostini *et al*., 2006; Spósito *et al*., 2008). By this mechanism, conidia of *G. citricarpa* can spread over relatively short distances (Kotzé, 1981; 1996; Kiely, 1948). This is further supported by studies conducted by Spósito *et al*. (2007) in Brazil in citrus orchards with 14-29 year-old trees of 3 m minimum height according to which, infected with CBS trees were aggregated in small foci, with a minimum radius of 24.7 m. Based on these results, the authors considered that among the biological, physical and environmental factors that could be involved in the aggregation of infected trees, the most important seemed to be the limitation of the pathogen inoculum dispersal over long distances (Spósito *et al*., 2007).

No other studies have been carried out on the distance or height above inoculum sources over which *G. citricarpa* conidia can be dispersed. However, studies carried out with other splash-dispersed fungi (Fitt *et al.,* 1989) have shown that in still air conidia covered with mucilage, like those of *G. citricarpa,* are splash-dispersed up to a height of not more than 50 cm or up to a distance of 1 m from the inoculum source with their numbers decreasing steeply with increasing height or distance. However, the dispersal pattern of conidia depends on the size of conidia and the velocity of the incident raindrop as well as on the presence of air currents. Rain tower experiments have shown that in still air large raindrops spread the conidia over shorter distances compared with the smaller drops (Fitt *et al.,* 1989). Generally, the size of raindrops that reach the ground are of 0.2-5 mm in diameter, since smaller drops evaporate rapidly, unless relative humidity is near to 100%, and larger drops break up when they fall at speeds approaching their terminal velocities. Nevertheless, rain with many large drops will effectively disperse these pathogens (Fitt *et al*., 1989).

In the presence of wind, conidia carried in small splash drops may also become wind-borne as an aerosol of fine spray (Fitt *et al*., 1989). The significance of wind in the dispersal of pathogens removed from the inoculum source in splash droplets becomes greater as the size of the inoculum particles becomes smaller. According to Fitt *et al*. (1989), small-sized conidia like those of *Phoma exigua* var. *foveata* (Foister) Boerema (7 × 3 μm), are more likely to be carried in small splash droplets, which become wind-borne. Therefore, under windy conditions, the small-sized *G. citricarpa* conidia (9.4-12.7 x 5-8.5 μm) (Baayen *et al.*, 2002) carried in small splash droplets could also become wind-borne and disperse over longer distances. Moreover, water drops formed on the leaves due to fog, dew, mist, overhead or sprinkler irrigation may cause drip-splash of *G. citricarpa* inoculum under canopies, which may be as important as direct rain-splash. These drip drops may have sufficient impact force for the dispersal of conidia in splash drops because they fall only short distances and thus, they are usually larger than 5 mm in diameter and less likely to break up compared to raindrops (Fitt *et al.*, 1989).

Commercial citrus orchards in the PRA area are usually irrigated during the dry periods (Eurostat, 2010). The irrigation systems used are drip- or mini-sprinklers (ARI, 2008; Colombo, 2004). In the absence of rain, dew, mist (commonly occurring during the night in the coastal citrus-growing areas), sprinkler irrigation or pesticide sprays using high pressure equipment may cause direct- and/or drip-splash of *G. citricarpa* inoculum produced on the leaf litter on the orchard floor. Citrus trees grown in private gardens are usually irrigated with a water hose, a practice which may also promote the splash-dispersal onto the tree canopy of *G. citricarpa* inoculum produced on leaf litter or discarded fruit or peel. The irrigation of citrus in the PRA area during the dry periods may also favour the maturation of pseudothecia and ascospores formed on leaf litter and the infection process by providing conducive microclimatic conditions even in citrus-growing areas where the climatic conditions are unfavourable.

Long distance spread of *G. citricarpa* may also occur by human activities, such as grafting of citrus trees using infected rootstocks, scions or budwood, disposal of infected plant material in the vicinity of susceptible hosts, etc. Infected citrus plant material for propagation purposes (rootstocks, scions, budwood) and fruit for human consumption could be freely distributed around the citrus-growing regions of the PRA area in the means of trade or movement by individuals. Such human-assisted distribution would lead to the spread of the organism from infested to non-infested regions within the citrus-growing EU-27. Latently infected plant material, such as rootstocks, scions, budwood and fruit, particularly fruit with pedicels and leaves, poses an even higher risk due to the absence of CBS symptoms. It is worth mentioning that in South Africa the organism is considered to have spread from Pietermaritzburg, where the disease was first reported in 1929 (Wager, 1952), to non-infested areas on latently infected nursery trees. It is also assumed that the disease was spread from South Africa to Zimbabwe on citrus budwood or nursery trees before restrictions were placed on plant material (Whiteside, 1965). Infected leaf and pedicel debris present in the conveyances used for the transportation of citrus fruit could also spread the pathogen from infested to non-infested regions within the citrus-growing EU-MSs.

The role of insects, birds, etc. in the spread of *G. citricarpa* has not been documented. However, Kiely (1948) considered that insects, birds etc. may be able to carry and deposit *G. citricarpa* conidia on the wounds they cause on the host.

2.3: Dispersal potential		
Rating	**Description**	**Probability Assignment [1]**
High	Pest has high biotic potential, e.g., many generations per year, many offspring per reproduction (“r-selected” species), AND evidence exists that the pest is capable of rapid dispersal , e.g., over 10 km/year under its own power; via natural forces, wind, water, vectors, etc., or human-assistance.	**35%**
Medium	Pest has either high reproductive potential OR the species is capable of rapid dispersal.	**65%**
Low	Pest has neither high reproductive potential nor rapid dispersal capability.	0%
	Check sum =	100%

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

2.4 Potential Consequences

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

Information / evidence:

Consequences on crop yield

CBS is a leaf-spotting and fruit-blemishing disease that does not kill the host plant. In citrus plant propagation material (rootstocks, scions, budwood), *G. citricarpa* is likely to be present as latent mycelium (aymptomatic infections) (Kiely, 1948a; Wager, 1952; Calavan, 1960; Whiteside, 1965; Kotzé, 2000; Timmer, 2004; Chung *et al*., 2009). Therefore, the direct effect that *G. citricarpa* might have on the yield and quality of nursery stock (rootstocks, scions, budwood) grown in the PRA area is expected to be negligible.

In the areas of its present distribution, *G. citricarpa* causes pre- and post-harvest crop losses. Pre-harvest losses arise when severely affected fruit drop prematurely in the orchard and go to waste, particularly in years favourable for disease development and when fruit is held on the trees past peak maturity (Wager, 1945; 1952; Kiely, 1969; Kotzé, 1981; 2000; Spósito, 2003; CABI, 2006). Already in 1895, CBS caused significant losses throughout Australia (Benson, 1895). Kiely (1970) reported that by 1970, losses due to CBS were so severe in New South Wales that it was seen as the most serious disease affecting citrus production in Australia. According to Seberry *et al*. (1967), if CBS is not controlled, it may cause total loss of the crop and therefore, in some areas, citrus production will be impossible without effective control programmes. Kiely (1949) also reported that the most serious type of CBS lesions is virulent spot, which appears when the fruit rind is fully mature. In this case two-thirds of the fruit surface may be affected by the irregular growth of a single spot within 4-5 days. Affected fruit fall readily and, in orchards where protective chemical sprays have not been applied, half of the crop drops within several days (Kiely, 1949). In studies carried out in 'Valencia' sweet orange orchards in Brazil during the period 2001-2002, the percentage of fruit drop due to *G. citricarpa* infection was reported to be on the unsprayed trees (control) 63.7% (Feichtenberger, 2010). Spósito (2003) showed that, in commercial 'Valencia' sweet citrus orchards in Brazil, the percentage of fruit drop due to *G. citricarpa* infection was linearly correlated with the disease incidence and that no fruit were harvested from trees with 97.9% disease incidence. In addition, *G. citricarpa* may cause considerable post-harvest losses, which are not always apparent, as latent, asymptomatic fruit at harvest may develop symptoms while in transit to the export market or in storage (Kiely, 1948b; Smith, 1962; Kotzé, 1963; 1981; Brodrick, 1969; 1975; Reuther *et al*., 1978; Agostini *et al*., 2006).

