Crop Protection 142 (2021) 105521

Contents lists available at ScienceDirect

Crop Protection

journal homepage: www.elsevier.com/locate/cropro

Probabilistic risk-based model for the assessment of *Phyllosticta citricarpa*-infected citrus fruit and illicit plant material as pathways for pathogen introduction and establishment

T.R. Gottwald [a,*], E.L. Taylor [a], L. Amorim [b], A. Bergamin-Filho [b], R.B. Bassanezi [c], G.J. Silva Jr. [c], G. Fogliata [d], P.H. Fourie [e,f], J.H. Graham [g], V. Hattingh [e,h], A.B. Kriss [i], W. Luo [a,j], R.D. Magarey [j], G.C. Schutte [e], M.B. Spósito [b]

[a] *Agricultural Research Service, US Department of Agriculture, US Horticultural Research Laboratory, Fort Pierce, Florida, 34945, USA*
[b] *Department of Plant Pathology, University of São Paulo, Escola Superior de Agricultura "Luiz de Queiroz", Piracicaba, São Paulo, 13418-900, Brazil*
[c] *Fund for Citrus Protection - Fundecitrus, Araraquara, São Paulo, 14807-040, Brazil*
[d] *Estación Experimental Agroindustrial-Obispo Colombres, Las Talitas, Tucumán, Argentina*
[e] *Citrus Research International, P.O. Box 28, Nelspruit, 1200, South Africa*
[f] *Department of Plant Pathology, University of Stellenbosch, Private Bag X1, Stellenbosch, 7602, South Africa*
[g] *Citrus Research and Education Center, University of Florida, Lake Alfred, Florida, 33850, USA*
[h] *Department of Horticultural Science, University of Stellenbosch, Private Bag X1, Stellenbosch, 7602, South Africa*
[i] *Syngenta Crop Protection, LLC, 410 S Swing Road, Greensboro, NC, 27409, USA*
[j] *North Carolina State University, Center for Integrated Pest Management, 1730 Varsity Drive, Suite 300, Raleigh, NC, 27606, USA*

ARTICLE INFO

Keywords:
Citrus black spot
Probabilistic model
International trade
Pest risk

ABSTRACT

Citrus Black Spot (CBS), caused by the ascomycete, *Phyllosticta citricarpa*, is a fruit, foliar, and twig spotting fungal disease affecting the majority of commercial cultivars of citrus. The disease causes cosmetic lesions, may cause fruit drop and *P. citricarpa* is considered a quarantine pathogen by some countries, impacting domestic and international trade of citrus fruit. Regulatory requirements affecting fruit trade exist even though there is no documented case of disease spread via infected fruit into previously disease-free areas. To clarify the risk of fruit as a potential pathway for the spread of CBS, we developed a quantitative, probabilistic risk assessment model. The model provides an assessment of all steps in the fruit pathway, including production, packinghouse handling, transportation, export-import distribution channels, and consumer endpoints. The model is stochastic and uses Monte Carlo simulation to assess the risk of *P. citricarpa* moving through all steps in the pathway. We attempted to use all available literature and information to quantitate risk at each point in the potential pathway and by sequentially linking all steps to determine the overall quantitative risk. In addition, we assessed climatological effects on incidence of diseased fruit at production sites and on fungal reproduction and infection, as well as criteria for establishment at endpoints. We examined ten case studies between exporting and importing locations/countries. Model results indicated fruit to be an epidemiologically insignificant means for CBS spread, even between producing countries where CBS occurs and CBS-free importing countries with disease-conducive climates. We created a second model to examine the introduction of infected plant material from countries where CBS occurs. This model demonstrated significant probability of introduction via such infected material. However, pathogen establishment and disease development was still restricted only to areas with conducive climatological conditions. We created a tool to quantitatively explore the viability of various potential pathways via combinations of CBS-present production sites and corresponding pathway endpoints, including environments conducive and non-conducive to CBS. The tool is provided to aid decision makers on phytosanitary risk relative to international trade of citrus fruit.

* Corresponding author.
E-mail address: citrusdoc1@gmail.com (T.R. Gottwald).

https://doi.org/10.1016/j.cropro.2020.105521
Received 19 June 2020; Received in revised form 17 December 2020; Accepted 18 December 2020
Available online 22 December 2020

1. Introduction

Citrus Black Spot (CBS), caused by the ascomycete, *Phyllosticta citricarpa* McAlp Van der Aa, (syn. *Guignardia citricarpa*) may cause cosmetic fruit lesions and crop losses under favorable conditions where it occurs in parts of Asia, Africa, Oceania, and Americas (Yonow et al., 2013). Fruit symptoms can be severe and lead to premature fruit drop in orchards where the disease is not well managed and the climate is highly conducive for CBS disease development (Spósito et al. 2007, 2008; Silva Junior et al., 2016; Lanza et al., 2018). The risk of introduction and spread of CBS to new, unaffected citrus-producing areas is a concern for citrus-producing areas attempting to remain free of the disease. To the authors' knowledge, it has never been demonstrated that harvested *P. citricarpa*-infected fruit have ever led to infections and/or subsequent disease in previously disease-free areas. Rather, spread to new climatologically suitable areas is likely due to movement of infected plant material as has previously been reported (Marchionatto 1926; Doidge 1929; Kiely 1948; Wager 1953). Nonetheless, phytosanitary legislation for the import of citrus fruit from countries where CBS occurs has been promulgated in the European Union and the United States (EFSA 2008; EFSA 2014a; EFSA 2014b; USDA 2002; USDA 2010; USDA 2011).

