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Risk assessment of *Citrus* spp. fruit as a pathway for the introduction of *Guignardia citricarpa* Kiely, the organism that causes Citrus Black Spot disease

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Executive Summary

This assessment considers scientific, expert, and empirical evidence to determine the significance of citrus fruit as a pathway for the introduction of *Guignardia citricarpa* Kiely, the causal agent of the fungal disease citrus black spot (CBS). The evaluation concludes that citrus fruit is not epidemiologically significant as a pathway for the introduction of *G. citricarpa*.

Disease occurrence depends on a specific set of biological, environmental and physiological conditions at the precise time that an infected citrus fruit is placed in direct proximity to a susceptible host. In the event that infected fruit enter a CBS-free area with susceptible hosts, the transmission of the pathogen and the establishment of the disease via this pathway requires a combination of conditions that are unlikely to occur. Based on substantial evidence, we conclude that the likelihood of the combination of conditions necessary for introduction and spread of *G. citricarpa* is not greater than the exposure represented by unregulated pathways.

The implementation of a disease management program for CBS, combined with normal commercial practices such as culling and washing fruit, further reduces the already low pest risk potential by reducing the prevalence of CBS affected fruit in commercial shipments.

Contents

I. Introduction

This document was prepared by the Plant Epidemiology and Risk Analysis Laboratory of the Center for Plant Health Science and Technology, USDA Animal and Plant Health Inspection Service (APHIS), Plant Protection and Quarantine (PPQ) in response to peer review and public comments received by the agency on its *Risk Assessment for the Importation of Fresh Lemon (*Citrus limon *(L.) Burm. f.) Fruit from Northwest Argentina into the Continental United States.*

International plant protection organizations, such as the International Plant Protection Convention (IPPC) of the United Nations, the Food and Agriculture Organization (FAO), and the North American Plant Protection Organization (NAPPO) provide guidance for conducting pest risk analyses. The methods used to initiate, conduct, and report pest risk analyses are consistent with guidelines provided by IPPC and NAPPO, specifically ISPM No 11, Pest Risk Analysis for Quarantine Pests, Including Analysis of Environmental Risks and Living Modified Organisms (IPPC, 2004). The use of biological and phytosanitary terms conforms to the Definitions and Abbreviations (Introduction Section) in the International Standards for Phytosanitary Measures, Section 1: Import Regulations Guidelines for Pest Risk Analysis (IPPC, 2004), the Glossary of Phytosanitary Terms and the Compendium of Phytosanitary terms (IPPC, 2004, 2007). These guidelines describe three stages of pest risk analysis: Stage 1, Initiation, Stage 2, Risk Assessment, and Stage 3, Risk Management. This document satisfies the requirements of IPPC Stage 1 and 2, and begins the discussion for Stage 3. Because this assessment examines fruit from any Citrus Black Spot (CBS) infected area, Stage 3 could not be completed fully as mitigation measures for specific countries and areas may vary.

This is a qualitative risk analysis; estimates of risk are expressed in terms of High, Medium, and Low pest risk potentials based on the combined ratings for specified risk elements related to the probability and consequences of pest introduction. For the purposes of this assessment High, Medium, and Low probabilities will be defined as:

High: Frequent events; more likely to occur than not occur
Medium: Moderate events; as likely to occur as not to occur
Low: Rare events; less likely to occur than not to occur

The appropriate risk management strategy for a particular pest depends on the risk posed by that pest. Identification of appropriate sanitary and phytosanitary measures to mitigate the risk for CBS is undertaken as part of the risk management phase discussed in this document.

II. Risk Assessment

2.1: Initiating Event

APHIS published a notice[1] in the Federal Register on August 13, 2007 (72 FR 45216-45217, Docket No. APHIS-2007-0112) in which the public was advised of the availability of the draft pest risk assessment (PRA) entitled: *"Risk Assessment for the Importation of Fresh Lemon*

[1] To view the notice, the draft PRA, and the comments APHIS received, go to http://www.regulations.gov/fdmspublic/component/main?main=DocketDetail&d=APHIS-2007-0112.

*(*Citrus limon *(L.) Burm. f.) Fruit from Northwest Argentina into the Continental United States."* APHIS solicited comments on this PRA for 60 days. On September 27, 2007, APHIS published another notice in the Federal Register (72 FR 54891-54892, Docket No. APHIS-2007-0112) in which APHIS extended the comment period on the draft PRA until December 11, 2007. APHIS received 21 comments by that date, from exporters, importers, domestic industry associations, researchers, and the national plant protection organization of Argentina. At the same time, APHIS submitted the draft PRA for external expert peer review in accordance with the Office of Management and Budget's bulletin on peer review (Final Information Quality Bulletin for Peer Review, Federal Register Vol. 70, January 14, 2005, 2664-2677).

APHIS received several comments from the public and peer reviewers regarding the status of fruit as a pathway for the introduction of *Guignardia citricarpa* Kiely, the causal agent of citrus black spot disease (CBS), as well as comments regarding other pests. The present assessment was initiated in response to comments to evaluate evidence associated with citrus fruit as a potential pathway for the introduction of *G. citricarpa* in the continental United States; other pests discussed in the PRA above are not addressed in this assessment.

2.2: Pest Categorization

CBS is caused by the fungus *Guignardia citricarpa* Kiely, 1948 (Ascomycetes, Dothideales) and its imperfect stage (anamorph) *Phyllosticta citricarpa* (McAlpine) Van der Aa. Historically, *G. citricarpa* Kiely was believed to include pathogenic and non-pathogenic variants. The "variants" of . *citricarpa* Kiely, which are morphologically identical, were examined molecularly and determined to be two distinct species of *Guignardia*, *G. citricarpa* Kiely (anamorph *P. citricarpa* (McAlpine) Van der Aa and *G. mangiferae* A.J. Roy (anamorph *P. capitalensis* Henn) (Baayen et al., 2002). *G. citricarpa* Kiely and the anamorph *P. citricarpa* (McAlpine) are considered to be quarantine pests for the United States (PestID, 2007).

In March, 2010, CBS disease was detected in a small area in Florida. To date, CBS disease has not been detected in other parts of Florida through multi-pest surveys conducted by Florida Department of Agriculture and Consumer Services (FDACS), Department of Plant Industry (DPI) as part of the Federal Citrus Health Response Program (CHRP). APHIS and FDACS DPI continue to delimit the area to determine the extent of the infestation (USDA, 2010). This document was originally written before the detection of CBS in Florida, and not as a response to this introduction.

The detection of CBS in Florida does not contribute to or detract from the evidence presented in this analysis that pertains to the evaluation of citrus fruit as a potential pathway for the introduction or establishment of *G. citricarpa*.

As mentioned above, the causal organism of citrus black spot disease has two stages: a sexual stage represented by the ascospores of *G. citricarpa*[2] Kiely and an asexual stage represented by

[2] Citrus black spot disease is properly identified as *Guignardia citricarpa* Kiely to correspond with the known sexual stage represented by ascospores (the teleomorph). The organism also has an asexual stage represented by the pycnidiospores of *Phyllosticta citricarpa* (McAlpine) (the anamorph) which is the stage found associated with fruit.

the pycnidiospores of *P. citricarpa* (McAlpine) Van der Aa (Appendix A). The peak production of these two stages is produced at different times, under different conditions, at different locations on the plant and result in different epidemiological dynamics.

Ascospores are produced in infected leaf debris from fungal structures called ascostroma that develop 40-180 days after infection (CABI, 2006). Alternate wet and dry periods aid in fungal growth, ascospore release. Released ascospores may infect susceptible host plants and result in disease development. Sequential and repeated wetting and drying of dead leaves is essential for ascostroma development (Kiely, 1948; Kotzé, 1981). Rainfall (or overhead irrigation) triggers the release of mature ascospores (Kotzé, 1963), but too much rainfall will disrupt ascospore discharge (Kotzé, 1981) and lead to the decomposition of the dead leaves destroying the substrate for *G. citricarpa* (Lee and Huang, 1973). In addition, excess rain reduces ascostroma formation as the leaves become colonized by competing saprophytes (CABI, 2006).

During rainfall, mature ascospores are forcibly ejected from the asci within the ascostroma up to a centimeter high (Kiely, 1948; Kotzé, 1963). Ascospores are subsequently spread by wind and water (Kiely, 1948; Kotzé, 1963; Whiteside, 1965). Upon contacting susceptible attached citrus leaves or fruit in a susceptible stage, ascospores germinate to form an appressorium. An infection peg then penetrates the cuticle, expanding into a small mass of mycelium located between the cuticle and epidermal wall to form a latent infection (Kotzé, 2000). Leaves are susceptible to infection up to 10 months of age (Truter et al., 2007), while fruit are susceptible for four to five months after petal fall independent of rainfall, temperature, or inoculum levels (Kotzé, 1963, 2000). Younger trees, less than 10 years old, appear to be less affected by CBS (Kiely, 1948) than older trees; in trees up to 10 years old the susceptible period of fruit is limited to 3 months, and CBS is more easily controlled (Kiely, 1969).

The infection in leaves typically remains latent with no symptom development until after the leaves die; although leaf spots occasionally occur on older leaves still attached to the tree (Kiely, 1948; Whiteside, 1965). In fruit, the infection typically remains latent until the fruit matures. Upon fruit maturation, the mycelium grows into the flavedo (outer rind tissue) producing circular lesions and occasionally pycnidia (the spore-producing structure of the asexual phase of the fungus). Whereas ascospores may initiate infections in fruit, ascostroma do not develop[3] on fruit on CBS affected trees or on artificially infected fruit (Kiely, 1948; Kotzé, 1963, 1981).

Lesions on leaves typically produce ascospores but may produce pycnidia (Kotzé, 1963, 2000; McOnie, 1967). The pycnidia produce pycnidiospores, also termed conidia, asexual spores in gelatinous masses (Korf, 1998). Mature pycnidiospores are embedded in a mucilaginous mass and emerge from pycnidia when wet. When the mass comes into contact with water, the mucilage dissolves and the spore suspension may then be splash-dispersed (Spósito et al., 2008). Pycnidiopsores are dispersed by water (Kiely, 1948; Whiteside, 1965). Pycnidiospores are not

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To be consistent with mycological conventions, *Guignardia citricarpa* Kiely is used throughout this document to refer to the CBS organism whether referring to either the teleomorph or the anamorph.