No adequate experimental data was found in the literature on the level of yield loss caused by the pathogen in the areas of its present distribution in the absence of control measures. Therefore, there is uncertainty concerning the effects that *G. citricarpa* might have on crop yield in the citrus-growing regions of the PRA area.

Consequences on crop quality

CBS affects the external quality of the citrus fruit by causing blemishes that make citrus fruit unsuitable for the fresh market (Calavan, 1960; Cobb, 1897; Kellerman and Kotzé, 1977; Kotzé, 1963, 1981). In 1945, 90% of the citrus fruit produced from unsprayed orchards in the northern provinces of South Africa were rendered unfit for export (McOnie, 1964d; Sutton and Waterston, 1966). Brodick (1969) reported that in the 1960s, 60% of the fruit produced in many citrus-producing areas in South Africa was unsuitable for export. According to Kotzé (1963), in 1960 and 1961, exports of citrus fruit through Durban and Capetown ports in South Africa stopped completely due to the development of CBS symptoms during the transportation of fruit from the orchards to the ports. There is only one report available on the effects of *G. citricarpa* on the internal quality of citrus fruit (Anonymous, 1988), according to which the acid content of the infected by *G. citricarpa* citrus fruit is much lower than that of the healthy ones.

However, there is no precise experimental data on the reduction that *G. citricarpa* causes in the quality of citrus fruit in the areas of its present distribution. Therefore, there is uncertainty with respect to the effects that CBS might have on the external quality of citrus fruit produced in the citrus-growing regions of the PRA area.

2.4: Potential consequences		
Rating	**Description**	**Probability Assignment** [1]
High	The pest has a severe impact on the standing crop with significant host mortality; losses in storage may be total. Intervention by growers may not be possible or would be essential and expensive to counter yield and /or quality losses.	0%
Medium	The pest has a moderate impact on the standing crop but host mortality is rare; losses in storage may occur. Threat to yield and /or quality changes would justify some intervention by growers to reduce losses.	**100%**
Low	The pest is likely to have no or only minor impact on a standing crop and little effect on stored products. Yield and /or quality changes are within range of natural variation. No intervention is likely to be needed.	0%
	Check sum =	100%

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

2.5 Environmental Impact

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

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

Information / evidence:

Biodiversity

G. citricarpa affects fruit and to a lesser extent leaves of *Citrus* spp. It is considered that the organism may also cause some damage to twigs, but this has not been documented (Calavan, 1960). As citrus trees are not killed by *G. citricarpa,* the effect of CBS on tree health is negligible. Therefore, it is expected that the establishment of the pathogen in the citrus-growing regions of the PRA area will not have any impact on the biodiversity with respect to the presence of host plants. However, in the areas of its present distribution, repetitive applications of fungicides in commercial orchards are required for the control of CBS during the 4-6 months of fruit susceptibility period (Kotzé, 2000). Therefore, the introduction of the organism into the citrus-growing regions of the PRA area may require specific chemical control programmes, which could negatively impact non-target pests.

Ecosystem services

In the CBS-infested areas, the organism has a serious environmental impact, as repetitive applications of fungicides, including copper-based fungicides, in commercial orchards are required for its control during the 4-6 months of fruit susceptibility period (Kotzé, 2000). Therefore, the introduction of the organism into the citrus-growing regions of the PRA area may require additional applications of copper-based fungicides, which can negatively impact the soil (i.e. copper toxicity) (Kotzé, 1963). No other effects of *G. citricarpa* on natural resources have been reported in the areas of its present distribution

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

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

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

Introduction Potential

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

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

With the EU legislation (EU Council Directive 2000/29/EC) in place, two pathways of entry of *G. citricarpa* into the PRA area (EU-27 Member States) are identified:

- Fresh citrus fruit, and
- Natural means (wind, rain, insects, birds, etc)

A third pathway, namely "Citrus plant material for propagation purposes (rootstocks, scions, budwood, etc) excluding seeds" is currently blocked because of the EU legislation (EU Council Directive 2000/29/EC, Annex III, Part A, point 16) that 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 Member States.

Of the above-mentioned two pathways of entry, the fresh citrus fruit pathway is considered as the major pathway for the entry of *G. citricarpa* into the PRA area. The natural means pathway (wind, rain, insects, birds, etc) is of minor importance, due to the great distance between the areas of the current distribution of the organism and the PRA area.

<u>Pathway</u>: Fresh citrus fruit

3.2.1 Quantity of commodity imported annually

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

Large quantities of citrus fruit are imported every year from CBS-infested Third countries into all the EU-27 MSs, including the citrus-growing MSs (i.e. Spain, Italy, Greece, Cyprus, France and Portugal) (Table 5) (Eurostat, 2010). In addition, some EU-MSs (e.g Belgium and The Netherlands) re-distribute within the EU large quantities of fresh citrus fruits imported from CBS-infested countries (Fig. 7) (FAOSTAT, 2010). For instance in 2009, Belgium imported almost 60,000 tonnes of fresh grapefruit (one third arriving from Florida and Argentina) and re-distributed almost half of these fruit to other EU-MSs. Netherlands is by far the main European citrus re-exporting country. In 2009, Netherlands imported around 450,000 tonnes of sweet orange and almost 170,000 tonnes of grapefruit from various CBS-infested countries (including Argentina, Brazil and Uruguay) and re-distributed almost 200,000 tonnes of sweet orange and 115,000 tonnes of grapefruit to other EU-MSs, including citrus-producing MSs (Eurostat, 2008).

The frequency of imports of citrus fruit into the PRA area varies between years, citrus species/varieties, exporting country and importing EU-MSs (Eurostat, 2010). Generally, citrus fruit consignments are imported into the EU-27 MSs throughout the whole year. However, the main import period is between March and November, when local produce is unavailable. (Fig. 8) (Eurostat, 2010).