Two spore types can be produced by *P. citricarpa*. Ascospores (teleomorph stage) are released from pseudothecia in leaf litter in the field and pycnidiospores (the conidial or anamorph stage) are released from pycnidia within certain types of lesions on fruit, twigs and leaves (Kiely 1948; Kotzé 1981). Sporulation, germination, and production of both of these spore types have been studied under various meteorological conditions (Reis et al., 2006; Shaw 2006; Timossi et al., 2003; Fourie et al., 2013; Dummel et al., 2015). Favorable environment such as level of humidity, intensity of solar radiation, temperature and leaf wetness among other factors such as orchard architecture and orientation, have a profound effect on incidence and severity of CBS (Andrade et al., 2009). In addition, studies from Brazil have been conducted to quantify the rate of CBS disease development and intensity over time (Spósito et al., 2004) and the rate of disease spread within citrus orchards (Spósito et al. 2007, 2008). These studies have shown that spread due to ascospores occurs at distances up to 25 m, whereas, pycnidiospores were disseminated downward within the tree only to a distance of 80 cm (Spósito et al., 2011). Notably some lesions types, such as false melanosis, do not produce pycnidia, which are only formed in hard spot, freckle spot and virulent spot (Kiely, 1948; Kotzé, 2000; FAO, 2014). In addition, pycnidiospores are water disseminated and do not disperse in dry air currents (Kiely 1948; Wager 1949; Kotzé 1963, 1981; Whiteside 1967). Pycnidiospores formed on fallen fruit and discarded peel lesions do not infect newly fallen leaves and leaf litter, and therefore do not contribute to the inoculum source (Truter et al., 2007).

Numerous studies evaluated fungicides for control of CBS in the orchard (Goes et al., 1990; Miles et al., 2004; Schutte et al. 1997, 2003; Schutte 2006; Silva Junior et al., 2016; Lanza et al., 2018). Other studies examined the use of chemical and horticultural methods to accelerate citrus leaf litter decomposition prior to ascospore release, thereby reducing the CBS inoculum potential (Bellotte et al., 2009; Kupper et al., 2006).

Packinghouse treatments and shipping or storage temperature conditions limit CBS lesion development, survival and inoculum viability (Seberry et al., 1967; Korf et al., 2001; Agostini et al., 2006; Schreuder et al., 2018). Packinghouse postharvest treatment of fruit for cleaning and sanitation, including chlorine wash, fungicide application, waxing and refrigeration, greatly reduce the viability of latent infections, lesions and pycnidiospores, sometimes to zero (Seberry et al., 1967; Agostini et al., 2006; Korf et al., 2001; Schreuder et al., 2018). Additionally, visual and computerized grading of fruit are also effective in eliminating fruits with obvious CBS lesions from being exported.

Despite the efficacy of pre- and postharvest CBS control measures, some trading partners are reluctant to import fresh fruit from production areas where CBS occurs and seek evidence that fruit is not an epidemiologically viable pathway for CBS spread to new areas. To this end, regulatory agencies have performed elaborate pest risk assessments (PRA) to amass and evaluate scientific literature and data on the CBS pathosystem that would pertain to the risk of introduction and potential spread of CBS (EFSA 2008; EFSA 2014a; Magarey and Borchert 2003; Magarey and Holtz 2009; USDA 2002). In addition to extensive literature on biology, etiology, infection and dissemination, these PRAs have also considered overarching epidemiological models that predict if *P. citricarpa* would establish and cause CBS disease in the new environment (Magarey and Borchert 2003; Magarey et al. 2005, 2007, 2015; Paul et al., 2005; Magarey and Holtz 2009; Fourie et al., 2013; Yonow et al., 2013). While these models predicted a lack of climate suitability for CBS disease in areas with a Mediterranean climate, such as California or southern coastal Europe, a model by Er et al. (2013) predicted CBS could potentially occur in California and parts of the southern European Union. However, the Er et al. model was shown to be flawed (Graham et al., 2014; Yonow and Kriticos 2014), underscoring the concern that all such models must be rigorously validated due to their expansive and severe trade implications. Recent surveys in Europe for the presence of fungi in the genus of *Phyllosticta* detected *P. citricarpa* in fallen leaves under 20-60 year-old trees in Italy, Portugal and Malta. However, there was no indication of the presence of CBS disease or spread from the isolated sites, indicating marginally suitable climatic conditions for the fungus to survive, but seemingly insufficiently conducive for CBS disease development (Guarnaccia et al., 2017).

Prior PRAs have been predominately qualitative and thus subjective in some aspects of their assessment of the CBS pathosystem. A European *P. citricarpa* PRA included a simplified quantitative analysis of entry, but did not consider the further steps necessary for establishment and disease development (EFSA 2014a). The study discussed here presents a quantitative, probabilistic assessment of the plant infection, pathogen survival and disease development risk of fruit and plant material throughout the pathways being examined. The fruit pathway includes production, packinghouse handling, transportation, distribution, export/import channels and consumer use, whereas the plant pathway is less complex. We attempted to include all available published information and expert opinion to quantify risk at each point in the pathway and by sequentially linking all steps, generate an overall quantitative risk. In addition, we assessed climatological effects on incidence of disease at production sites as well as on fungal replication, establishment and disease development at endpoints. It was our intent to create a model to quantitatively explore the viability of various infected fruit and plant material trade routes which consist of production sites where CBS occurs and various consumer endpoints, which may also be disease-free areas.

2. Materials and methods

2.1. Probabilistic risk-based model framework, methods and data analyses

We explore two potential pathways for spread of CBS from CBS-present areas into disease-free locales. The first is the commercial citrus fruit trade pathway. The second is an illicit plant material pathway. The commercial citrus fruit trade pathway can be broken into three main phases (production, packinghouse combined with shipping and cold storage, and marketing channels to consumer endpoint), each composed of a number of discrete steps (Fig. 1A).

The second model addresses the illicit plant material pathway and is considerably less complex, but can still be broken into discrete steps (Fig. 1B). Both pathways utilize the same probabilistic risk-based model framework developed in Excel (Microsoft Corporation, Redmond, Washington, USA) utilizing @RISK software (Palisade Corporation, New York, USA) for decision-making and risk analysis via Monte Carlo simulation.