[3] Based on the large amount of scientific research, technical reports, and observations conducted on *G. citricarpa* and CBS disease, ascospores have never been observed to develop on citrus fruit. Therefore, considering the relative weight of this information we make the scientific judgment that ascospores do not develop on fruit.

airborne and must be released from pycnidia to infect and cause disease in young susceptible fruit (Kotzé, 1963), leaves and twigs (Agostini et al., 2006), usually within the same tree (Kotzé, 1981, 2000).

Even under ideal conditions (25°C, 90% RH, 14:10 light/dark cycle), pycnidiospores are not capable of infecting non-susceptible host tissue (Truter et al., 2007). Leaf litter, including both old and newly detached green leaves, are not susceptible to infection by pycnidiospores. Truter *et al.* (2007), "demonstrated that viable pycnidiospores from a culture, symptomatic fruit or peel were not able to infect and colonize freshly detached green leaves or natural leaf litter from Eureka lemon under controlled and field conditions. Even after exposure of the leaves to high inoculum pressure under highly favorable environmental conditions, pycnidiospores of *G. citricarpa* Kiely did not colonize the leaves. As Eureka lemon is the most susceptible cultivar to CBS, we can assume that similar results will be achieved on other susceptible cultivars." The pathogen strain used in this study was tested for pathogenicity in a concurrent study where the same methods and pathogen strain were used to infect leaves of different ages. Leaves, one to ten months old were successfully infected with this strain under the same experimental conditions (Truter et al., 2004). Colonization of the leaves in the Truter *et al.* (2007) study was determined through microscopic examination of the leaf tissue for the presence of *G. citricarpa*-like pycnidia or perithecia. PCR was then performed to confirm the presence of *G. citricarpa* or *G. mangiferae*.

Adequate hours of wetness, temperatures, and inoculum must be present simultaneously for infection to occur (Huang and Chang, 1972; Kotzé, 1981; Lee and Huang, 1973). For ascospores, a minimum of 15-38 hours of ascostroma wetness is required for successful production and germination (Kotzé, 1963), while pycnidiospore germination and appressoria formation require a minimum of 12 hours of wetness (Noronha, 2002). Temperature affects several aspects of CBS epidemiology. Ascostroma formation is optimal at temperatures between 21-28°C (Lee and Huang, 1973). Ascostroma formation ceases below 7°C and above 35°C (Lee and Huang, 1973). Under laboratory conditions, ascospores are ejected at a higher rate at higher temperatures (25°C) than at lower temperatures, but can still be expelled at temperatures as low as 5°C (Kotzé, 1963). In the field, the release of ascospores is more closely correlated to leaf wetness than temperature (Reis et al., 2006). For pycnidiospore germination and appressorium formation may occur between 10-40°C, but the extreme temperatures 10 and 40°C) require a longer wetting period (Noronha, 2002). Lesion development on fruit is correlated with temperature; as temperatures increase, lesion development also increases (Kotzé, 1981). However, four to five months after petal fall fruit become resistant to infection, independent of rainfall, temperature, or inoculum levels (Kotzé, 1963, 2000).

Latent infections on fruit are common and symptoms typically develop after fruit attains full size or becomes mature. Symptomatic citrus fruit may display four types of symptoms: hard spot, false melanose spot, freckle spot, and/or virulent spot (Kiely, 1948; Kotzé, 1963, 2000). The type of symptoms that develop is related to the temperature and stage of fruit maturity (Kotzé, 1963). Hard spot lesions typically develop pre-harvest, and are characterized by circular depressed lesions. Pycnidia are usually present in these lesions, but not always (Kiely, 1948; Kotzé, 2000). False melanose spot lesions typically develop on green fruit and do not contain pycnidia (Kotzé, 1963, 2000). Freckle spot lesions are typically round, light brown to red

(orange) and depressed lesions. Pycnidia may be found within these lesions (Kotzé, 1963, 2000). Virulent spot lesions develop on either fruit reaching maturity or fully mature fruit and are typically irregular in shape. Virulent spot lesions may contain numerous pycnidia, depending on environmental conditions (Kiely, 1948; Kotzé, 1963, 2000). Occasionally, two additional types of spots have also been reported (EPPO, 2009). Lacey spot lesions typically develop on green fruit and are smooth superficial lesions yellow to brown in color. These spots are most likely a variant of false melanose spot and do not contain pycnidia. Finally cracked spot lesions occasionally appear on fruit older than six months, these lesions are slightly raised and cracked and do not produce pycnidia. These lesions have been associated with the citrus rust mite (EPPO, 2009). Ascospores do not develop on fruit (Kiely, 1948; Kotzé, 1963, 1981).

Leaves may also harbor latent infections of CBS with symptoms developing three to ten months after infection (Wager, 1952). Symptomatic leaves typically display small pin-point dots that may develop into larger, perfectly round lesions (Wager, 1952). Development of lesions on leaves is rare unless disease pressure is high. Leaf lesions are most commonly observed on highly susceptible varieties, such as Eureka lemons (Kotzé, 1981).

The sexual stage, ascospores, are the primary source of tree to tree spread (Kotzé, 2000; McOnie, 1964d, 1965). The correlation between ascospore discharge and fruit infection, as well as the distribution of infected fruit throughout the tree showed that the asexual stage, pycnidiospores do not play a significant role in the disease cycle (McOnie, 1964d). Ascospores are responsible for pathogen introduction into new areas, while the secondary cycle of the disease or asexual stage may be responsible for increasing the disease within the tree canopy (Kotzé, 1981, 2000).

2.3: Assessment of the likelihood of introduction

This portion of the assessment assumes that fruit have not undergone any mitigation in the field or packinghouse.

2.3.1: Likelihood of entry of a pest

Potential Pathway: Natural spread
Once established in an area, *G. citricarpa* can spread readily in the natural environment with adequate rainfall, conducive temperatures, and adequate inoculum, resulting in increased incidence of CBS. Ascospores are spread by wind and water (Kiely, 1948; Kotzé, 1963). Wind is known to spread ascospores over short distances (Whiteside, 1965) to new areas. In mature groves, ascospores move less than 100 feet per season. A recent study of the spatial distribution of CBS in orchards in Brazil revealed highly aggregated patterns of disease, where the vast majority of new infections occur within 20 m of prior infections (Spósito et al., 2007). However, weather events such as hurricanes are known to carry propagules of other citrus diseases very long distances (Gottwald et al., 2002a), and may also spread CBS over long distances. There are no documented reports that *G. citricarpa* is established, or CBS occurs, in Canada, Mexico, or the Caribbean (CABI, 2006). Therefore, there is little likelihood that CBS could enter into the United States through natural spread from these areas.

Potential Pathway: Propagative Material

Long distance dissemination of the casual agent of CBS is most likely through the movement of infected nursery stock and budwood (Whiteside, 1965). The introduction of *G. citricarpa* into new areas in South Africa occurred through the movement of infected nursery stock with invisible latent infections (EPPO, 1997). Latent infections on leaves and fruit are common. Leaves may harbor latent CBS infections with symptoms developing three to ten months after infection (Wager, 1952).

The movement of citrus nursery stock into the United States is regulated by Quarantine 19 (7CFR §319.19, *Citrus Canker and Other Citrus Diseases*), which prohibits the importation of citrus plants or plant parts except fruit into the United States. We assume and expect that these regulations are strictly enforced. Infected citrus imported for propagation has been intercepted 11 times between 1985 and 2007 at U. S. ports-of-entry, with none of these interceptions coming from commercial shipments (PestID, 2007). The total incidence of smuggled *G. citricarpa* infected nursery stock entering the United States was not estimated in this review, but is likely to occur at some low level. Due to the current regulations there is a low likelihood that CBS will enter the United States through the movement of regulated commercial propagative material.

Potential Pathway: Leaves as contaminants
Citrus fruit may be imported into the United States under permit and strict guidelines, which typically include requirements that the shipments be free of leaf and stem debris. We assume that these regulations are enforced. Despite restrictions, leaves may move with non-commercial fruit at a low level. From 1985-2007 CBS-affected leaves were intercepted at US ports-of-entry with fruit for consumption eleven times. The interceptions also occurred with non-commercial fruit found in baggage and mail (PestID, 2007).

Leaves may harbor latent infections of CBS with symptoms developing three to ten months after infection (Wager, 1952). Ascospores, the sexual stage, are the primary source of inoculum (Kotzé, 2000; McOnie, 1964d, 1965). The correlation between ascospore discharge and infection onset demonstrates that ascospores play a significant role in the disease cycle of CBS (McOnie, 1964d). Ascospores produced on decomposing leaves may be responsible for pathogen introduction into new areas (Kotzé, 1981, 2000), in addition to the infected nursery stock.

Leaves infected with *G. citricarpa* may play an important epidemiological role in disease spread. Due to the restrictions in place preventing the movement of leaves with fruit shipments and the low number of interceptions over the past 23 years, there is a medium likelihood of *G. citricarpa* entering the United States via leaves contaminating fruit shipments.

Potential Pathway: Fruit
Citrus fruit infected with *G. citricarpa* and showing CBS lesions is likely entering the United States at a high rate. Numerous years of interception data (1985-2007) at US ports-of-entry show that *G. citricarpa* infected fresh fruit has been intercepted 4,694 times[4] (PestID, 2007). These

[4] Prior to 2002, *G. citricarpa* could not be reliably differentiated from *G. mangiferae* (Baayen et al., 2002). It is likely that a portion of these intercepted fruit were not infected with *G. citricarpa* but rather *G. mangiferae.* Therefore, the number of interceptions that pertain specifically *G. citricarpa* infected fruit are likely lower than what

interceptions indicate that inspectors can detect diseased fruit, although the total number of CBS affected fresh fruit that enters the United States undetected or not inspected is uncertain. We assume that the number of undetected or not inspected fruit is larger than the total number of fruit detected, as is the case with diseases on other commodities (*e.g.* Meissner et al., 2009; Meissner et al., 2003).

The majority of CBS interceptions involved fresh fruit from non-commercial shipments, with only 23 of recorded interceptions occurring in commercial shipments (permit cargo) (Table 1) (PestID, 2007). This indicates that infected fruit move more frequently in unregulated and/ or non-commercial pathways.