Table 5. Quantities of citrus fruit imported into the PRA area from CBS-infested countries in 2008 and 2009 (Eurostat, 2010)

Country of Origin	Quantities of citrus fruit imported into the EU-27 MSs (in tonnes)	
	2008	**2009**
South Africa	676,519	526,988
Argentina	424,803	307,890
Uruguay	99,045	103,616
Brazil	78,142	73,130
China	68,234	75,415
USA	89,823	62,964
Zimbabwe	18,104	15,519
Australia	2,697	3,983
Mozambique	0	285
TOTAL	**1,457,367**	**1,169,790**

Table 6. Quantities of citrus fruit (oranges, lemons, limes, mandarins and grapefruit) imported into the citrus-growing EU-Member States from South Africa, Argentina and Uruguay in 2008 and 2009

EU-Member State	Quantities of citrus fruit imported into the citrus-growing EU-27 (in tonnes)					
	South Africa		**Argentina**		**Uruguay**	
	2008	**2009**	**2008**	**2009**	**2008**	**2009**
Spain	61289.7	35462.3	87856.4	28406	19748.9	17346.5
Italy	49006.2	48257.3	65195.4	38773.6	8608.7	7605
Greece	8161.2	7817	28822.1	26024.9	196.3	382
France	13639.5	10638.7	10748.1	4326.1	597.9	265.7
Portugal	15753.4	8186	11073.4	1632.3	6904.2	4534.8
Cyprus	144.2	179.6	1553.5	1278.4	0	0
TOTAL	**147,223.2**	**109,696.9**	**205,248.9**	**100,441.3**	**36,056**	**30,134**

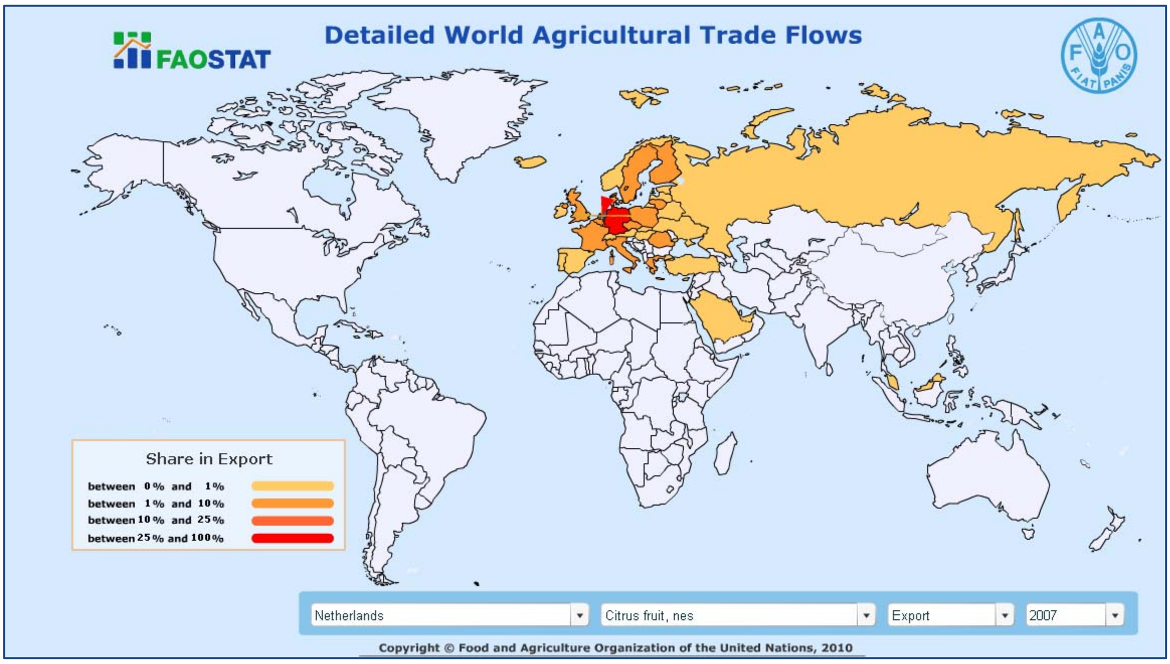

Figure 7. Flow of citrus fruit imported into the Netherlands and distributed to other EU-MSs. (Source: FAOstat, 2010; http://faostat.fao.org/DesktopModules/Faostat/WATFDetailed2/watf.aspx?PageID=536)

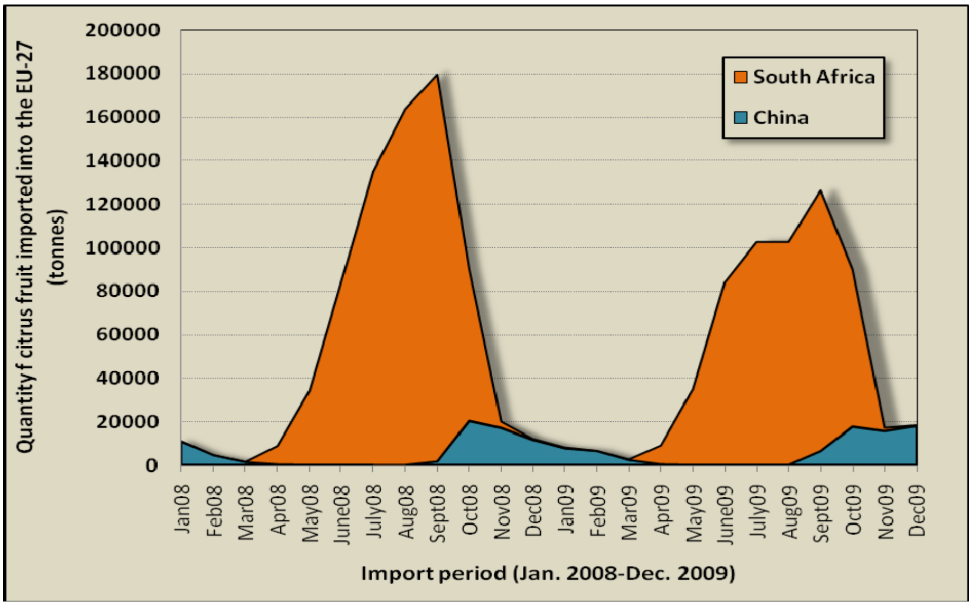

Figure 8. Quantities of citrus fruit (oranges, mandarins, lemons, grapefruit) imported monthly into the EU-27 MSs from South Africa and China during the period January 2008-December 2009 (Eurostat, 2010)

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

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

3.2.2 Survive postharvest treatments

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

The combination of cultural practices and the pre- and post-harvest application of fungicides used for the management of CBS in the areas of the pathogen's present distribution may reduce the disease level in the orchard or delay symptom development in storage but they do not completely eliminate quiescent infections on citrus fruit (CABI, 2006; Goes, 2002; Goes *et al.*, 2000; Kellerman and Kotzé, 1977; McOnie, 1964a; Nam *et al*., 1993; Miles *et al.*, 2004; Schutte, 2002; Agostini *et al*., 2006; Seberry *et al.,* 1967; Andrade *et al*., 2001a; 2001b; Agostini *et al.*, 2006).

Latently infected fruit may remain asymptomatic for 2 to 12 months (Kellerman and Kotzé, 1977; McOnie, 1967). Six different types of lesions are caused by *G. citricarpa* on citrus fruit, depending on the temperature and fruit maturity (Kiely 1949a; 1949b; 1960; Kotzé, 1981; 2000; Goes *et al*., 2000; Goes, 2001; Aguilar-Vildoso *et al*., 2002) (see revised datasheet for details). Under favourable environmental conditions, on some of these types of fruit lesions (e.g. hard spot, virulent spot), pycnidia of the anamorph, *P. citricarpa,* may develop (Kotzé, 1981; 2000).

Latently infected, asymptomatic fruit at harvest may develop symptoms during transport or storage depending on the temperatures and light intensities in the packinghouse and during transport and storage [low temperatures (i.e. 8°C) delay symptom development, while high temperatures (27°C) and 24 h fluorescent lighting speed up symptom development] (Kiely, 1948; Kotzé, 1963; 1981; Brodrick and Rabie, 1970; Brodrick, 1975; Reuther *et al*., 1978; Korf, 1998; Agostini *et al*., 2006; CABI, 2006).

Physical treatments of citrus fruit in the packinghouse (e.g. immersion in hot water, waxing, etc) may reduce or delay the post-harvest development of CBS symptoms, but they will not eliminate the pathogen (Agostini *et al.,* 2006; Korf *et al*., 2001; Seberry *et al*., 1967). Brushing of citrus fruit in the packinghouse will most probably remove any conidia present on its surface as contaminants, but it will not affect the latent mycelium or the conidia embedded within pycnidia.