The hypotheses assumed for this study were *$H0_1$: Fruit are a pathway*

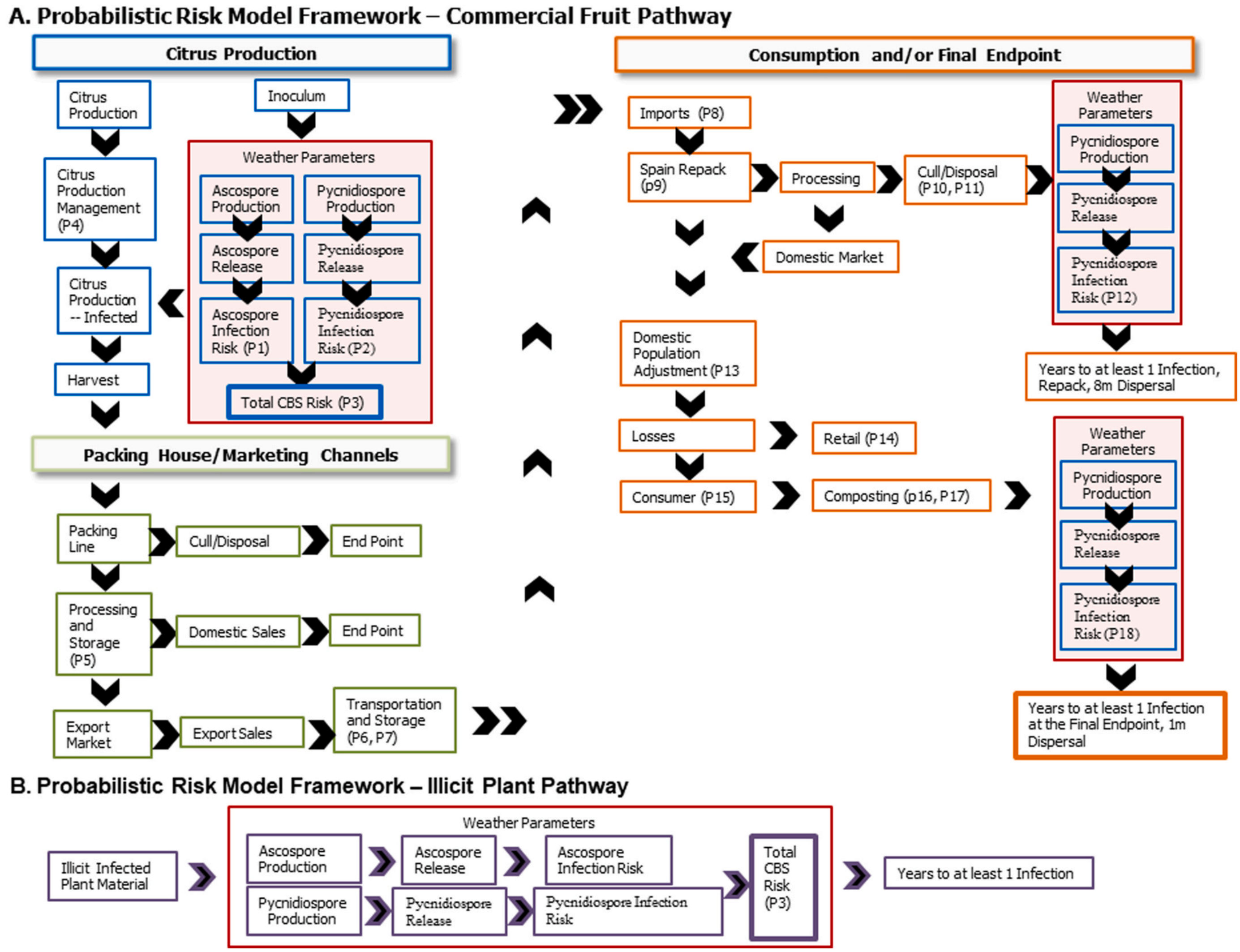

Fig. 1. Model flow chart indicating steps in the pathway for A) commercial fruit and B) illicit plant introduction pathways from point of origin to endpoint. References in parenthesis correspond to steps in the model as indicated in Table 1.

for CBS spread from the production area to the endpoint, and *$H0_2$: Plant material is a pathway for CBS spread.* To test the validity of these hypotheses, we used Monte Carlo simulation via the flexible stepwise probabilistic model to explore and assess each of a series of variables (steps in the pathway) independently and sequentially in the pathway. For each pathway, we utilized model parameters as described in Table 1 and in the following sections.

2.1.1. *The commercial citrus fruit trade pathway model*

For each production area (origin) considered, we used yearly average of 2005–2011 fruit production or export records obtained from various industry organizations and tracked the total fruit exported, converted to total number of individual pieces of fruit exported, relative to each pathway examined. Monthly export volume data were used for those areas where available, else we assumed a uniform monthly export volume over the season. Based on the method in 2.2.1 below we calculated the proportion of fruit exported that was potentially infected with *P. citricarpa*. The model tracks potentially infected fruit through each step in the entire pathway on a monthly basis for each citrus commodity considered, i.e., orange, grapefruit, mandarin, and lemon (Appendix A). Where appropriate the pathway branches if more than one path is possible with each branch populated by its own data and resulting distribution. For example, Spain performs repacking (~40%) and processing for juice (20%) of a portion of the total fruit imported (EFSA 2014a). Some fruit going to repacking are culled and all fruit residue after juicing are discarded (EFSA 2014a). These discards bypass the marketing and consumer endpoint portions of the model and move directly to the environmental assessment of establishment. The remaining whole fruit, repacked and non-repacked fruit, move through the marketing and consumer steps of the model prior to environmental assessment of establishment steps. To capture this bifurcation in the pathway, different distribution functions represent the efficacy of the repack/juice and non-repack branches. When the branches converge prior to the next step in the pathway, the resulting number of whole fruit is recombined. Introductions into new locales must be combined with favorable endpoint climatological conditions for infection, establishment and disease development.