Table 1: Interceptions of CBS infected fresh fruit from 1985 to 2007.

Type of shipment where CBS infected fresh fruit was intercepted	**Number of interceptions**
Permit cargo	23
Baggage	2008
General cargo	12
Holds (supplemental cargo areas)	7
Mail	31
Miscellaneous	6
Quarters (crew area)	222
Stores (non-cargo storage)	2385

In addition to these interceptions, CBS affected dried fruit was intercepted 1,034 times[3], and interceptions that did not indicate the host part that was infected, but were declared as fruit were intercepted 1,168 times[3] (PestID, 2007). If these interceptions are taken into account, CBS affected fruit imported as fruit has been intercepted 6,896 times from 1985 to 2007. Therefore there is a high likelihood that *G. citricarpa* will enter the United States via unregulated and/ or non-commercial infected fruit.

Summary

Four potential pathways that *G. citricarpa* may enter the United States are identified above: natural spread, the importation of propagative material, the importation of leaves as contaminants, and the importation of fruit. *G. citricarpa* is not known to be established anywhere in Canada or Mexico, therefore, it is not likely that *G. citricarpa* could enter into the United States through natural spread. The importation and domestic movement of propagative material is currently regulated by PPQ, therefore, there is a low likelihood that *G. citricarpa* will enter the United States via this pathway. Due to the restrictions in place preventing the movement of leaves with fruit shipments and the low number of interceptions over the past 23 years of leaves contaminating shipments of citrus, there is a medium likelihood of *G. citricarpa* entering the United States via contaminated leaves. Based on years of interception data, there is a high likelihood that *G. citricarpa* will enter the United States via unregulated and/or non-commercial infected fruit.

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is reported. The number of infected fruit misidentified as *G. citricarpa* is unknown, therefore, we conservatively assume that all interceptions were correctly identified as infected with *G. citricarpa* for this analysis.

The epidemiological significance of the entry of *G. citricarpa* infected fruit will be explored in the next section of the risk assessment, which explores the likelihood of establishment of CBS disease from the fruit pathway.

2.3.2: Likelihood of establishment

2.3.2.1 Availability of suitable hosts in PRA area

CBS is primarily a disease of fruit (Kotzé, 2000), although leaves and stems are also infected (CABI, 2006). All commercially grown *Citrus* spp. are susceptible to CBS, with the exception of sour orange (*C. aurantium* L.) and its hybrids. Lemons (*Citrus limon*) are particularly susceptible to CBS (Kotzé, 1981).

Erroneous reports exist in the literature suggesting CBS affects non-citrus hosts (Kiely, 1948), such as almonds (*Prunus dulcis*), avocados (*Persea americana*), *Eucalyptus* spp., guavas (*Psidium guajava, P. montanum*), mangoes (*Mangifera indica*), passionfruits (*Passiflora edulis*), *Rubus* spp., *Caesalpinia pulcherrima, Callistemon citrinus, Camellia japonica, Dendrobium speciosum*, holly (*Ilex aquifolium*), *Magnolia* sp., *Smilax* sp. cardamoms (*Elettaria cardamomum*), *Cola nitida, Dioscorea pentaphylla, Eucalyptus deglupta*, and sugarcane (*Saccharum officinarum*) (EPPO, 1997). While CBS-like symptoms appear to occur on several non-citrus hosts, several *Guignardia* spp.-caused symptoms on non-citrus hosts closely resemble *G. citricarpa* Kiely (McOnie, 1964b). Recent molecular studies have demonstrated that *G. citricarpa* does not infect hosts outside of the genus *Citrus*. A cosmopolitan *Guignardia* (*Phyllosticta*) species, which is not pathogenic, occurs on a wide range of hosts and has previously been misidentified as *G. citricarpa* Kiely (Baayen et al., 2002).

Summary
Multiple host range studies, including molecular examination of questionable hosts, have confirmed the host range of *Guignardia citricarpa* with a high level of certainty. All of the hosts occur within a single genus, *Citrus,* limiting the number of suitable hosts in the United States. Furthermore, citrus is only grown in U.S. plant hardiness zones 8-10 (BLCN, undated; Wright, 2001) and imported fruit is distributed in the United States according to market demands through commercial distribution areas, meaning much of the fruit goes to areas where citrus is not grown; further reducing the likelihood of *G. citricarpa* finding a suitable host. Based on the above information there is a low likelihood that *G. citricarpa* will find a suitable host in the United States.

2.3.2.2 Suitability of environment

CBS occurs in tropical and subtropical regions with abundant rainfall in the summer, including Argentina, Bhutan, Brazil, Hong Kong, China, India, Indonesia, Kenya, Nigeria, Mozambique, Philippines, Swaziland, Taiwan, Uruguay, Venezuela, West Indies, Zambia, and Zimbabwe, parts of South Africa and coastal areas of Australia (EPPO, 1997; Paul et al., 2005). Reports of CBS occurring in Japan and New Zealand are conflicting and there is no definitive report clarifying the status of *G. citricarpa* or CBS in those countries (Paul et al., 2005).

Factors such as rainfall and temperature, the amount of available inoculum, and the susceptibility of the fruit influence the survival, sporulation, and dispersal of the pathogen. Wetting and drying of leaves is essential for ascostroma development (Kiely, 1948; Kotzé, 1981). Rainfall is positively correlated with disease development during susceptible periods and disease development is negatively correlated with rainfall after petal fall, when infection occurs (Kiely, 1950). For ascospores a minimum of 15-38 hours of wetness is required for production and germination (Kotzé, 1963), while pycnidiospore germination and appressorium formation require a minimum of 12 hours of wetness (Noronha, 2002).

Temperature affects several aspects of CBS epidemiology. Ascostroma formation is optimal at temperatures between 21-28°C (Lee and Huang, 1973). Ascostroma formation ceases below 7°C and above 35°C (Lee and Huang, 1973). Under laboratory conditions, ascospores are ejected at a higher rate at higher temperatures (25°C), but can still be expelled at temperatures as low as 5°C (Kotzé, 1963). In the field, the release of ascospores is more closely correlated to leaf wetness than temperature (Reis et al., 2006). For pycnidiospores, appressorium formation may occur between 10-40°C, with appressorium formation at the extreme temperatures requiring a longer wetness period (Noronha, 2002).

All *Citrus* spp. are susceptible to CBS (EPPO, 1997; Farr et al., 2007). Citrus is only grown in U.S. plant hardiness zones 8-10 (BLCN, undated; Wright, 2001). Fruit are susceptible to infection for four to five months after fruit set (Kotzé, 1963, 2000), while leaves are susceptible up to 10 months of age (Truter et al., 2007).

There are several factors that limit the global distribution of CBS. Cold stress appears to be the major factor limiting CBS distribution, but heat stress and insufficient moisture levels are also limiting factors (Paul et al., 2005).

A climate suitability model developed by Magarey and Holtz (2009) using NAPPFAST examined the potential distribution of *G. citricarpa* if it were to be introduced into the United States. The NAPPFAST global database was derived from NCEP gridded data (Kalnay et al. 1996) and resampled to 32 km. Station data from the ISHS network was used to supplement the grid data. The combination of both grid and station data sources improves the quality of predictions in areas with sparse or lower quality weather networks. The database includes both native variables (e.g. air temperature) and derived variables (leaf wetness) (Magarey et al. 2007). This climate suitability model improved upon an earlier NAPPFAST model (Magarey and Borchert, 2003) by including international validation data. Model parameters were taken from EFSA (2008) and assumed to be widely accepted values for each parameter, Ascospore: minimum temperature = 15°C, maximum temperature = 35°C, optimum temperature = 27°C, minimum wetting hours = 15 h, maximum wetting hours = 38 and precipitation > 2mm; Pycnidiospores: minimum temperature = 10°C, maximum temperature = 35°C, optimum temperature = 25°C, minimum wetting hours = 12 h, maximum wetting hours = 35 and precipitation > 2mm. Predictive maps were developed for the months during periods of fruit susceptibility to CBS disease (April through August). According to the suitability maps (Figures 1 and 2), *G. citricarpa* is likely to be a threat to citrus production in Florida and to a lesser extent Gulf Coast production. It is unlikely to be a concern in California (Magarey and Holtz, 2009). The NAPPFAST infection model did not include wetness interruption, as this feature is not yet available in the NAPPFAST infection model. In addition, the NAPPFAST infection model used a 32 k grid data set which is relatively coarse and

may not perform as well in areas with mountainous topography. Due to the structure of the climate suitability model there is the possibility that small areas with microclimates suitable for CBS disease development may occur in areas generally deemed as unsuitable, such as California. If such microclimates do exist, and assuming *G. citricarpa* can successfully establish in those areas, it is not likely that *G. citricarpa* would spread from those small areas as the larger surrounding area is not suitable for CBS disease development.

A model predicting geographic distribution of CBS produced by Paul et al. (2005) yielded similar results as the Magarey and Holtz (2010) model. The Paul et al. (2005) model found that favorable climates exist in Florida and Texas, while California has a climate unsuitable for establishment of *G. citricarpa*.

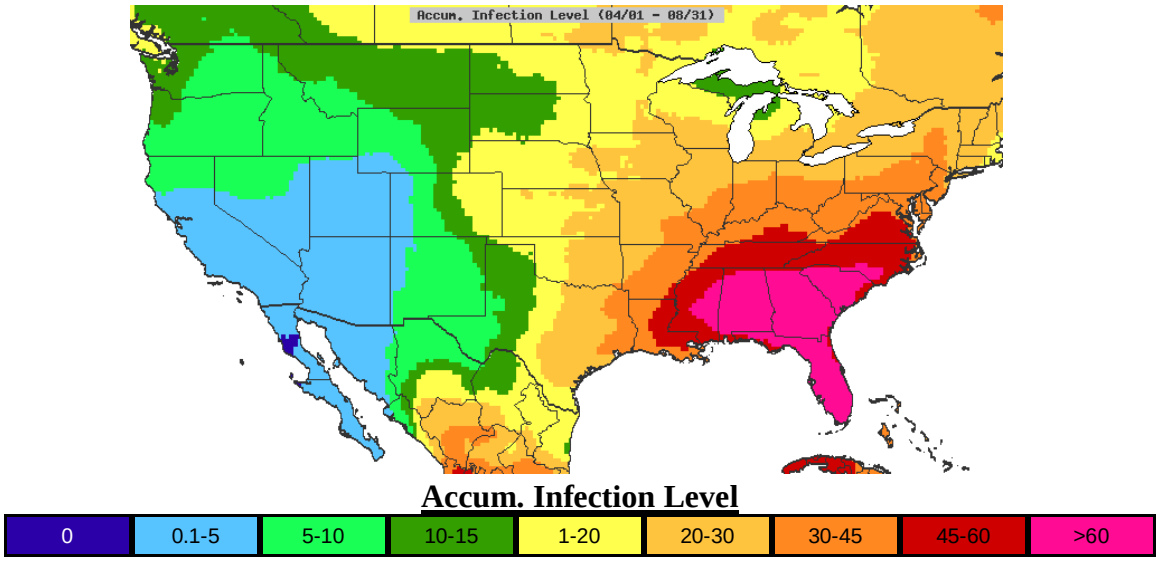

Figure 1. The average accumulated number of days suitable for *G. citricarpa* ascosporic infection between April and August based on 10 years of climate data (Magarey and Holtz, 2009).