Culling of infected citrus fruit in the packinghouse is unlikely to be effective, as (i) CBS symptoms on fruit are quite variable in appearance (see datasheet for details) and, with the exception of hard spot with pycnidia, lesions are very small (< 1 mm or 1-3 mm, in diameter) and similar to those caused by other citrus pathogens, mechanical or insect damage (Kotzé, 2000; Snowdon, 1990), (ii) two out of the six different types of fruit symptoms, freckle spot and virulent spot, appear after colour break (Bonants *et al.,* 2003; Kotzé, 1981), at the end of the season and in storage (Kotzé, 2000), respectively, (iii) symptomless citrus fruit at harvest may develop symptoms during transport or storage (Agostini *et al*., 2006; Kotzé, 1963), and (iv) fruit harvested green and subjected to de-greening (Terblanche, 1999) may have not developed CBS symptoms at harvest time. In addition, culling will not detect latently infected fruit. Reliable detection and identification of the organism on citrus fruit can be made only after laboratory examination.

The current EU legislation does not require the citrus fruit originating in Third countries to be treated post-harvest before their importation into the EU-27 MSs. Only plant health inspection and pre-harvest treatments are considered in the EU Council Directive (Annex IV, Part A, Section I, point 16.4(d)).

3.2.2: Likelihood of surviving post harvest treatments		
Rating	**Description** (likelihood of survival is ....)	**Probability Assignment** [1]
High	> 10% (greater than one in ten survive)	**10%**
Medium	Between 0.1% - 10% (between one in one thousand to one in ten survive)	**60%**
Low	< 0.1% (less than one in one thousand survive)	**30%**
	Check sum =	100%

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

3.2.3 Survive shipment

Estimate survival during shipment; assume standard shipping conditions

Citrus fruit are usually transported under cool conditions (Wills *et al*., 1998). More specifically, shipping temperatures for oranges and mandarins are fairly standard at 1°C and 4°C respectively, whereas lemons and limes are normally shipped at 10°C, as they are sensitive to chilling injury. Grapefruit temperatures range from 10 to 15°C depending on the time of the year and conditions of the trees at harvest. The cooler temperature provides better decay control while the warmer protects against chilling injury (Wardowski, 1981).

Low transport and storage temperatures may prolong the survival of *G. citricarpa* conidia on citrus fruit. As Korf *et al.* (2001) showed, mature *G. citricarpa* conidia produced on infected citrus fruit were still viable after 3 weeks storage of the fruit at 4.5 or 10ºC, but they lost their viability when the fruit were stored at 25ºC. Kiely (1948) showed that the viability of freshly exuded conidia incubated at 25ºC was decreased by 60 and 100% after four days and three months, respectively. Conidia of *G. citricarpa,* similarly to other splash-dispersed conidia, are surrounded by mucilage that protects them from desiccation and loss of viability during dry conditions (Marconi, 1964; 1967; Fournet, 1969).

Low temperatures during transport and storage of citrus fruit are unlikely to affect the survival of latent mycelium of the pathogen either, as CBS symptoms quickly develop when the fruit with quiescent infections are transferred to higher temperatures (Wager, 1952; Kotzé, 1988). *G. citricarpa* could be readily isolated from infected citrus fruit stored for more than 40 days under various moisture conditions at 8ºC or ambient temperatures (15-25ºC) (Agostini *et al.*, 2006). Agostini *et al*. (2006) also reported that, although the viability of *G. citricarpa* tended to decline with time of fruit storage, the pathogen was still viable long after the fruit had passed the stage where it would be marketable.

The low temperatures and light intensities prevailing during transport of citrus fruit suppress symptom development in CBS latent infected citrus fruit but they do not affect the survival of the pathogen (Wager, 1952; Brodrick and Rabie 1970; Kotzé, 1988; Agostini *et al.*, 2006). This is further supported by the studies of Agostini *et al.* (2006) according to which, the survival of *G. citricarpa* in CBS lesions on whole citrus fruit or peel was not affected by the various temperatures and moisture levels during storage. However, upon arrival at the destination place, it is very likely that fruit will be subjected to higher temperatures for marketing. The increase in temperature can dramatically increase the development of symptoms whereas, symptomatic fruit can be a source of conidia for several months, as the sporogenous layers of pycnidia are regenerative and can produce numerous crops of conidia following regular wetting of the fruit (Wager, 1952; Kiely, 1948; Truter *et al.,* 2007).

The main mode of transport to the EU of citrus fruit originated in CBS-infested areas is by sea (Eurostat, 2010). In 2008 and 2009, 817,446 and 650,494 tonnes of citrus fruit, respectively, originated in South Africa, Uruguay, Brazil, Argentina and China were shipped to the EU compared with 431 and 590 tonnes, respectively, transported by air (Table 7). There is not exact data on the time taken for citrus fruit to be transported from intercontinental countries to the PRA area. According to Terblanche (1999), citrus fruit produced in South Africa are shipped under refrigeration and reach the EU market after three weeks or longer.

No data exists on the prevalence of the pest on citrus fruit consignments (% infected fruit in each consignment) imported into the PRA area, as under the Council Directive 2000/29/EC, a citrus fruit consignment is intercepted even one a CBS-infected fruit is detected in it (“zero tolerance”).

Table 7. Quantities (tonnes) of citrus fruit imported by sea and air into the EU-27 from CBS-infested areas in 2008 and 2009 (Eurostat, 2010)

Country of origin	**Transported by sea**		**Transported by air**	
	2008	**2009**	**2008**	**2009**
South Africa	396,639	311,788	93	194
Argentina	287,326	209,844	110	219
Uruguay	63,610	66,509	0	3
Brazil	36,243	34,671	223	174
China	33,628	27,682	5	0
Total	**817,446**	**650,494**	**431**	**590**

Therefore, in terms of duration and conditions of transport and storage, *G. citricarpa* in the form of (i) conidia within pycnidia in fruit lesions and/or (ii) latent mycelium present in asymptomatic fruit, will not be affected by the transport and storage conditions, although its activity may be reduced due to the low temperatures and light intensities occurring during transport and storage of citrus fruit. This is further supported by the fact that living stages of

G. citricarpa continue to be intercepted on citrus fruit consignments imported into the EU-27 (EYROPHYT, 2010). According to EUROPHYT (2010), during the period 2005-2010 there have been 560 notifications of interceptions of *G. citricarpa* on citrus fruit originated from CBS-infested Third countries. In the Netherlands alone, 20 consignments of citrus fruit infected with *G. citricarpa* have been intercepted in 2010. The average size of these consignments was 20 tonnes (source: NPPO of the Netherlands, 2010).

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

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

3.2.4 Not be detected at the port of entry

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

The pathogen may be present on the citrus fruit pathway originated from CBS-infested areas and imported into the PRA area as (i) latent mycelium on asymptomatic fruit and although the mycelium is not by itself infective, it is capable of producing lesions in which pycnidia with conidia may develop following the exposure of fruit to high temperatures (27^{o}C) and 24 h fluorescent lighting (Kiely, 1948; Brodrick and Rabie, 1970), and (ii) conidia within pycnidia produced in lesions of symptomatic fruit.