2.1.2. *The illicit plant material pathway model*

The illicit plant material model begins with the assumption of 100 infected plants per month introduced from a CBS-present area directly into a CBS-free area. The pathway terminates with the subsequent planting of the infected plants on residential properties and it is only necessary to consider climatological suitability for potential establishment of the pathogen and disease development at the endpoint (Fig. 1B).

Table 1
Parameters used in the sequentially linked steps in the probabilistic model from CBS risk at source (P1 to −3), effects of mitigation steps or losses in production (P4), packinghouse, cold storage and export to and repacking in destination countries (P5 to −10), adjustment for population, retail and consumer loss and household composting (P14 to 16) and pycnidiospore dispersal and infection risk at end points (P12-13 and P17-18).

Model component	Factor	Description	Source
P1	Ascospore infection risk	Site specific weather data are utilized to calculate the probabilities associated with ascospore formation, release, and germination based on published biological criteria.	ZedX: altitude-adjusted CSFR database (Saha et al., 2010; Magarey et al., 2015)
P2	Pycnidiospore infection risk	Site specific weather data are utilized to calculate the probabilities associated with pycnidiospore formation, release, and germination based on published biological criteria.	ZedX: altitude-adjusted CSFR database (Saha et al., 2010; Magarey et al., 2015)
P3	Total CBS risk	P1 and P2 are summed and regressed against uncontrolled disease incidence (DI) levels from field trials in regions/areas with CBS, providing a field calibration index for conversion of P1+P2 to proportion of fruit potentially infected.	Calculated from P1, P2 and DI regression analysis
P4	Field spray control	Published CBS control studies provided the CBS control relative to unsprayed plots.	Schutte et al. (1997, 2003, 2012), Schutte (2006), Miles et al. (2004), Fogliata et al. (2001)
P5	Viability of infection—Packinghouse/Cold storage	Control of latent infections following standard packinghouse procedures and cold storage.	Schreuder et al. (2018)
P6	Export transshipping losses	The losses in each class of citrus relative to transshipment.	Buzby et al. (2009)
P7	Pycnidia formation – Cold storage reduction on viability	Reduction of pycnidium formation following standard packinghouse procedures, shipping & cold storage conditions.	Schreuder et al. (2018)
P8	Proportion of exports going to endpoint country	Proportion of citrus exports from the originating country going to the endpoint country	FAO
P9	Repack in Spain	The proportion of imports entering Spain that are repacked.	EFSA (2014a)
P10	Repack Losses	Indicates the losses associated with repacking, and includes oranges that are processed for juice and removed from the retail marketing channel.	EFSA (2014a)
P11	Water Dispersal Risk	Calculated hypothetical 1.0 m water dispersal risk associated with composting citrus waste	Sposito et al. (2011)
P12	Pycnidiospore infection risk	Site specific weather data is utilized to calculate the probabilities associated with pycnidiospore formation, release, and germination based on published biological criteria.	ZedX: altitude-adjusted CSFR database (Saha et al., 2010; Magarey et al., 2015)
P13	Population adjustment	A demand adjustment factor allocating fruit to the retail market of interest. Based on a PERT distribution from zero (0) to two (2) times the population of the retail location.	
P14	Retail Loss	Citrus losses occurring at the retail level.	Personal communication, Paris fruit broker
P15	Consumer loss	Citrus losses occurring at the consumer level.	Buzby et al. (2009)
P16	Households Composting	Proportion of households composting	Neeley and Marnell (2011)
P17	Water Dispersal Risk	Calculated from the hypothetical 1.0 m water	Sposito et al. (2011)

(continued on next page)

Table 1 (*continued*)

Model component	Factor	Description	Source
		dispersal risk associated with composting citrus waste.	
P18	Pycnidiospore infection risk	Site specific weather data is utilized to calculate the probabilities associated with pycnidiospore formation, release, and germination based on published biological criteria.	ZedX: altitude-adjusted CSFR database (Saha et al., 2010; Magarey et al., 2015)

2.2. Trade route scenarios examined

We applied the model to a selected group of commercial fruit and plant production/distribution trade routes (Table 2). Some of these pathway endpoints were considered to be conducive for CBS disease while others were considered to be non-conducive based on prior climatological suitability studies (Yonow et al., 2013; Magarey et al., 2015) (Table 3). Not all scenarios are current and/or actual trade routes, but serve to illustrate their effect on pathogen introduction, establishment and disease development. The trade route used for initial model development and as an example is the export of commercial fruit from South Florida, USA to Barcelona, Spain.

2.2.1. Influence of production area climate on incidence of infected fruit

The citrus crop within a region is subjected to varying climatological conditions resulting in the potential for accumulation of fruit infections during each month of fruit susceptibility over the 6-month period following fruit set (Kiely 1948, 1950; Kotzé 2000; Lanza et al., 2018). The fruit were tracked through the model via a linked series of monthly matrices. For each citrus type we applied infection models for the spore types independently (ascospore and pycnidiospore) to determine if the climate conditions were met during the appropriate fruit susceptibility period (April 1 through September 30 in the northern hemisphere and October 1 through March 31 in the southern hemisphere). For both ascospores and pycnidiospores, infection was considered to occur only if conditions for the three steps, i.e., spore formation, spore release and infection occurred in temporal sequence without breaks in time that would preclude progression to the next step. The infection models estimate monthly probabilities of infection, but only on days predicted

Table 2
Potential pathways explored by probabilistic risk model for commercial citrus fruit and illicit plant material from export origin to import destination (endpoint), with the "years to infection" metric indicated for the retail scenario.