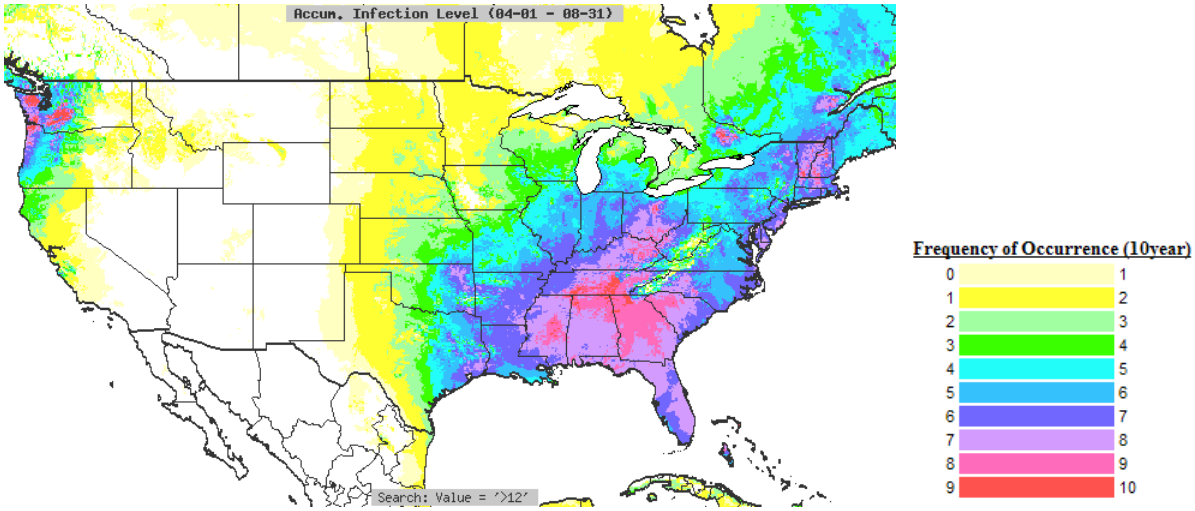

Figure 2. The probability of more than 12 days suitable for *G. citricarpa* ascosporic infection between April and August (Magarey and Holtz, 2009).

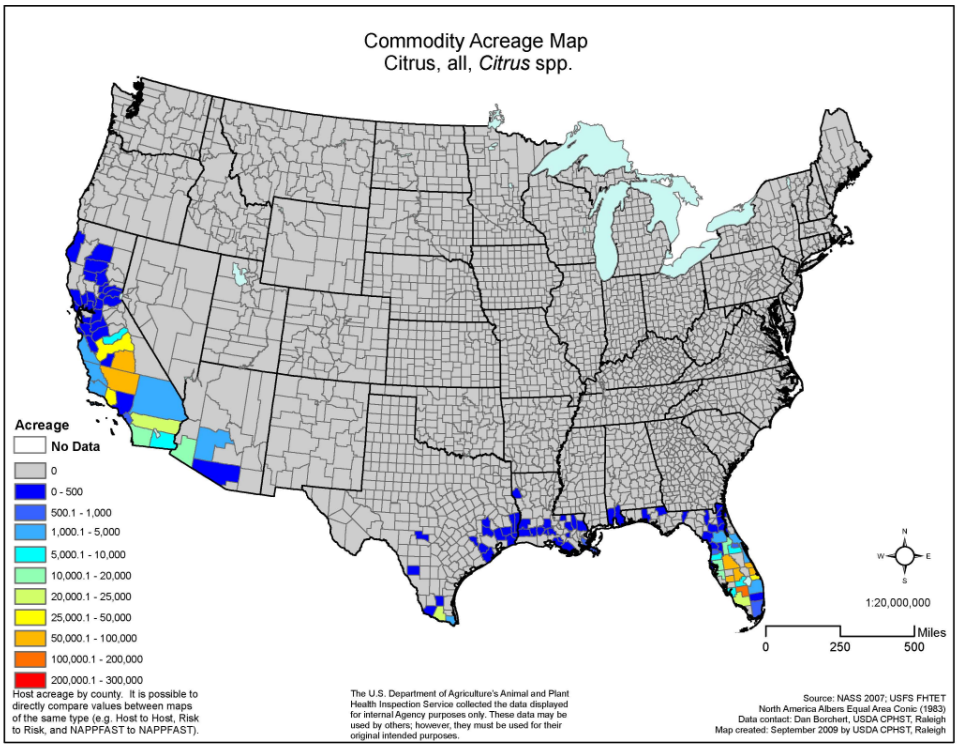

Figure 3. Acreage of citrus grown in the United States by county.

There is an inherent level of uncertainty associated with the use of models to predict suitable areas of establishment, particularly for plant pathogens because strains of bacterial and fungal plant pathogens can vary genetically and may have varying tolerances to biotic and abiotic environmental factors. While no model can accurately capture all potential genetic variants of any organism, the two models used in this analysis to estimate the regions in the United States that *G. citricarpa* could establish based on ecological parameters currently experienced in other countries, use slightly different parameters yet conclude similar forecasts of suitability.

Summary

The areas of the United States deemed suitable for *G. citricarpa* establishment are limited primarily to the Southeast portion of the United States. Based on the above information there is a medium likelihood that CBS would encounter a suitable environment to establish in the United States.

2.3.2.3 Likelihood that the pest, *G. citricarpa*, will find a suitable host and incite disease

For successful transmission of *G. citricarpa*, the causal agent of CBS, from fruit with lesions to susceptible hosts, several events must occur:

1. Infected fruit must arrive in an area with hosts available and conducive for infection and disease development;
2. The host needs to be in a susceptible physiological stage for infection to occur;
3. Spores of the causal organism must be produced on the fruit;

4. Fruit with lesions containing the causal organism, *G. citricarpa*, in a stage that can cause infection leading to disease must be released from the lesions;
5. Water contaminated with pycnidiospores must be brought into contact with susceptible host tissue in a susceptible stage for infection; and
6. Enough time must elapse with the relevant weather conditions remaining conducive for infection to occur.

All of these events must occur for successful transmission and establishment. The events are assessed in further detail below.

1. Infected fruit must arrive in an area with hosts available and conducive for infection and disease development. Hosts susceptible to CBS are exclusively in the genus *Citrus* (Baayen et al., 2002). Citrus is only grown in U.S. plant hardiness zones 8-10 (BLCN, undated; Wright, 2001). United States demographics and the distribution of markets are strong indicators of the ultimate destination of imported fruit. It is assumed that the distribution of the U.S. population according to the 2000 U.S. Census (Figure 4) describes the likely patterns of imported fruit destined for human consumption (US Census Bureau, 2001). Comparing the susceptibility maps (Figures 1 & 2), the commercial acreage of citrus (Figure 3) and the corresponding areas on the U.S. Census map (Figure 4) is a useful tool in estimating the likelihood of imported fruit being moved to a suitable environment for disease development.

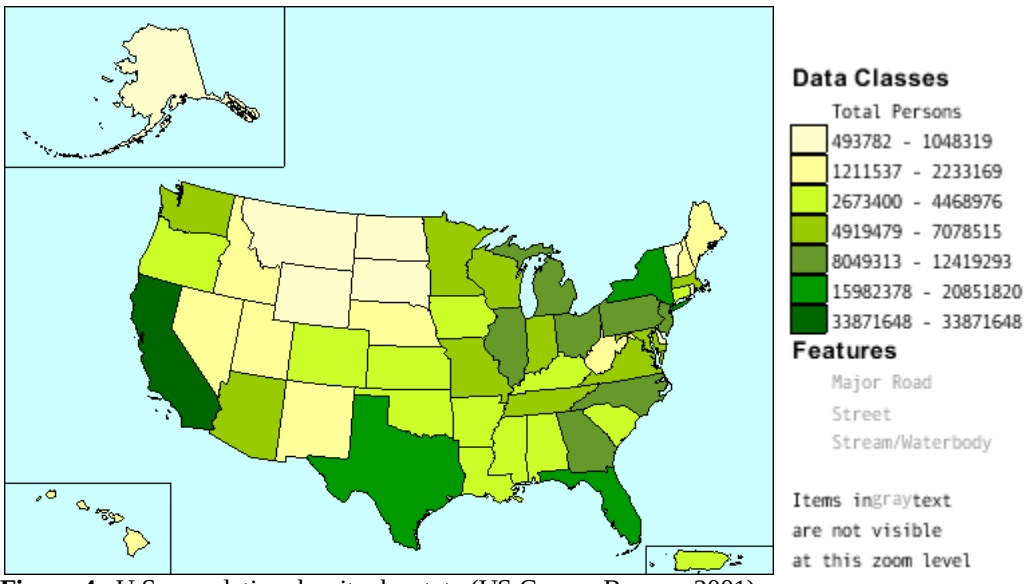

Figure 4. U.S. population density, by state (US Census Bureau, 2001)

Three of the four most populous States in the United States- Florida, Texas, and California-are in the southern tier of the United States (Figure 4) (US Census Bureau, 2001), where the climate most closely resembles the native climates for *G. citricarpa* and CBS occurrence (US Census Bureau, 2001). If we assume that citrus is proportionally distributed across the United States in accordance with the population then it is reasonable to assume that imported fruit will be shipped to these States; however, only a small portion of each State actually produces citrus (Figure 3), and an even smaller portion has a climate suitable for CBS disease development (Figure 2).