Quiescent infections of fruit are common and pre-harvest symptoms develop after fruit attains full size or becomes mature (Kotzé, 1963, 1981, 2000; McOnie, 1967). The development of CBS symptoms on fruit prior to harvest depends on (i) the environmental conditions: high temperatures, high sunlight intensities and drought favour symptom development (Kotzé, 1981), (ii) the fruit maturity: the more mature the fruit becomes, with the rind colour changing from green to yellow or orange, the better the chances for symptom development, and (iii) the age and physiological condition of the citrus tree: the disease is more severe on old trees than on healthy, young trees (Kotzé, 1981).

Latently infected, asymptomatic fruit at harvest may develop symptoms during transport or storage causing considerable losses (Kiely, 1948; Kotzé, 1963; 1981; Brodrick, 1975; Reuther *et al*., 1978; Agostini *et al*., 2006). Post-harvest development of symptoms on fruit is greatly influenced by the temperatures in the packinghouse and during transit and storage (Kotzé, 1981). Low temperature (8^{o}C) delay symptom development (Agostini *et al.*, 2006), while high storage temperatures (27°C) and 24 h fluorescent lighting speed up symptom development (Brodrick and Rabie, 1970; Korf, 1998; CABI, 2006).

CBS symptoms on fruit (i) are quite variable in appearance (see updated datasheet for details) and, with the exception of hard spot with pycnidia, lesions are very small (< 1 mm or 1-3 mm, in diameter), (ii) are similar to those caused by other citrus pathogens, mechanical or insect damage (Kotzé, 2000; Snowdon, 1990), and (iii) may also appear post-harvest, in transit or storage (e.g. the virulent spot type) (Kotzé, 2000). Moreover, fruit harvested green and subjected to de-greening (Terblanche, 1999) and low transport temperatures may have not developed CBS symptoms by the time they arrive at the border.

Under the current EU legislation, citrus fruit can be imported into the EU-27 MSs only if they originate in a pest-free country or a pest-free area or a pest-free field of production. In addition it is required that citrus fruit consignments originating in Third countries shall be subjected to a plant health inspection in the country of origin or the consignor country, before being permitted to enter the EU Community (Annex V, Part B, point 3) and they shall be free of *G. citricarpa* (Annex IV Part A, Section I, point 16.4).

Detection of the pathogen in consignments is based on inspection, sampling and testing. General procedures for inspection and sampling of consignments are described in ISPMs No. 23 and 31, respectively (FAO, 2005). Special sampling procedures for citrus fruit consignments are described in the Work Plan for USDA Preclearance Inspection and Cold Treatment of South African Citrus Fruit designated for export to the USA (APHIS, 2006).

However, the numerous interceptions of *G. citricarpa* (560 notifications during the period 2005-2010) that continue to be made by the EU-MSs on imported citrus fruit consignments originated in CBS-infested countries (EUROPHYT, 2010), indicate that the pathogen survives pest management procedures applied at the place of origin, including inspection and detection.

Therefore, under the current EU legislation, the likelihood of *G. citricarpa* to escape border detection on the citrus fruit pathway is rather medium with a high uncertainty.

3.2.4: Likelihood pest will not be detected at port of entry		
Rating	**Description** (likelihood of no detection is ....)	**Probability Assignment** [1]
High	> 10% (greater than one in ten will not be detected)	**30%**
Medium	Between 0.1% - 10% (between one in one thousand to one in ten will not be detected)	**60%**
Low	< 0.1% (less than one in one thousand will not be detected)	**10%**
	Check sum =	100%

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

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

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

Large quantities of citrus fruit are imported every year from CBS-infested Third countries into all the EU-27 MSs, including the citrus-growing MSs (i.e. Spain, Italy, Greece, Cyprus, France and Portugal) (Eurostat, 2010). In addition, some EU-MSs (e.g Belgium and The Netherlands) re-distribute within the EU large quantities of fresh citrus fruits imported from CBS-infested countries (Fig. 7) (FAOSTAT, 2010).

Fresh citrus fruit are destined for human consumption and thus, once fruit consignments enter the PRA area, they are destined to packinghouses, wholesale and retail fresh fruit markets before being sold to the consumers.

Most of the citrus fruit consignments imported into the PRA area arrive at ports as they are transported by sea (Table 7) (Eurostat, 2010; EUROPHYT, 2010).

Citrus species susceptible to CBS are widely grown in the Southern EU-MSs (Eurostat, 2010, Tables 1-3, Fig. 3) in commercial orchards and nurseries, smallholdings, private gardens for family consumption and as ornamentals in public gardens and along the roadsides both in urban and rural regions. Commercial citrus orchards and nurseries are located mainly in coastal areas, next to rivers and in some cases in close proximity to the ports.

The risk with citrus fruit imported into the PRA area from CBS-infested Third countries is mainly associated with the discarded unmarketable whole fruit or peels, derived from packinghouses, fresh fruit markets, households, etc. and its management.

Symptomatic citrus fruit/peel can be a source of *G. citricarpa* conidia, which may remain viable for several months (Kiely, 1948; Truter *et al.*, 2007; Wager, 1952) or until decomposed by other organisms (Agostini *et al.*, 2006). Wager (1949) showed that *G. citricarpa* conidia produced on a slowly mummified and dried up infected orange fruit could survive for up to 4-5 months. Symptomatic fruit removed from the field and washed for pycnidiospores revealed that only 1% of the conidia germinated (Kiely, 1948). The same symptomatic fruit was then held in the lab and submerged in water for 1 h at 22^{o}C, then held for 24 hours at 25^{o}C. When this cycle was repeated for 4 days, it increased the percentage of germinated conidia from less than 1% to 10%, 25%, 48% and 78%, respectively (Kiely, 1948). Based on this study, Kiely (1948) concluded that successive crops of conidia are produced by the pycnidia on symptomatic citrus fruit and displace those which had been formed earlier under less favorable environmental conditions. However, Kiely (1948) and Korf (1998) reported that conidia of *G. citricarpa* are short-lived, failing to germinate under laboratory conditions after 3-14 days contingent on the procedure employed.

Packinghouses are usually located within the citrus-growing regions of the PRA area and in close proximity (<500 m) to commercial citrus orchards (NPPO of Italy, 2010). In response to the questionnaire distributed to the EFSA Network on Plant Health, the NPPO of Italy reported that the citrus fruit/peels that are discarded during the packinghouse process are used for pectin extraction or pet food (NPPO of Italy, 2010). In the latter case, discarded citrus fruit/peels are dried, but no further details are provided on the method used for drying (e.g. exposure to the sun, hot air treatment, etc). No information is available on the management of citrus fruit/peel waste derived from packinghouses in the other citrus-producing EU-MSs or that from the fresh fruit markets, households, etc.

Based on the above, it may be concluded that, following their import into the PRA area, citrus fruit are widely distributed in both citrus-growing and non-citrus-growing EU-MSs. Unmanaged citrus fruit waste derived from packinghouses and fresh fruit markets located in rural areas is very likely to be disposed in citrus-growing regions where the environmental conditions are suitable for *G. citricarpa* to complete its life cycle (see section 3.1.5) and susceptible hosts are widely grown commercially. However, uncertainties exist on the quantities and frequency of fresh fruit waste disposed in such environments.

3.2.5: Likelihood commodity that will be moved to suitable environment for pest survival (same as % of commodity moved to suitable environment)		
Rating	**Description** (likelihood, or amount moved to suitable environment is ....)	**Probability Assignment** [1]
High	> 10% (greater than one in ten)	**25%**
Medium	Between 0.1% - 10% (between one in one thousand to one in ten)	**70%**
Low	< 0.1% (less than one in one thousand)	**5%**
	Check sum =	100%

3.2.6 Come into contact with host material suitable for reproduction

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

CBS-infected citrus fruit imported into the risk assessment area may carry the pathogen in the form of conidia within pycnidia in lesions and/or latent mycelium, which, under certain environmental conditions, is capable of producing lesions in which pycnidia with conidia may develop following the exposure of fruit to high temperatures (27^{o}C) and 24 h fluorescent lighting (Kiely, 1948; Brodrick and Rabie, 1970).