Commercial citrus fruit trade pathways			"Years to infection"[A]				
Pathway	Export origin	Endpoint (import destination)	Orange	Mandarin	Grapefruit	Lemon	Appendix
F1	South Florida, USA	Barcelona, Spain	∞	∞	∞	∞	A
F2	South Florida, USA	Addo, South Africa	∞	∞	∞	∞	B
F3	Addo, South Africa	South Florida, USA	∞	∞	∞	∞	C
F4	Nelspruit, South Africa	Valencia, Spain	2,500	48,709	13,919	∞	D
F5	Nelspruit, South Africa	South Florida, USA	∞	∞	∞	∞	E
F6	Limeira, Sao Paulo, Brazil	Seville, Spain	97,465	NA	NA	NA	F
F7	Limeira, Sao Paulo, Brazil	Andravida, Greece	∞	NA	NA	NA	G
F8	Tucuman, Argentina	Tulare, California, USA	∞	∞	∞	∞	H
F9	Nelspruit, South Africa	Citrusdal, South Africa	∞	∞	∞	∞	I
F10	Emerald, Australia	Tulare, California, USA	∞	∞	∞	∞	J
Illicit plant material pathway							
	Export origin	Endpoint (import destination)	"Years to infection"				
P1	South Florida, USA	Barcelona, Spain	1				L
P2	Addo, South Africa	South Florida, USA	1				M
P3	Nelspruit, South Africa	Citrusdal, South Africa	1				N
P4	Limeira, Sao Paulo, Brazil	Addo, South Africa	1				O

[A] ∞, value is undefined, approaching infinity in limit.

Table 3
Citrus Black Spot occurrence and predicted climate suitability of locations used as export origin or import destinations in the probabilistic risk model.

Location	Observed CBS presence/absence	EI[a]	Infection period scores[b]		Average fruit infection during 6-month fruit susceptibility period (FIR)[c]				Plant infection probability (PIP) index[d]		
			Asc	Pyc	Asc	Pyc	FIR index	Disease incidence	Score	Establishment and disease	Predicted suitability
Addo, South Africa	Present	4	13.4	48.4	0.4529	0.4704	0.9233	0.5461	1.2694	Yes	Marginal
Andravida, Greece	Absent	1	20	34.7	0.1520	0.1988	0.3508	0.0614	1.1578	No	Not suitable
Barcelona, Spain	Absent	2	2.3	15.6	0.2477	0.3293	0.5771	0.3073	0.7386	No	Not suitable
Citrusdal, South Africa	Absent	0	1.6	11.3	0.0988	0.2087	0.3075	0.0285	0.7245	No	Not suitable
Emerald, Australia	Present	–	22.3	43.0	0.6574	0.3457	1.0031	0.5777	1.2023	Yes	Suitable
Limeira, Sao Paulo, Brazil	Present	–	–	–	3.0386	2.2155	5.2540	0.7804	6.3318	Yes	Suitable
Nelspruit, South Africa	Present	–	34.5	94.2	1.3575	1.1309	2.4885	0.7503	2.7272	Yes	Suitable
Seville, Spain	Absent	1	0.9	7.8	0.0315	0.0966	0.1281	0.0000	0.4414	No	Not suitable
South Florida, USA	Present	50	53.5	187	3.1168	1.2604	4.3772	0.7765	5.6209	Yes	Suitable
Tucuman, Argentina	Present	–	–	–	1.5483	1.4872	3.0355	0.7629	3.2760	Yes	Suitable
Tulare, California, USA	Absent	0	0	0.1	0.0000	0.0116	0.0116	0.0000	0.0904	No	Not suitable
Valencia, Spain	Absent	1	4.9	15.9	0.1160	0.1411	0.2571	0.0068	0.3787	No	Not suitable

[a] Ecoclimatic index reported by Yonow et al. (2013).
[b] Infection period scores from ascospores (Asc) and pycnidiospores (Pyc) as reported by Magarey et al. (2015).
[c] Average sum of monthly proportion of days suitable for Asc and Pyc infection during the 6-month fruit susceptibility period, the sum of Asc and Pyc giving the Fruit Infection Risk (FIR) index, and disease incidence as predicted using the FIR index on the logistic regression equation of FIR index values and uncontrolled disease incidence levels from CBS field trials.
[d] Suitability of location predicted by the PIP index as determined in this study (PIP index < 1.2 is not suitable and >1.2 is regarded as suitable. [c].

suitable for ascospore and pycnidiospore availability and dispersal (Fourie et al., 2013; Magarey et al., 2015) (Appendix A – P1-2). Climatological data for each locale were obtained for the 2005–2011 timeframe (ZedX Corporation, Bellefonte, PA 16823) and corrected via an elevation adjustment algorithm (Magarey et al., 2015).

Pycnidiospore production is initiated by high temperatures, RH and free moisture but the precise conditions necessary to form pycnidia are not known. Therefore, the model assumes (overestimates) the continuous presence of mature pycnidia. Pycnidiospore release occurs following precipitation >0.2 mm (Magarey et al., 2015). Subsequent infection occurs when daily temperatures range between 10 and 35 °C combined with >12 h leaf wetness (Magarey et al., 2015). For pycnidiospore infection, I_{pycn} eq. (1), we used the surface response model by Noronha (2002) for percentage appressorium formation based on temperature (t) and hours of wetness (m) (Noronha 2002, see model formula page 49)

$$I_{pycn} = (((0.15(t-9.8)^{0.37})\ ((43.34-t)^{0.73})\ (20.42))/(1+(10.36)\mathrm{Exp}(-(0.14)m)))/71.42)/378.29. \quad \text{eq. 1}$$