While California is the most populated state, and therefore, could reasonably receive the highest number of infected fruit only a small portion of the state has a climate suitable for CBS development based on the Magarey and Holtz (2010) model (Figure 2) and the majority of commercial citrus grown in California is not grown in that area (Figure 3). Therefore, there is a low likelihood that infected citrus will end up in areas with environmental conditions conducive for *G. citricarpa* infection and CBS development in California. Assuming that fruit is distributed based on population patterns, Texas has high likelihood of receiving *G. citricarpa* infected fruit, however, only a very small portion of the state grows citrus fruit commercially (Figure 3), including areas with environmental conditions conducive for *G. citricarpa* infection and CBS development are found within a CBS susceptible climate (Figure 3). Therefore, there is a medium likelihood that that infected citrus will end up in CBS susceptible areas in Texas. In Florida, another highly populated state (Figure 4), there is a high likelihood that the state could receive *G. citricarpa* infected citrus fruit. Florida produces a large amount of commercial citrus (Figure 3), and the entire state has a very conducive climate for CBS development (Figure 2). Therefore, there is a high likelihood that infected citrus will end up in areas with environmental conditions conducive for *G. citricarpa* infection and CBS development in Florida.

2. The host needs to be in a susceptible physiological stage for infection to occur. Leaves are susceptible up to 10 months of age (Truter et al., 2007), while fruit are susceptible four to five months after petal fall, independent of rainfall, temperature, or inoculum levels (Kotzé, 1963, 2000). Younger trees, less than 10 years old, appear to be less susceptible to CBS (Kiely, 1948). In trees up to 10 years old, the susceptible period is limited to 3 months (Kiely, 1969). In Florida the citrus trees would be most susceptible to infection in the spring around mid-March. Sixty percent of new leaf flush occurs at this time (Mossler and Aerts, 2009). New leaves produced during this flush would be highly susceptible to infection. The life span of a leaf before it falls is variable depending upon several environmental factors (Erickson, 1968). In oranges, the average life span varied from seventeen to twenty four months (Wallace et al., 1954).

Even under ideal conditions (25^{o}C, 90%RH, 14:10 light/dark cycle), pycnidiospores are not capable of infecting non-susceptible host tissue (Truter et al., 2007). Leaf litter, including both old and newly detached green leaves, are not susceptible to infection by pycnidiospores. Truter *et al.* (2007), "demonstrated that viable pycnidiospores from a culture, symptomatic fruit or peel were not able to infect and colonize freshly detached green leaves or natural leaf litter from Eureka lemon under controlled and field conditions. Even after exposure of the leaves to high inoculum pressure under highly favorable environmental conditions, *G. citricarpa* Kiely did not colonize any of the leaves. As Eureka lemon is the most susceptible cultivar to CBS, we can assume that similar results will be achieved on other susceptible cultivars." The pathogen strain used in this study was tested for pathogenicity in a concurrent study where the same methods and pathogen strain were used to infect leaves of different ages. Leaves 1 to 10 months old were successfully infected with this strain under the same experimental conditions (Truter et al., 2004). Colonization of the leaves in the Truter *et al.* (2007) study was determined through microscopic examination of for the presence of *G. citricarpa* like pycnidia or perithecia. PCR was then performed to confirm the presence of *G. citricarpa* or *G. mangiferae*.

Harvested mature *G. citricarpa*-infected fruit with CBS lesions do not spread pycnidiaspores to other harvested mature uninfected fruit even when placed in direct contact (Korf et al., 2001a).

Germination of pycnidiospores on mature leaves is possible under laboratory conditions. In a dissertation by Nozaki (2007), that examined the production of pycnidiospores under laboratory conditions, she was able to achieve a low level of pycnidiospore germination. In the experiment mature leaves were autoclaved and a high concentration of conidia (10^3-10^5 conidia/mL) was applied in one spot on the leaf. Those leaves were then placed under optimum conditions (25^{o}C, 12 hours light/ 12 hours dark) for pycnidiospore germination in a growth chamber. Under these conditions a 10-25% germination occurred at 7 hours of incubation. The conditions in which germination occurred are highly contrived under laboratory conditions and are unlikely to occur in nature. In addition there was no indication that the leaf tissue became infected.

As citrus trees are not susceptible to *G. citricarpa* infection year round and pycnidiospores are not capable of infecting non-susceptible host material under natural conditions, there is a medium likelihood that *G. citricarpa* will encounter a host in a susceptible physiological stage for infection to occur.

3. Spores of the causal organism must be produced on the fruit. *G. citricarpa*, the causal organism of CBS, has two life cycle stages: a sexual stage represented by the ascospores of *Guignardia citricarpa* Kiely and an asexual stage represented by the pycnidiospores of *Phyllosticta citricarpa* (McAlpine) (Appendix A). These two stages are produced at different times, under different environmental conditions, at different locations on the plant and result in different epidemiological dynamics.

Ascospores, the sexual stage, are the primary source of spread of the pathogen (Kotzé, 2000; McOnie, 1964d, 1965). Ascospores are responsible for pathogen introduction into new areas, while the secondary cycle of the disease or asexual stage may be responsible for increasing the incidence and/or disease within the same or nearby plants (Kotzé, 1981, 2000). The correlation between ascospore discharge and infection onset showed that pycnidiospores, the asexual stage, do not play a significant role in the disease cycle. “The correlation between ascospore discharge and the onset of infection indicates, therefore, that pycnidia on the dead leaves do not play a significant role” (McOnie, 1964d).

Ascostroma do not develop on fruit (Kiely, 1948; Kotzé, 1963, 1981). Ascospores are not considered to be epidemiologically significant on fruit. *G. citricarpa* infected fruit are only known to produce pycnidiospores, that do not play a significant role in the disease cycle (McOnie, 1964d), and are not considered a primary means of introducing *G. citricarpa* into new areas (Kotzé, 1981, 2000).

G. citricarpa infected symptomatic fruit produce pycnidia, the asexual phase of the fungus. Latent infections on fruit are common and symptoms often develop after fruit attains full size or becomes mature (Kotzé, 1963, 1981, 2000; McOnie, 1967). Without lesions with pycnidia that produce pycnidiospores, there is no documented way for *G. citricarpa* to spread from infected fruit. Therefore, it is assumed that asymptomatic *G. citricarpa* infected fruit do not spread *G. citricarpa*.

After harvest, *G. citricarpa* infected fruit may develop lesions during packing, shipping, or upon arrival at the final destination. Storage temperatures influence the rate of disease development. Low temperatures (~8^{o}C) delay symptom development (Agostini et al., 2006), while high storage temperatures (~27^{o}C) and 24-hour fluorescent lighting increase the rate of symptom development (Brodrick and Rabie, 1970; CABI, 2006; Korf, 1998).

Upon fruit maturation, fungal mycelia grow into the rind (but not into the juice vesicles), producing necrotic lesions in the peel, some of which produce pycnidia (Kotzé, 1963, 2000; McOnie, 1967). The pycnidia produce pycnidiospores, asexual spores in gelatinous masses (Korf, 1998). Mature pycnidiospores are embedded in a mucilaginous mass and emerge from pycnidia when wet. When the mass come in contact with water, the mucilage dissolves and the spore suspension may then be splash-dispersed (Spósito et al., 2008). For pycnidiospores, appressorium formation may occur between 10-40^{o}C, with the extreme temperatures requiring a longer wetting period (Noronha, 2002). Lesion development on fruit is correlated with temperature; as temperatures increase, lesion development also increases (Kotzé, 1981). Pycnidiospores may be dispersed by water (Kiely, 1948; Whiteside, 1965), but are not dispersed over great distances (0-3m) (Madden, 1997).

Symptomatic citrus fruit may display four types of lesions: hard spot, false melanose spot, freckle spot, and/or virulent spot, not all of which produce pycnidia (Kiely, 1948; Kotzé, 1963, 2000). The type of lesions that develop is influenced by the temperature and stage of fruit maturity (Kotzé, 1963). Hard spot lesions typically develop pre-harvest, and are characterized by circular depressed lesions. Pycnidia are usually present in these lesions, but not always (Kiely, 1948; Kotzé, 2000). Melanose spot lesions typically develop on green fruit and do not contain pycnidia (Kotzé, 1963, 2000). Freckle spot lesions are typically round, light brown to red (orange) and depressed lesions. Pycnidia may be found within these lesions (Kotzé, 1963, 2000). Virulent spot lesions develop on either fruit reaching maturity or fully mature fruit and are typically irregular in shape. Virulent spot lesions may contain numerous pycnidia, depending on environmental conditions (Kiely, 1948; Kotzé, 1963, 2000). Occasionally, two additional spots have also been reported (EPPO, 2009). Lacey spot lesions typically develop on green fruit and are smooth superficial lesions yellow to brown in color. These spots are most likely a variant of false melanose spot. Finally cracked spot lesions occasionally appear on fruit older than six months. These lesions are slightly raised and cracked and do not produce pycnidia. These lesions have been associated with the citrus rust mite (EPPO, 2009).

Despite substantial research and observation, there is no documented mechanism for asymptomatic *G. citricarpa* infected dried or fresh fruit to serve as a source of inoculum for disease development. Therefore, even if *G. citricarpa* infected asymptomatic fruit comes in contact with a host in a susceptible stage there is no mechanism for the pathogen to spread from the fruit to the host. *G. citricarpa* infected fruit can develop lesions that may contain pycnidia typically sometime after harvest. The onset of lesion development varies, based primarily on temperature. Symptomatic fruit may produce pycnidospores. Ascospores, the primary source of *G. citricarpa* spread, do not develop on fruit. Lesions that do develop on fruit only produce pycnidiospores, although not all lesions will produce pycnidiospores. Pycnidiospores are not considered a primary means of introducing *G. citricarpa* into new area. Based on the above

information, there is a medium likelihood that the causal organism will be produced to cause disease onset on any given piece of fruit.