The risk with citrus fruit imported into the PRA area from CBS-infested Third countries is mainly associated with the waste (discarded whole fruit or peel), derived from packinghouses, households, fresh fruit markets and its management. Symptomatic citrus fruit/peel can be a source of *G. citricarpa* conidia, which may remain viable for several months (Kiely, 1948; Truter *et al.*, 2007; Wager, 1952) or until decomposed by other organisms (Agostini *et al.*, 2006). Wager (1949) showed that *G. citricarpa* conidia produced on a slowly mummified and dried up infected orange fruit could survive for up to 4-5 months. Symptomatic fruit removed from the field and washed for conidia revealed that only 1% of the conidia germinated (Kiely, 1948). The same symptomatic fruit was then held in the lab and submerged in water for 1 h at 22^{o}C, then held for 24 hours at 25^{o}C. When this cycle was repeated for 4 days, it increased the percentage of germinated conidia from less than 1% to 10%, 25%, 48% and 78%, respectively (Kiely, 1948). Based on this study, Kiely (1948) concluded that successive crops of conidia are produced by the pycnidia on symptomatic citrus fruit and displace those which had been formed earlier under less favorable environmental conditions. However, Kiely (1948) and Korf (1998) reported that conidia of *G. citricarpa* are short-lived, failing to germinate under laboratory conditions after 3-14 days contingent on the procedure employed.

In the risk assessment area, citrus packinghouses are mainly located within the citrus-growing regions and in close proximity (<500 m) to commercial citrus orchards (NPPO of Italy, 2010). In Italy, the discarded citrus fruit/peels are dried in order to be used as pet food or for pectin extraction (NPPO of Italy, 2010). However, no information is available on the management of citrus fruit/peel waste in the other citrus-producing EU-MSs.

In case infected citrus fruit or fruit peel with pycnidia is disposed in the vicinity of host plants in the PRA area, the mature conidia exuded from pycnidia under wet conditions may be splash-dispersed by rain or irrigation water (overhead or sprinkler irrigation) upward from the orchard floor onto the lower reaches of the tree canopy infecting young leaves and fruit that are at the susceptible stage (Kiely, 1948; Kotzé, 1981; Spósito *et al*., 2008; Wager, 1952; Gottwald *et al.,* 2002). This is further supported by the studies of Schinor *et al.* (2002) and Spósito *et al.* (2008), according to which, the disease incidence (% of symptomatic fruit) was higher in the lower part of the canopy, which indicates that infection occurred by splash-dispersed conidia produced on leaf litter on the orchard floor. The splash-dispersal mechanism of conidia can be triggered by rain and rain events occur during the period of import of citrus fruit into the citrus-growing regions of the PRA area (April-November), particularly in spring (April-May) and autumn (September-November). During the dry periods, dew or mist, commonly occurring during the night in the coastal citrus-growing regions, irrigation (sprinkler or overhead) or application of pesticides using high pressure equipment may cause direct and/or drip-splash of the conidia from the infected citrus fruit or fruit peel discarded on the orchard floor onto the low hanging young leaves and fruit that are at the susceptible stage.

Water hoses, commonly used for the irrigation of citrus trees grown in private gardens and residential regions in the PRA area, may also trigger the splash-dispersal of conidia from the discarded fruit/peel onto the tree canopy. According to Fitt *et al*. (1989) drip-splash may be as efficient as direct rain-splash for the dispersal of mucilaginous conidia. Moreover, application of pesticides using high pressure equipment may also trigger the splash dispersal mechanism of conidia.

The distance between the low hanging susceptible tissues (leaves, fruit) of a citrus tree and the orchard floor in the commercial orchards in the PRA area varies from 0 to 80 cm, depending on the cultivation technique. In addition, during the last few years there is a trend for shorter citrus trees by grafting onto dwarfing rootstocks (Colombo, 2004; Regione Puglia, 2007) (Fig. 9).

In still air, conidia covered with mucilage are splash-dispersed up to a height of not more than 50 cm or up to a distance of 1 m from the inoculum source with their numbers decreasing steeply with increasing height or distance (Fitt *et al.,* 1989). Therefore, conidia of *G. citricarpa* present on citrus fruit/peel discarded underneath a citrus tree could be splash-dispersed by rain or irrigation water to infect young leaves and fruit hanging at the lower reaches of the tree canopy.

However, under windy conditions, the small-sized *G. citricarpa* conidia (9.4-12.7 x 5-8.5 µm) (Baayen *et al.*, 2002) carried in small splash drops could potentially become windborne as an aerosol of fine spray, spreading *G. citricarpa* conidia over longer distances (Fitt *et al.,* 1989). Although not yet documented, *G. citricarpa* conidia produced on discarded CBS-infected fruit/peels could be transported by insects, birds, etc and deposited on susceptible hosts grown in the region (Kiely, 1948) (see above, section *Means of dispersal/spread*).

Large quantities of citrus fruit are imported into the risk assessment area from Third countries mainly during the period March to November when local citrus produce is not available. As mentioned above, leaves, particularly lemon leaves, are susceptible to infection by *G. citricarpa* for 10 months following their emergence and citrus fruit are susceptible from petal fall up to 4–6 months later.

Leaves, particularly lemon leaves, are susceptible to infection by *G. citricarpa* for 10 months following their emergence and citrus fruit are susceptible from petal fall up to 4–6 months later. Although the number of citrus leaf flushes per year depends on the variety, the cultural practices (e.g. pruning, fertilisation, irrigation, etc.), the rootstock, etc, in the citrus-growing regions of the PRA area, the main citrus leaf flushing and flowering period is from early April to early May with the exception of lemon trees, which have one or two additional leaf flushing and flowering periods from July to September (Guardiola, 1997; Krezdorn, 1986; Colombo, 2004, Italy NPPO, 2010). Based on the above, it may be concluded that the period of imports of citrus fruit into the EU-27 MSs (March-November) and particularly into the citrus-growing regions (Southern EU-MSs) coincides with the period (April-September) where susceptible plant tissues (young leaves and fruit) are present in citrus trees grown in the risk assessment area.

Figure 9. Commercial citrus orchards in Greece (left) and Spain (right). In the latter case, the lower reaches of the trees are in contact with the orchard floor, due to the dwarfing rootstocks used.

Courtesy (photo on the right): Dr Antonio Vicent, Centro de Proteccion Vegetal y Biotechnologia, IVIA, Spain)

Based on the above, it may be concluded that if CBS-infected citrus fruit or peels with pycnidia were discarded underneath or in close proximity to susceptible citrus trees, the pathogen is likely to be transferred by natural means (rain or irrigation water, insects, birds, etc.) to susceptible hosts present in the citrus-growing regions of the PRA area. However, there are uncertainties concerning (i) the prevalence of *G. citricarpa* on infected citrus fruit imported into the PRA area, (ii) the frequency and quantity of infected fruit/peel being discarded in close proximity to a host in the citrus-growing regions of the PRA area, and (iii) the time taken for discarded asymptomatic whole fruit or peel to produce conidia before it is decomposed by other organisms.