For ascospore production, we used the output of the temperature model described in Fourie et al. (2013), with pseudothecia regarded as mature at $P = 0.5$ corresponding to a cumulative degree day temperature > 10 °C of 767.9 starting July 1 in the southern hemisphere and January 1 in the northern hemisphere. Ascospore release was considered to occur on days with minimum temperature >13.6 °C (1st percentile of daily T_{min} at which ascospores were trapped) and maximum temperature < 35.5 °C (95th percentile of T_{max} at which ascospores were trapped) with a coincident RH of >50.5% (1st percentile RH_{ave}) (Fourie et al., 2013). Ascospore germination and infection was considered to occur if daily temperatures were between 15 and 35 °C; if there was more than 15 h of leaf wetness over the given day and prior day (total 48-h period); and daily precipitation was >0.2 mm (Magarey et al., 2015). For ascospore infection, we used the percentage ascospore germination data from Kotze (1963) and McOnie (1967) to construct a surface response model for an index of ascospore infection risk I_{asco} based on temperature and hours of wetness,

$$I_{asco} = \mathrm{Exp}(-39.08 + 2.81t - 0.05t^2 + 0.16w)/(1 + \mathrm{Exp}(-39.08 + 2.81t - 0.05t^2 + 0.16w)) \quad \text{eq. 2}$$

where t is temperature (°C) and w is hours of wetness.

For each spore type the daily infection risk index values were summed for each month and expressed as the proportion of days in the month that were suitable for infection. The proportions for each spore type were summed to calculate the total fruit infection risk index (FIR) for each production season. The regression described in the fruit susceptibility index section (Eq. (3)) was used to convert FIR to proportion of fruit potentially infected. These fruit were then subjected to reductions in fruit disease incidence as calculated by the disease control step described in section 2.2.2 below.

Calculation of the CBS incidence: To estimate the proportion of infected fruit in CBS-present production areas, combined disease incidence data in the absence of CBS controls were obtained from citrus plantings in multiple locations, Mpumalanga and Eastern Cape in South Africa, São Paulo state in Brazil, Queensland in Australia, and Tucuman in Argentina (Schutte et al. 1997, 2003, 2012; Schutte 2006; Miles et al., 2004; Fogliata et al., 2001). These disease incidence values were regressed against the FIR for the 6-month fruit susceptibility period. The functional form utilized in the regression analysis was consistent with the biology of disease processes, demonstrating the curvilinear relationship of disease progression, resulting in a sigmoidal (logistic) relationship. The R^2 of the regression analysis of 0.9675 was indicative of the high and significant correlation between disease incidence and predicted *P. citricarpa* infection based on environmental conditions during the period when fruit is most susceptible to infection. The resulting equation was utilized (Appendix A - P3) to quantify the proportion of total fruit produced at each location that could potentially be infected:

$$p(\text{Total CBS Risk}) = EXP\left(4.369 + \frac{-0.3142}{(FIR)^2}\right). \quad \text{Eq.3}$$

2.2.2. Efficacy of field disease control measures at the place of fruit production

Published and unpublished CBS disease control data collected from

South Africa, Brazil, Argentina and Australia (Schutte et al. 1997, 2003, 2012; Schutte 2006; Miles et al., 2004; Fogliata et al., 2001) were very consistent and therefore the pooled data were fitted to a Pert distribution with input parameters of an absolute minimum (85.04% control), absolute maximum (95.04%) and most likely (90.04%) control value (Appendix A – P4). This distribution estimates the adjusted number of infected fruit for each of the four citrus types (Appendix A – V3).

The number of potentially infected fruit (*n*) and the probability that an individual fruit is infected (*p*) were then estimated through a binomial distribution. The probability (*p*) is the respective probability of reduction in infection for that step in the model as defined by the Monte Carlo distribution. The mean of the binomial distribution ($n*p$) is reflected in each matrix in the Appendix as the average number of potentially infected fruit.

2.2.3. *Packinghouse treatment, handling, and transport effects*

Two main disease-mitigating steps occur in the packinghouse. First, fruit are subjected to standard packinghouse handling and treatment practices, which mostly include disinfectant wash, fungicide dip/drench, drying and waxing (Korf et al., 2001; Schreuder et al., 2018). Second, fruit are culled for defects and blemishes, size, shape, and other non-desirable characteristics, which also include any fruit with visually detectable CBS lesions. The model did not consider (underestimated) this second risk reduction measure. The reduction of lesion viability due to packinghouse treatment such as washing, fungicide application, waxing, combined with cold handling during shipping and cold storage is 52.5% for lemons and 82.2% for oranges (Schreuder et al., 2018). Reductions for mandarins and grapefruit were based on the most conservative value, 52.5%. The reductions are represented in the model by a Pert distribution and adjusted by a binomial model as described above (Appendix A – P5). Korf et al. (2001) was not able to recover viable pycnidiospores from lesions present at the time of packinghouse treatment. However, the model did not consider (underestimated) this risk reduction component.

USDA Economic Research Service estimates the proportion of fruit lost during shipment due to damage as 3 ± 1% for oranges, 5 ± 1% for mandarins, 4 ± 1% for lemons, and 3 ± 1% for grapefruit, which are represented in the model as Pert distributions (Appendix A – P6).

Schreuder et al. (2018) reported that only 10%–15% of lesions developing from infections that survived packing and shipping/storage conditions, formed pycnidia. This reduced reproductive capability is estimated as 80.67% for oranges and 99.63% for lemons. As before, the most conservative value was assumed for mandarins and grapefruit (80.67%) and represented by the appropriate Pert distributions (Appendix A – P7). The monthly total number of pieces of fruit with potentially viable infections were adjusted downward by the packinghouse and transportation mitigations and losses indicated above. This provides an estimate of the monthly total number of pieces of fruit, with potentially viable *P. citricarpa* infections, arriving in the importing country.

2.2.4. *Post import and consumer related effects*

2.2.4.1. Direct versus fruit commodity repack effects. Citrus export and import data were extracted from the yearly United Nations Development Program, Food and Agriculture Organization (UNDP-FAO) cumulated trade information (FAO, 2000-2009). For the purposes of our example (Southwest Florida to Barcelona trade route) we used the UNDP-FAO data for the average proportion of US exports that are imported into Spain by month and by citrus commodity. We proportionately reduced the monthly potentially infected fruit imports into Spain (Appendix A – P8).