4. Fruit with lesions containing the causal organism, *G. citricarpa*, in a stage that can cause infection leading to disease must be released from the lesions and germinate. Dry lesions with pycnidiospores on infected fruit cannot infect susceptible plant tissue, as the pycnidiospores do not spread from lesions in the absence of water. Pycnidiospores cannot be released from lesions on dry symptomatic fruit (Kiely, 1948). Pycnidiospores are only spread from the lesions through wetting (Kotzé, 1963). "Pycnidiospores have no special mechanism of release into the atmosphere, and those occurring on dead leaves, on the ground can reach the susceptible fruits only by splashing of raindrops" (Kotzé, 1981). Water-splashed conidia typically do not travel far (approximately 0-3m) from the source (Madden, 1997). Pycnidiospores in lesions on dry mature fruit are not capable of infecting other uninfected mature fruit even when placed in direct contact (Korf et al., 2001a). Symptomatic fruit hung in wire baskets in the field in susceptible trees were not capable of causing infection in those trees when the fruit remained dry (Wager, 1949).

The initial wetting of fruit kept dry throughout the shipping process may release pycnidiospores which have a very small chance of germinating. When symptomatic fruit were removed from the field and washed to collect pycnidiospores revealed that only 1% of the pycnidiospores germinated (Kiely, 1948). The same symptomatic fruit was then held in the lab and submerged in water for 1 hour at 22^{o}C then held for 24 hours at 25^{o}C. This cycle was repeated for 4 days and had a remarkable effect on the germination success as it increased the success from less than 1% to 10%, 25%, 48% and 78% respectively (Kiely, 1948). The conclusion of this study stated "…fresh crops of pycnidiospores are produced and displace the spores which had been formed earlier under less favorable conditions …moisture conditions at the time of pycnidiospore formation influence germination ability, and that such pycnidiospores in any case are short-lived" (Kiely, 1948). Another study concluded that pycnidiospore germination and appressoria formation requires a minimum of 12 hours of wetness (Noronha, 2002). While small amounts of pycnidiospores that are able to germinate are released upon the first wetting, optimum pycnidiospore germination requires multiple wetting and drying cycles (Kiely, 1948).

Infected fruit with lesions would need to be discarded or held in a situation where it would come into direct contact with water in order for the pycnidiospores to be spread from the lesions. Pycnidiospores released upon the first wetting of dry fruit have a very low germination success rate. Pycnidiospore germination increases with multiple wetting and drying cycles. Therefore, fruit would need to be held or discarded in a manner that it would be exposed to multiple wetting and drying cycles. Based on the above information, there is a low likelihood that fruit with lesions containing the casual organism in a stage that can cause infection resulting in disease development will be released from the lesions and germinate.

5. Water contaminated with pycnidiospores must be brought into contact with a susceptible host tissue in a susceptible stage for infection. The only mechanism for pycnidiospores to spread to susceptible hosts is through the movement of pycnidiospore contaminated water (Kotzé, 1981). "Pycnidiospores have no special mechanism of release into the atmosphere, and those occurring on dead leaves, on the ground can reach the susceptible fruits only by splashing of raindrops" (Kotzé, 1981). Therefore, it is assumed that fruit would need to be held or

discarded in a manner that would facilitate pycnidiospores washed from the lesions to come into contact with susceptible hosts with tissue in a susceptible stage for infection. Infected tissue would need to be within close proximity to a susceptible host at a susceptible stage and then wetted to cause infection (Agostini et al., 2006; Kotzé, 2000). It is assumed that citrus fruit is imported for consumption and it is unlikely that fruit will be discarded within close proximity to susceptible citrus trees. Based on the above information there is a low likelihood that water contaminated with pycnidiospores from infected fruit will be brought into contact with a susceptible host in a susceptible stage.

6. Enough time must elapse with the relevant weather conditions for infection to occur.
Once a host is infected with CBS, the fungus remains dormant until conditions for renewed growth and symptom development are appropriate. The fungus in infected leaves remains dormant typically until the leaves fall from the tree. Ascospores are produced in infected leaf debris from ascostroma that develop 40-180 days after infection (CABI, 2006). After deposition on attached leaves or fruit in a susceptible stage, ascospores germinate to form an appressoria. An infection peg then penetrates the cuticle, expanding into a small mass of mycelium between the cuticle and epidermal wall to form a latent infection (Kotzé, 2000). Approximately 12 to 15 months separate the initiation of the primary infection (ascospore development in leaves) and the development of pycnidiospores for secondary infection (Kiely, 1948). Infection of the fruit occurs with the actual penetration of the fruit rind by the germinating fungus spore. The fungus then forms a resting body within the rind that remains dormant until the fruit matures and temperature conditions are appropriate. (PPQ, 2002). Once a host is infected a considerable amount of time passes before symptoms appear. During that time it is unlikely that the infected asymptomatic host will be removed or the disease eradicated. Therefore, there is a high likelihood that enough time will elapse for key host-pathogen interactions resulting in infection to occur.

Summary
For successful transmission of *G. citricarpa* from fruit with lesions to susceptible hosts, all of the above events must occur. The likelihood of any given event occurring varies based on the individual parameters of the event. In summary, there is a low likelihood that infected citrus will end up in California, a medium likelihood that they will end up in Texas, and a high likelihood that they will end up in Florida; in areas with environmental conditions for *G. citricarpa* infection and CBS development. There is a medium likelihood that *G. citricarpa* will encounter a host in a susceptible physiological stage for infection to occur. In addition, there is a medium likelihood that the causal organism will be produced to initiate disease development on any given piece of fruit. There is a low likelihood that fruit with lesions containing the casual organism, in a stage that can initiate infection resulting in disease development will be released from the lesions and germinate. There is a low likelihood that water contaminated with pycnidiospores from infected fruit will be brought into contact with a susceptible host in a susceptible stage. Finally, there is a high likelihood that enough time will elapse for key host-pathogen interactions for infection to occur.

As successful transmission of *G. citricarpa* from infected fruit into the environment is dependent on the success of all six events. As there is a low likelihood that two events will occur (fruit with lesions containing the casual organism in a stage that can initiate infection resulting in disease

development will be released from the lesions and germinate; and water contaminated with pycnidiospores from infected fruit will be brought into contact with a susceptible host in a susceptible stage), the overall likelihood that the pest will find a suitable host with susceptible tissue and incite disease is also low.

2.3.2.4 Biological attributes that affect likelihood of establishment in the United States once the causal organism is transferred to a susceptible host

Reproductive potential

The causal organism of CBS has two distinct reproductive stages: a sexual stage represented by the ascospores of *G. citricarpa* Kiely and an asexual stage represented by the pycnidiospores of *Phyllosticta citricarpa* (McAlpine) Van der Aa (Appendix A). Ascospores initiate a disease cycle by developing in infected leaf debris. Infection of attached leaves typically remains latent, with no symptom development until after the leaves die, although leaf spots occasionally occur on older leaves while still attached to the tree (Kiely, 1948; Whiteside, 1965). Dead leaves may produce ascospores for several months, even when the leaves are in an advanced stage of decomposition (Kiely, 1948). On fruit, the infection remains in the latent stage until the fruit matures. Upon fruit maturation, the infection progresses into the rind, producing spots and pycnidia (the asexual phase of the fungus). Under favorable environmental conditions, pycnidiospore production is continuous (Kiely, 1948). Ascospores do not develop on fruit (Kiely, 1948; Kotzé, 1963, 1981).

The sexual stage, ascospores, are the primary source of inoculum (Kotzé, 2000; McOnie, 1964d, 1965) and are responsible for pathogen introduction into new areas, while the secondary cycle of the disease or asexual stage (conidia) may be responsible for increasing the disease within the same or nearby plants (Kotzé, 1981, 2000).

The production of ascospores on an infected tree greatly increases the likelihood of establishment of CBS in the United States. Ascospores are easily spread by wind and water (Kiely, 1948; Kotzé, 1963) and are considered the primary source of inoculum.

Specific environmental limitations to disease cycles

Alternate wet and dry periods aid in disease development. Wetting and drying of leaves is essential for ascostroma development (Kiely, 1948; Kotzé, 1981). Rainfall (or overhead irrigation) triggers the release of mature ascospores from mature ascrostoma (Kotzé, 1963), but too much rainfall will disrupt ascospore discharge (Kotzé, 1981) and lead to the decomposition of the dead leaves destroying the CBS causal agent (Lee and Huang, 1973). In addition, excess rain prevents ascostroma formation as the leaves become colonized by competing saprophytes (CABI, 2006). Alternatively, rapid drying of the leaves kills the latent mycelium in the leaf tissue, resulting in the failure of pycnidia to be produced on leaves (Kiely, 1948).

Adequate wetness, conducive temperatures, and sufficient inoculum must be present for infection to occur (Huang and Chang, 1972; Kotzé, 1981; Lee and Huang, 1973). For ascospores a minimum of 15-38 hours of wetness is required for germination (Kotzé, 1963), while pycnidiospore germination and appressorium formation requires a minimum of 12 hours of

wetness (Noronha, 2002). Temperature affects several aspects of CBS epidemiology. Ascrostoma formation is optimal at temperatures between 21-28°C (Lee and Huang, 1973). Ascrostoma formation ceases below 7°C and above 35°C (Lee and Huang, 1973). Under laboratory conditions, ascospores are ejected at a higher rate at high temperatures (25°C) than at lower temperatures, but can still be expelled at temperatures as low as 5°C (Kotzé, 1963). In the field, the release of ascospores is more closely correlated with leaf wetness than temperature (Reis et al., 2006). For pycnidiospore germination and appressorium formation may occur between 10-40°C, but the extreme temperatures (10 and 40°C) require a longer wetness period (Noronha, 2002). Lesion development on fruit is correlated with temperature; as temperatures increase, lesion development also increases (Kotzé, 1981).

The specific environmental condition an infected tree encounters will greatly affect the likelihood of establishment of *G. citricarpa* in the United States. If conditions are favorable, *G. citricarpa* will develop and produce sufficient ascospores that will then greatly increase the likelihood of establishment. If environmental conditions do not promote the establishment of *G. citricarpa* and disease development, pathogen spread may be hindered or prevented entirely, greatly decreasing or eliminating the likelihood of establishment.