3.2.6: Likelihood pest will transfer to host material where it can reproduce		
Rating	**Description** (likelihood of pest transfer is ....)	**Probability Assignment**
High	> 10% (greater than one in ten)	5%
Medium	Between 0.1% - 10% (between one in one thousand to one in ten)	10%
Low	< 0.1% (less than one in one thousand)	85%
	Check sum =	100%

Pathway 3: Natural means (wind, rain, insects, birds, etc)

In addition to vertical dissemination through infested host plant material, *G. citricarpa* can be disseminated horizontally by ascospores and/or conidia, which can be vectored by a variety of agents such as wind, rain, insects, etc. Although information from areas were the pest currently occurs clearly demonstrate that wind and rain are effective means for the dissemination of *G. citricarpa* ascospores and conidia, respectively, the contribution of these means to the entry of the organism into the PRA area is considered to be negligible, due to the large distance. The distance over which *G. citricarpa* propagules (conidia, ascospores) can be dispersed by wind or in water splash was suggested to be at 24.7 m away from a point source (Sposito *et al*., 2007). *G. citricarpa* is not present in the European continent. Therefore, the intercontinental spread of *G. citricarpa* by abiotic factors, such as rain and wind is very unlikely.

The role of insects, birds, etc in the spread of *G. citricarpa* has not been documented. However, according to Kiely (1948) insects, birds etc. may be able to transfer and deposit conidia of *G. citricarpa* on the wounds they cause on the host. Although it is well known that some insects are capable for long distance flight (Greenslade, 1991), no evidence is available that insects may contribute to the entry of the pathogen into the PRA area. However, insects carried or transported unintentionally (hitchhikers) are regularly found on goods not normally subject to quarantine (Crowe, 2001). Therefore, although the possibility that insects would contribute to the entry of *G. citricarpa* into the PRA area is considered to be negligible, it cannot be fully excluded.

However, as the pathway of entry of *G. citricarpa* into the PRA area by natural means is of minor significance, it will not be analysed further.

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

3.7 Potential for introduction via individual pathways

Figure 10 summarises the results of combining individual questions and scores (3.1. to 3.6.) relating to likelihood of *G. citricarpa* introduction on the fresh citrus fruit pathway, using the Bayesian Belief network (BBN) model. The model suggests that the likelihood of *G. citricarpa* introduction via the citrus fruit pathway originating in CBS-infested Third countries is **medium** (66% likelihood) to **high** (33% likelihood).

3.8. Overall Risk for individual pathways

<u>Pathway . Fresh citrus fruit</u>

In Figure 10, the overall risk for *G. citricarpa* introduction into the PRA area on the fresh citrus fruit pathway, under the current EU legislation, is summarised by combining the consequences of introduction (2.1. to 2.5) with the likelihood of introduction, using the Bayesian Belief Network model. The model suggests that the risk presented by *G. citricarpa* to the PRA area is **medium** (62% likelihood) to **high** (30% likelihood).

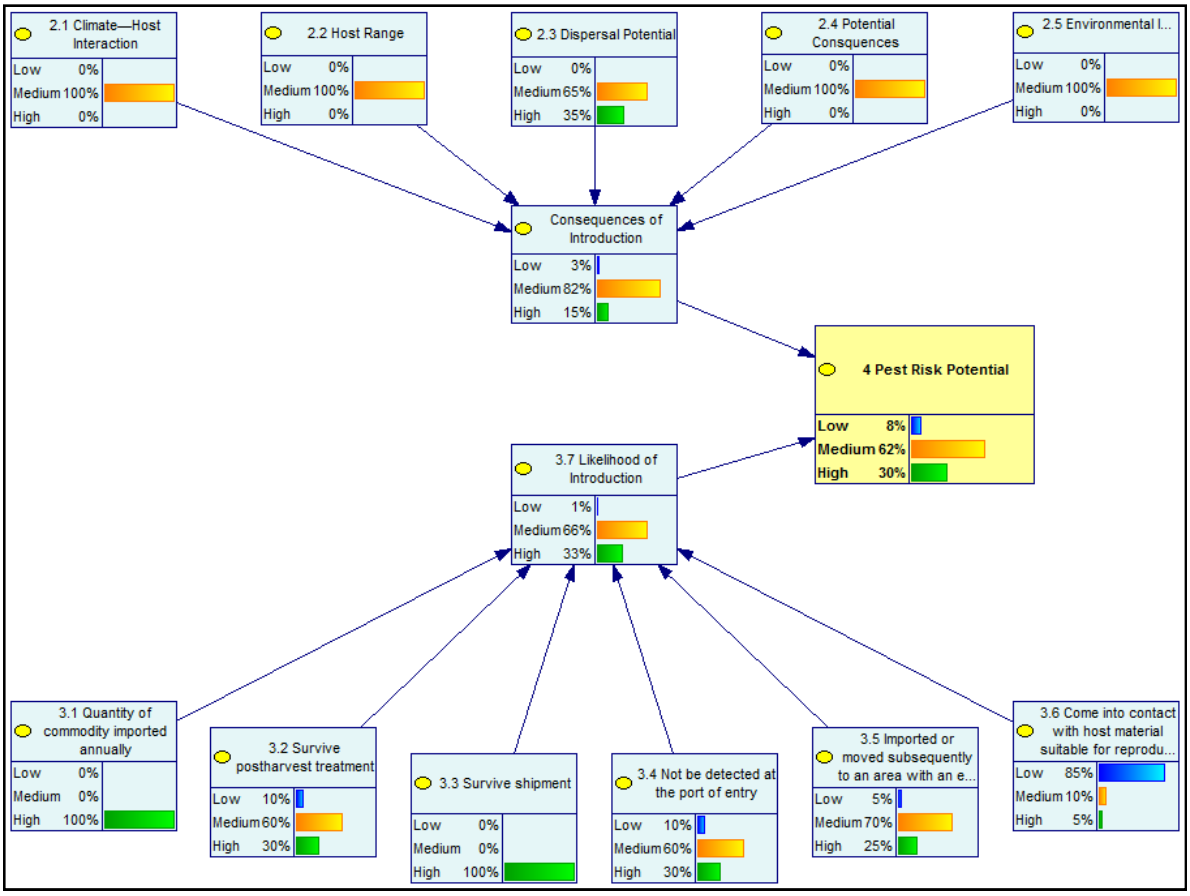

Figure 10. Graphical representation of combining scores using a Bayesian Belief Network (BBN) model to give the potential for introduction and the overall risk rating for *G. citricarpa* potentially entering the PRA area on the fresh citrus fruit pathway, assuming that the EU legislation (EU Council Directive 2000/29/EC) is in place.

Conclusion

G. citricarpa is not reported to be present in the PRA area (EU-27 MSs) and is considered a harmful organism under the Council Directive 2000/29/EC. Hosts of *G. citricarpa* are exclusively species of the genera *Citrus, Poncirus* and *Fortunella*. All citrus species and cultivars are susceptible to *G. citricarpa* with the exception of sour orange (*C. aurantium* L.) and Tahiti lime (*C. latifolia* Tan.), which are considered to be tolerant. Susceptible citrus species/varieties are extensively grown in the Southern EU-MSs in commercial orchards, nurseries, private and public gardens, roadsides, etc. both in rural and urban areas.

Three pathways of entry of *G. citricarpa* into the PRA area exist:

- Citrus plant material for propagation purposes (rootstocks, scions, budwood, etc), excluding seeds.
- Fresh citrus fruit, and
- Natural means (wind, rain, insects, birds, etc)

Of the above mentioned pathways the citrus plant material for propagation purposes pathway is blocked because of the current EU legislation (EU Council Directive 2000/29/EC, Annex III, Part A, point 16) that 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 Member States. In addition, the natural means pathway (wind, rain, insects, birds, etc) is of minor importance, due to the great distance between the areas of the current distribution of the organism and the PRA area.