Imports into Spain pass through either of two branches: 1) the proportion of fruit commodities that are directly marketed [oranges = 50%, mandarins = 30%, lemons = 92.5%, and grapefruit = 92.5% (no losses were considered with this activity)]; and 2) the balance are repacked in Spain. Additionally, 20% of the repacked oranges are diverted to juicing. Repacking is accomplished by multiple facilities in Spain. Repack losses are estimated as oranges = 3%, mandarins = 5%, lemons = 4%, and grapefruit = 3%. The two branches converge providing the sum of fruit entering the Spanish market (Appendix A – P9-10).

2.2.4.2. Within country distribution and marketing losses. To determine the proportion of imported fruit distributed to individual communities, we adjusted fruit volumes by the population of the community as a proportion of the total population of the importing country (Appendix A – P14). Average retail fruit losses in the marketplace due to damage, postharvest rot, excessive fruit age, etc., for oranges are 11.6%, mandarins 20.4%, lemons 7%, and grapefruit 12.8% and is diverted to solid waste (Personal communication, Paris fruit broker) (Appendix A – P15).

2.2.5. *Consumption and loss adjustments*

Once in the hands of a consumer, there are various endpoints for citrus fruit purchased for consumption. Consumer losses post retail due to poor storage, rot, or failure to consume are estimated to be 36, 52, 54, and 44% for oranges, mandarins, grapefruit and lemons, respectively (Buzby et al., 2009). The Pert distribution of each class are ±1 as estimated via a Monte Carlo simulation (Appendix A – P16). Loss at the consumer level includes non-edible share and cooking loss or uneaten food or plate waste. Two possible endpoints are considered. If consumed, we assumed that the fruit peel is then discarded, whereas if not consumed, the entire fruit is considered to be discarded. In either case, we consider the peel or entire fruit are disposed of either via solid waste, which is 1) bagged and terminates in a landfill, or 2) is composted. Solid waste is a nonviable endpoint, as infected fruit are not available to the environment in close proximity of a citrus host for further potential pathogen dispersal. The proportion of households that compost organic waste is estimated to be 5.43% (Neeley and Marnell 2011). It is only composted fruit peel or whole fruit that are considered for their potential role in pathogen introduction (Appendix A – P17).

2.2.6. *Composting and probability of sporulation and water dispersal*

2.2.6.1. Water dispersal for consumer discarded fruit. For this portion of the pathway we began with the estimated proportion of *P. citricarpa*-infected fruit harboring potentially viable pycnidia that have transcended all prior steps in the pathway, have been discarded by the consumer, and now reside in a compost pile. We assumed that lesions with pycnidia have formed on fruit discarded by the consumer in the month following export. We examined the probability for spore release, assuming that 50% of either the fruit peel and/or intact fruit resides on the compost pile with the pycnidia-laden lesion in an upright orientation for potential spore dispersal. This is an overestimate as oblique orientation of the lesion would lead to diminished potential for dispersal. It is furthermore unlikely that all the fruit/peel would rest on the upper surface layer of the pile. We also assumed approximately 2 weeks viability of pycnidia in fruit/peel prior to decay. For discarded citrus peel, this is an overestimate, since it was demonstrated that peel segments exposed to direct sunlight under natural conditions remained a viable substrate for pycnidiospore release for a less than 6 h (Schutte et al., 2014). By applying the above risk probability, we estimate the pieces of composted fruit/peels in a compost pile that would potentially be available to release pycnidiospores by month and by commodity (Appendix A – V15).

Water dispersal of *P. citricarpa* pycnidiospores from symptomatic fruit or twigs, including infection, was studied in São Paulo, Brazil (Sposito et al., 2011). The farthest distance water dispersed infections occurred was 0.8 m from an infected fruit within the tree and in a downward direction; no more than 0.4 m lateral spread was observed. No upward, or lateral dispersal from ground level was reported. Even so, as a conservative assumption (overestimate), we use a potential 1-m lateral spread in the water dispersal model, indicating probability of dispersal:

$$\text{Dispersal}_{1\text{m}} = \text{Exp}\,(-3.6 * s), \quad \text{eq. 4}$$

where s is the distance from the inoculum source.

Based on available data from Florida, California, Texas, Spain, and South Africa, it appears that as many as 60% of the households in a commercial citrus producing area may have residential citrus trees. Considering the average size of residential properties in Barcelona, we used a stochastic process to virtually place both compost piles and potential target citrus trees within the property boundaries. By this method we estimated the proportion of properties that would have a citrus tree with a canopy that would reside within the potential dispersal distance of 1 m from a randomly placed compost pile. Monte Carlo simulation was utilized to determine the probability of pycnidiospore dispersal based upon historical Barcelona weather (Appendix A – V15). Monte Carlo simulation was subsequently utilized to determine the sequential probability of germination followed by infection based upon suitability of endpoint weather by month and citrus commodity (Appendix A – P18).

2.2.6.2. Water dispersal for repacking/processing discarded fruit. There are 440 packing facilities in the Valencia Spain area alone, of which 78 conduct repacking of fruit from imported fruit (EFSA 2014b). Repacking results in some additional fruit loss (Appendix A P9-10), while the pulp from processed oranges are also culled. We consider processing pulp of 1 infected fruit as 1 fruit continuing in the model; this is an overestimate, particularly since processed pulp has never been shown to be a substrate for pycnidiospore formation. The citrus waste from these facilities, as well as waste and pulp from commercial citrus processing (juicing) facilities is spread in a thin layer to solar dry for animal feed and biofuel production (EFSA 2014a). Such facilities could possibly be in close vicinity of commercial citrus plantings, thus placing potentially infected fruit in proximity to susceptible citrus trees (EFSA 2014a). For the purpose of our model, we assumed that all drying facilities are adjacent to citrus plantings, similar to the facility illustrated in Fig. 2 (GIS latitude, longitude 39.1749, −0.4716), despite this being an overestimate scenario and contrary to the EPPO standard for disposal of biological waste potentially containing quarantine pests (EPPO 2008). However, these facilities are unlikely to be within the 1 m lateral dispersal distance (used in the model) from citrus trees, which renders the pathway as a dead-end, since longer distance pycnidiospore dispersal leading to infection has never been reported (Spósito et al., 2011). However, as a hypothetical alternative to the pathway dead-end, we also calculated the proportion of the citrus residue that fell within 8 m of commercial citrus trees (see What-if scenario 2.3.4 below).