Summary

The reproductive potential and environmental limitations of *G. citricarpa* affects the likelihood of establishment of CBS within the United States. Successful ascospore production by an infected host increases the likelihood of establishment. The specific environmental conditions encountered by the infected tree will affect the likelihood of CBS establishment. Favorable environmental conditions will increase the likelihood of establishment, while unfavorable conditions will decrease or eliminate the likelihood of establishment.

2.4: Assessment of potential consequences

2.4.1 Direct pest effects

CBS may affect the leaves, twigs, and fruit of host plants but its effects are most marked on the fruit. Unappealing lesions typically develop as fruit reach maturity or postharvest. Symptomatic citrus fruit may display four types of symptoms: hard spot, melanose spot, freckle spot, and/or virulent spot (Kiely, 1948; Kotzé, 1963, 2000). Hard spot lesions typically develop pre-harvest, and are characterized by circular depressed lesions. Melanose spot lesions typically develop on green fruit (Kotzé, 1963, 2000). Freckle spot lesions are typically round, light brown to red (orange) and depressed lesions (Kotzé, 1963, 2000). Virulent spot lesions develop on either fruit reaching maturity or fully mature fruit and are typically irregular in shape (Kiely, 1948; Kotzé, 1963, 2000).

Symptomatic fruit that are still attached to the tree fall readily (Kiely, 1969), fruit left on CBS-affected trees drop typically after harvest. Some yield losses due to fruit drop may occur in years favorable for disease development, when fruit is held on the trees past peak maturity (CABI, 2006).

Control of CBS in the field is costly as several prophylactic applications of fungicide are typically required throughout the growing season (Brodrick and Rabie, 1970). Large economic losses have been recorded in groves not treated with prophylactic sprays to control CBS. In South Africa in 1946, up to 90% of unprotected fruit were commonly deemed unsuitable for export (McOnie, 1964d). The introduction of *G. citricarpa* into a new area may require specific chemical control programs which can negatively impact non-target pests and the environment. A number of fungicides, such as copper products, dithiocarbamates, benzimidazoles, and strobilurins, are effective in preventing CBS (Goes et al., 1990; Kellerman and Kotzé, 1977; Kotzé, 2000). Prophylactic sprays of fungicides are administered to protect fruit during periods of high susceptibility (Kellerman and Kotzé, 1977), between fruit set and four to five months after petal fall (Kotzé, 1963, 2000). Prophylactic sprays of copper products are already administered in Florida to combat citrus canker disease (Dewdney and Graham, 2010).

According to the U.S. Fish and Wildlife Threatened and Endangered Species System (TESS), there are no threatened or endangered species of citrus in the United States that CBS would impact.

Based on the above information there is a high likelihood that the introduction of G. *citricarpa* and subsequent CBS development in the United States would lead to direct negative impacts on the United States citrus industry.

2.4.2 Indirect pest effects

The most significant effect of CBS is the loss of domestic and foreign commercial markets due to regulatory restrictions and unmarketable fruit. The unappealing lesions that develop postharvest render the fruits unacceptable and unmarketable for export (EPPO, 1997; Kotzé, 1963, 1981). Loss of revenue is likely to include losses due to lower revenue from diverting fresh market fruits to processing, and from trade embargos. Fresh citrus is worth considerably more than processed citrus in most markets (prices vary based on fruit type and state). For example on average a box of fresh oranges is worth $13.89, and the price of a box of oranges for processing is $7.76. The average price of fresh tangerines and mandarins is worth $23.47, and the price of a box of tangerines and mandarins for processing is $0.75 (NASS, 2009b). It is assumed that the introduction of *G. citricarpa* and subsequent disease development in the United States would likely lead to the loss of some foreign markets or the addition of mandatory mitigation due to quarantines imposed by trading partners. Currently, the European Union and twelve additional countries (Brazil, Chile, Croatia, Ecuador, Israel, Jamaica, Morocco, New Zealand, Peru, Syria, Turkey, and Uruguay) have listed *Guignardia citricarpa* Kiely as a quarantine pest (CERIS, 2010).

Based on the above information there is a high likelihood that the introduction of CBS into the United States would lead to indirect negative impacts on the United States citrus industry.

2.4.3 Market effects

The value of production for citrus fruit produced during the 2008-09 season in the United States reached $2.74 billion, with 12 million tons of utilized production (NASS, 2009a). In 2002, an economic assessment was conducted to evaluate the potential economic impacts that may be

associated with the introduction of *G. citricarpa* and the establishment of CBS. The 2002 assessment assumed[5] the following: 1) the joint response of the lead regulatory agency (USDA/APHIS/PPQ), State departments of agriculture, and producer groups would follow a comprehensive eradication program and USDA emergency response guidelines; 2) due to the long latency period, it is assumed that once CBS is detected on fruit, disease incidence would become widespread in Florida; and 3) the assessment only considers losses that may occur in Florida, as that is the most likely area of establishment (USDA, 2002).

The assessment concluded that without implementation of disease control strategies, the establishment of *G. citricarpa* in the United States is predicted to cause between $116[6] and $847[7] million in losses. Controlling CBS in the United States was estimated to cost at least $220[8] million annually (USDA, 2002). However, prophylactic sprays of copper products are already administered in Florida to combat citrus canker disease (Dewdney and Graham, 2010), therefore the actual cost may not be nearly as high as that predicted value in the economic assessment.

Based on the above information there is a high likelihood that the introduction of *G. citricarpa* and the establishment of CBS in the United States would impact U.S. citrus industry markets negatively.

2.4.4 Non-market effects

The introduction of *G. citricarpa* is likely to have minimal effects on the U.S. ecosystems and biodiversity. CBS does not kill affected host tress (CABI, 2006). There are no threatened or endangered species of citrus in the United States that CBS would impact. In addition, copper sprays typically used to control CBS (Agostini et al., 2006; Fogliata et al., 2001; Schutte, 2002) are already in use in Florida to control citrus canker (Dewdney and Graham, 2010), and therefore, would not likely cause additional effects on the environment.

Based on the above information there is a negligible likelihood that the introduction of *G. citricarpa* and the establishment of CBS in the United States would lead to any negative impacts on non-market citrus facets.

[5] These assumptions were made by the authors of USDA (2002) and are not considered applicable to other areas of this document (i.e. Risk assessment of *Citrus* spp. fruit as a pathway for the introduction of *Guignardia citricarpa* Kiely, the organism that causes Citrus Black Spot disease).

[6] The value reported in USDA (2002) of $95 million has been changed to $116 million to account for inflation between 2002 and 2010. Adjustment calculation based on The Federal Reserve Bank of Minneapolis http://www.minneapolisfed.org/index.cfm.

[7] The value reported USDA (2002) of $694 million dollars has been changed to $847 million to account for inflation between 2002 and 2010. Adjustment calculation based on The Federal Reserve Bank of Minneapolis http://www.minneapolisfed.org/index.cfm.

[8] The value reported in USDA (2002) of $180 million has been changed to $220 million to account for inflation between 2002 and 2010. Adjustment calculation based on The Federal Reserve Bank of Minneapolis http://www.minneapolisfed.org/index.cfm.

2.5 Degree of Uncertainty

There is substantial uncertainty around the rate of entry of CBS infected fruit based on the data obtained from US port-of-entry interceptions. This is because interception records do not also provide information regarding the total number of fruit imported, inspected, or infected. The interception data is useful however to provide evidence that the pathway for entry exists and that CBS is intercepted relatively frequently. We reasonably assume that the actual approach rate for CBS is at least greater than what is indicated by interceptions; an important conclusion for establishing that CBS infected fruit are entering.

There is an inherent level of uncertainty associated with the use of models to predict suitable areas of establishment, particularly for plant pathogens. Strains of bacterial and fungal plant pathogens can vary genetically and may have varying tolerances to biotic and abiotic environmental factors. Likewise, environmental factors such as climate may vary considerably with the possibility for isolated areas with micro-climates that may be very different from surrounding areas. While no model can accurately capture all potential genetic variants of any organism or every possible climatic niche, the two models used in this analysis to estimate the regions in the United States that *G. citricarpa* could establish based on ecological parameters currently experienced in other countries, use slightly different parameters yet conclude similar forecasts of suitability. We view this as evidence that our conclusions based on these models have a higher degree of certainty than if the model outputs disagreed significantly.

Guignardia citricarpa, the causal organism of CBS, which has a sexual stage represented by the ascospores of *G.citricarpa* Kiely and an asexual stage represented by the pycnidiospores of *Phyllosticta citricarpa* (McAlpine), has been studied in some detail over the past 60 years. These studies have resulted in a substantial amount of information on the basic biology, hosts, ecological parameters, mechanisms of spread and strategies for management of CBS in different areas of the world where it occurs. The quantity and quality of data available for our analysis on the likelihood of establishment significantly decreases the uncertainty around our conclusions in this aspect of the analysis.

Uncertainty regarding the overall impact of CBS in the United States is increased by the lack of reports on actual consequences from the recent introduction of the disease in Florida.

2.6 Risk Potential

The pest risk potential is a single rating which represents an overall estimate of the risk posed by *G. citricarpa* Kiely. The pest risk potential is arrived at by examining the overall likelihood of introduction, and the overall potential consequences. The significant conclusions of each section are as follows:

Likelihood of introduction:

Likelihood of entry: Four potential pathways that *G. citricarpa* may enter the United States were identified: natural spread, the importation of propagative material, the importation of leaves as contaminants, and the importation of fruit. *Guignardia citricarpa* is not known to be established anywhere in Canada or Mexico, therefore, it is not likely that *G. citricarpa* could

enter into the United States through natural spread. Long distance dissemination of *G. citricarpa* is most likely through the movement of infected nursery stock and budwood. The importation and domestic movement of propagative material is currently regulated by PPQ, therefore, there is a low likelihood that *G. citricarpa* will enter the United States via this regulated pathway. Based on the distribution of *G. citricarpa* and CBS and current regulations regarding the movement of citrus plants, the two likely pathways that *G. citricarpa* may enter the United States is through the unregulated and/ or non-commercial importation of leaves contaminating shipments of citrus fruit (medium likelihood) and the unregulated and/ or non-commercial importation of fruit (high likelihood).

Likelihood of establishment: Suitable hosts exist in the United States that aid the likelihood of establishment. *Guignardia citricarpa* hosts on which CBS develops are limited to the *Citrus* genus, which only has the potential to grow in Plant Hardiness Zones 8-10 or twenty-four percent of the area of the United States. Based on the restricted host range and limited area of the United States where hosts are found, there is a low likelihood that *G. citricarpa* will find a suitable host in the United States.