Therefore, under the current EU legislation, the fresh citrus fruit pathway is considered as the only pathway for the entry of *G. citricarpa* into the PRA area.

The current EU legislation (EU Council Directive 2000/29/EC, Annex IV, Part A. Section I, paragraph 16.4; Annex IV, Part A, Section I, point 16.1.; Annex V, Part B, point 3) requires that fruit of *Citrus* L., *Poncirus* Raf. and *Fortunella* Swingle as well as their hybrids originating in Third countries:

- shall be subjected to a plant health inspection in the country of origin or the consignor country, before being permitted to enter the Community
- shall be free from peduncles and leaves and the packaging shall bear an appropriate origin mark, and
- will originate in a country recognised as being free from *Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*), in accordance with the procedure laid down in Article 18, or in an area recognised as being free from *Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*), in accordance with the procedure laid down in Article 18, and mentioned on the certificates referred to in Articles 7 or 8 of this Directive, or no symptoms of *Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*), will 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 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.

Citrus fruit imported into the PRA area from Third countries are mainly destined for human consumption. Citrus fruit originating in citrus black spot (CBS)-infested areas may carry the pathogen in the form of (i) conidia within pycnidia in lesions (symptomatic fruit) and/or (ii) latent mycelium (asymptomatic fruit). However, the pathogen has a long latent period on citrus fruit, whereas fruit symptoms are variable in appearance and similar to those caused by other pathogens, mechanical or insect damage. Moreover, *G. citricarpa* has the ability to survive cultural practices, pre- and post-harvest treatments as well as transport and storage conditions and remain undetected on latently infected fruit (asymptomatic) and fruit with low disease incidence and severity.

Susceptible citrus species and varieties are extensively grown in the Southern EU-MSs in commercial orchards and nurseries, private gardens for family consumption, and as amenity trees in public gardens, roadsides, etc. both in rural and urban areas.

So far *G. citricarpa* has established in areas with various climatic conditions and particularly in coastal areas that belong to the warm temperate climatic zone, as do most of the citrus-growing regions in the PRA area. Climatic comparisons and modelling (EFSA, 2008) suggest that, although the climatic conditions in the citrus-growing regions of the PRA area are marginally suitable for the establishment of the pathogen, the microclimatic conditions occurring in spring (April-May) and autumn (September-October) in these regions would greatly favour the establishment of *G. citricarpa*. In addition, it is unlikely that the existing management practices applied in commercial orchards and nurseries in the PRA area would prevent establishment. The long latent period of the pathogen, the widespread occurrence of lemon, which is the most susceptible species and thus, the first to be infected in a new area, the presence of citrus in semi- and/or unmanaged environments, where cultural practices and chemical control measures are not usually undertaken (e.g. private and public gardens, roadsides, etc) and the absence of enemies/antagonists could greatly facilitate the establishment of the pathogen in the PRA area. Irrigation (drip or sprinkler) applied to citrus orchards during the dry periods would promote the maturation of *G. citricarpa* ascocarps produced on leaf litter and provide favourable conditions for the infection of susceptible plant tissues. Moreover, the existence of citrus species/varieties with overlapping crop periods (i.e. co-existence of mature and young fruit on the same tree) and more than one leaf flushing and flowering periods in the same year (e.g. lemon, 'Valencia' sweet orange, etc) are likely to extend the period during which citrus species/varieties grown in the PRA area will be susceptible to the pathogen.

The risk of introduction of *G. citricarpa* on the citrus fruit pathway is mainly associated with the fruit waste (whole symptomatic fruit or peels) derived from packinghouses, households and fresh fruit markets, and its management. In case this waste is not properly managed, the pathogen has the ability to spread by natural means (wind, rain or irrigation water, etc) to susceptible hosts grown in close proximity.

Given the end-use of the fresh citrus fruit (consumption) and the numerous interceptions (560 during the period 2005-2010) of living stages of the pathogen on citrus fruit consignments imported into the PRA area from CBS-infested areas, it is considered that, under the current EU legislation, the likelihood of *G. citricarpa* introduction into the PRA area, as estimated by the BBN model, is **medium** (66% likelihood) to **high** (33% likelihood). However, there are uncertainties with respect to (i) the concentration of *G. citricarpa* on the fresh citrus fruit imported into the PRA area, particularly on the latently infected (asymptomatic) fruit, (ii) the volume and frequency of infected fruit being disposed in the vicinity of susceptible hosts in the citrus-growing regions of the PRA area (i.e. Southern EU-MSs), and (iii) the time taken for discarded asymptomatic whole fruit or peel to produce pycnidia and conidia before being decomposed by other organisms.

Spread of *G. citricarpa* within the citrus-growing regions of the PRA area is very likely. The pathogen has the ability to spread by natural means (wind, rain or irrigation water, etc) and by human activities (grafting of citrus trees using infected rootstocks, scions or budwood, movement of infected plant material, disposal of infected plant or fruit waste, etc). Rain and/or irrigation water are the means by which *G. citricarpa* can spread over relatively short distances (within the tree or between neighbouring trees). Spread of the pathogen over longer distances may occur by wind and human activities. However, uncertainties exist due to a lack of information on the distance over which *G. citricarpa* spores could be disseminated by wind or wind-driven rain and the role of insects, birds in the dispersal of *G. citricarpa* spores.

G. citricarpa has negligible or no effect on citrus tree health. In the areas of its present distribution it causes pre- and post-harvest fruit losses. Pre-harvest losses arise when severely affected fruit drop prematurely in the orchard and go to waste, particularly in years favourable for disease development and when fruit is held on the trees past peak maturity. CBS also affects the external quality of the citrus fruit by causing blemishes that make citrus fruit unsuitable for the fresh fruit market. Nevertheless, it is expected that in case *G. citricarpa* was introduced in the PRA area, the copper-based fungicides, currently applied in commercial citrus orchards for the control of other pathogens, would most probably reduce the disease incidence and severity and subsequently the consequences of CBS on crop yield and quality. The introduction of the pathogen in the citrus-growing regions of the PRA area will have no consequences on the biodiversity with respect to the presence of citrus. However, the introduction of the organism may require specific chemical control programmes and additional copper-based fungicide sprays, which could negatively affect non-target pests and soil (i.e. copper toxicity), respectively.

In the absence of any control measures, the overall consequences on citrus production as a result of the introduction of *G. citricarpa* into the citrus-growing regions of the PRA area (i.e. Southern EU-MSs) is estimated by the BBN model as **medium** (59% likelihood) to **high** (34% likelihood). However, uncertainties exist around the level of yield/quality reduction as a result of the introduction and spread of the pathogen in the citrus-growing regions of the PRA area, as there are only a few experimental data from the CBS-infested areas on the yield/quality losses that the pathogen causes to citrus production.

Based on the above, it can be concluded that *G. citricarpa* satisfies the IPPC definition of a harmful organism for the EU

Supporting documentation (separate files)

- **Annex 1:** References
- **Annex 2:** Uncertainties
- **Annex 3:** EFSA (2008). Pest risk assessment and additional evidence provided by South Africa on *Guignardia citricarpa* Kiely, citrus black spot fungus – CBS. Scientific Opinion of the Panel on Plant Health (Question No EFSA-Q-2008-299). *The EFSA Journal* (2008) 925: 1-108