Suitable environmental conditions exist in the United States that increases the likelihood of establishment of *G. citricarpa*. Suitable environmental conditions are primarily limited to the Southeastern United States. There is a medium likelihood that *G. citricarpa* would encounter a suitable environment in which to become established in the United States.

Several factors for successful transfer of *G. citricarpa* from fruit to hosts in the United States were examined: 1) Infected fruit must arrive in an area with hosts available and conducive for infection and disease development; 2) The host needs to be in a susceptible physiological stage for infection to occur; 3) Spores of the causal organism must be produced on the fruit; 4) Fruit with lesions containing the causal organism, *G. citricarpa,* in a stage that can cause infection leading to disease must be released from the lesions; 5) Water contaminated with pycnidiospores must be brought into contact with susceptible host tissue in a susceptible stage for infection; and 6)Enough time must elapse with the relevant weather conditions remaining conducive for infection to occur . The successful transmission of *G. citricarpa* from infected fruit into the environment to cause infection of susceptible citrus tissue is dependent on the success of all six events. The assessment concluded that there is a low likelihood that two events will occur (fruit with lesions containing the causal organism in a stage that can cause disease onset will be released from the lesions and germinate; and water contaminated with pycnidiospores from infected fruit will be brought into contact with a susceptible host in a susceptible stage), therefore, the overall likelihood that the pest will find a suitable host and incite disease is also low.

The reproductive potential and environmental limitations of CBS greatly impacts the likelihood of establishment of CBS within the United States. Upon successful production of ascospores by an infected tree, the reproductive potential of *G. citricarpa* will greatly increase the likelihood of establishment of the pathogen and subsequent disease development. The specific environmental conditions encountered by the infected tree will greatly impact the likelihood of establishment of the pathogen and subsequent disease development. Favorable environmental conditions will greatly increase the likelihood of establishment of the pathogen and subsequent disease

development, while unfavorable conditions will greatly decrease or eliminate the likelihood of establishment of the pathogen and subsequent disease development.

Potential consequences: The assessment considered direct, indirect, market, and non-market effects of *G. citricarpa* in assessing the consequences of CBS establishing in the United States. The establishment of *G. citricarpa* and widespread occurrence of CBS in the United States has a high likelihood of negatively impacting the United States citrus industry.

Pest risk potential: While there is a high likelihood that unregulated and/ or non-commercial *G. citricarpa* infected fruit will enter the United States and establishment of *G. citricarpa* and widespread occurrence of CBS in the United States has a high likelihood of negatively impacting US citrus industries, the likelihood of establishment is low for the fruit pathway. The low likelihood that infected fruit will release the pycnidiospores that will successfully germinate from lesions, and that those pycnidiospores will be brought into contact with susceptible hosts in a susceptible stage leads to an overall low likelihood that *G. citricarpa* and CBS will become established from infected fruit. Based on the low likelihood of establishment the overall risk potential is low.

III. Risk Management

This assessment assumed that the imported infected fruit has not undergone any treatment either in the field or packinghouse. It is assumed that infection of fruit is likely if fruit originates in areas where *G. citricarpa* Kiely occurs but the magnitude of the hazard depends in large part on the proportion of infected fruit. The incidence of infected fruit depends primarily on the variety, environmental conditions, and field management. This section briefly describes common mitigation practices.

There are several effective field management programs that reduce the incidence of CBS in the field. It has been reported that mechanically removing leaf litter from the grove floor reduces the amount of available *G. citricarpa* ascospores (CABI, 2006), and that removing mature fruit left on the tree also prevents *G. citricarpa* pycnidia from spreading from old infected fruit (EPPO, 1997; Kotzé, 1981). Prophylactic fungicide applications in the field throughout the fruit growing season reduce the incidence of CBS development postharvest (Agostini et al., 2006). Several fungicides are effective in reducing CBS prevalence, such as copper products, dithiocarbamates, benzimidazoles, mancozeb, iprodione, thiophanate methyl, and strobilurins (CABI, 2006; Goes, 2002; Goes et al., 2000; Kellerman and Kotzé, 1977; McOnie, 1964a; Miles et al., 2004; Rodriguez and Mazza, 2004; Schutte, 2002).

Post-harvest treatments are effective in reducing the viability of pycnidiospores in lesions on symptomatic fruit. Routine packinghouse treatments such as chlorine dips, warm water bath, or chemical tank dip (1000 µg/ml guazatine, 503 µg/ml imazalil sulphate, 500 µg/ml 2,4-D sodium salt) and combinations of these treatments reduce the viability of the conidia to zero on fruit kept at 4.5^{o}C-25^{o}C (Korf et al., 2001a). In addition, wax treatments will further reduce the viability of conidia (Korf et al., 2001a; Seberry et al., 1967).

Summary

Current disease management strategies employed to control and treat CBS disease in the field and packinghouse appear to be generally effective. While it behooves growers to employ CBS disease management programs there is no universal program or guarantee that such programs are undertaken. If CBS disease management programs are undertaken, the pest risk potential rating will be lowered further. Disease management programs and commercial harvesting and packing practices reduce the prevalence of *G. citricarpa* infected or CBS affected fruit in commercial shipments of fruit thus further lowering the overall risk.

IV. Conclusion

The risks associated with fruit as a pathway for the introduction of *Guignardia citricarpa* Kiely, the causal agent of CBS, were repeatedly questioned in peer review and public comments received in relation to the *Risk Assessment for the Importation of Fresh Lemon (*Citrus limon *(L.) Burm. f.) Fruit from Northwest Argentina into the Continental United States.* This assessment was initiated in response to those comments to evaluate evidence associated with citrus fruit as a possible pathway for the introduction of *G. citricarpa* Kiely, the causal agent of the fungal disease CBS.

This assessment used scientific, government and other documents to examine the likelihood of entry, the likelihood of establishment, and the potential consequence of establishment to arrive at a pest risk potential rating.

The pest risk potential for *G. citricarpa* Kiely is low for the fruit pathway. Furthermore this assessment examined the current disease management strategies employed to control and treat CBS disease in the field and packinghouse. Common treatments further reduce the risk of fruit as a pathway for CBS.

The conclusion of this risk assessment is that fruit is not epidemiologically significant as a pathway for the introduction of *G. citricarpa* or establishment of CBS disease.

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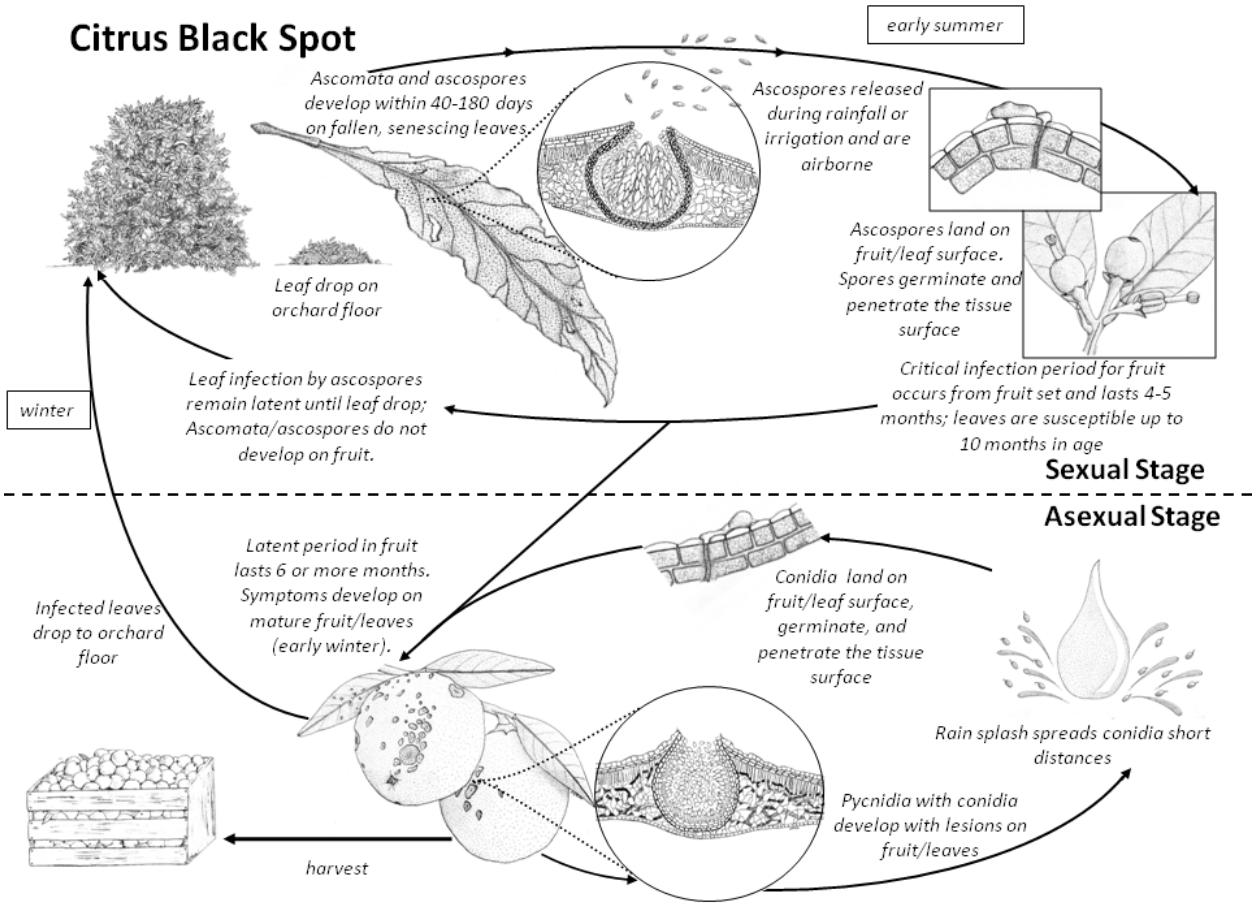
Appendix A: Life cycle of citrus black spot



Life cycle of *G.citricarpa*, the pathogen causing citrus black spot disease (by Heather Hartzog USDA-APHIS-PPQ-CPHST-PERAL).