2.2.7. Estimation of establishment and disease development via plant infection probability index

Pathogen establishment and disease development does not necessarily result simply from an initial infection. To accomplish establishment and disease development in a new area, there must be successive cycles of spore production, spore release, germination and infection. Weather conditions must be consistently suitable to allow multiple sequential cycles of disease from year to year. Situations may occur where the climate is marginally suitable, sufficient for establishment, but not sufficient for disease development (Magarey et al., 2015; Guarnaccia et al., 2017). Magarey et al. (2015) used frequency of occurrence of suitable conditions to compare regional climatic suitability for establishment and disease development; they calibrated their model output on known distribution of the disease. Subsequent discovery of *P. citricarpa* establishment and persistence without disease in parts of Europe (Guarnaccia et al., 2017) indicates the occurrence of marginal suitability supporting establishment but without CBS disease development.

In this portion of the model, establishment and disease development was determined by the plant infection probability index, $\text{PIP}_{\text{index}}$, which is the yearly sum of the monthly averages of ascospore and pycnidiospore infection index values associated with each location. A $\text{PIP}_{\text{index}} < 1.2$ indicates *P. citricarpa* will not establish and disease will not develop for a given climate × location combination, whereas a $\text{PIP}_{\text{index}} \geq 1.2$ is indicative of conditions sufficiently suitable for establishment and disease development. This $\text{PIP}_{\text{index}}$ threshold was based on examining multiple commercial citrus areas in South Africa and Australia where continual introduction of *P. citricarpa* over multiple years has led to establishment and disease development, or cases where CBS disease did not establish (results not shown). The calculation of probability of establishment is essentially the endpoint of the commercial fruit pathway model, and determines whether or not the pathway is epidemiologically viable, if the pathway did not reach a dead-end at an earlier stage.

An additional quantitative metric, termed "years to infection", was calculated to aid in comparing the relative risk of one pathway versus another. The pycnidiospore infection index values of all monthly assessments were adjusted (multiplied) by the final monthly volume of infected fruit in the pathway, and were summed to calculate the mean

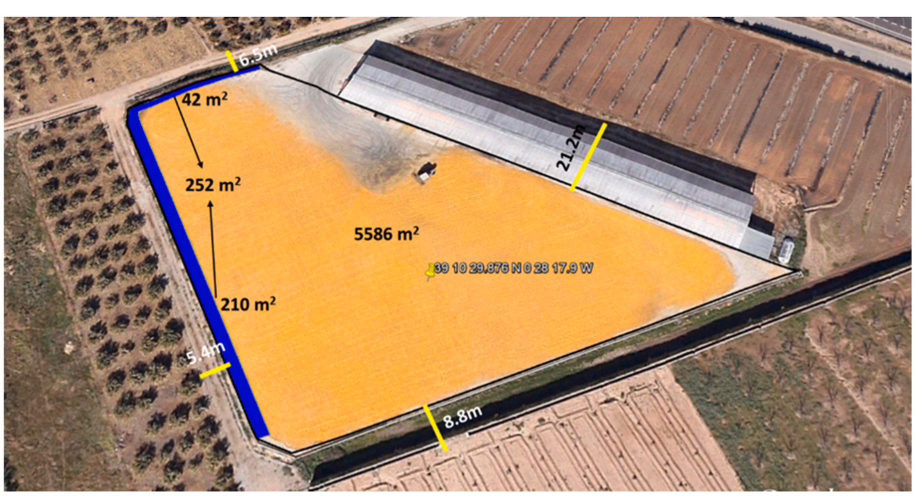

Fig. 2. Citrus waste drying facility near Valencia Spain showing citrus waste spread over surface and proximity to surrounding citrus orchards. All citrus trees were more than 1 m from the edge and the blue region lies within 8.0 m of the adjacent citrus. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

probability of infection for each of the respective citrus commodities (oranges, mandarins, grapefruit, and lemons) during a one-year period. The “years to infection” estimate was calculated by using the negative binomial (Appendix A –V16). Although this is expressed as “years to infection” this is not an actual quantification of real time to first infection, it is merely the number of sampling periods before the model output generates a similar outcome. This metric aids in assessing the potential viability of the pathway and comparisons between pathways.

2.3. Examination of the commercial fruit pathway model to explore “what-if” scenarios

The commercial fruit pathway model provides a methodology to explore various changes in yield, climate, disease control, etc., on probability of introduction, establishment in new areas currently free of the disease. Four *“What-if”* scenarios were examined.

2.3.1. What-if scenario: effect of dramatic increases in fruit exports from origin countries

The volumes of fruit exported influence the probability for introduction, if fruit were to be a viable pathway. To explore the effect of increasing international trade in citrus fruit, we used an extreme of 10 × production increase in the exporting region/country and resulting import into the endpoint region/country (Appendices P - Y). It is unlikely that even after many decades such an increase in citrus fruit trade would occur, but testing these scenarios provides a useful example of the effect of large increases in citrus fruit trade on the potential viability of the pathway.

2.3.2. What-if scenario: effect of disease conducive climate change at endpoint countries

We investigated a scenario where climate changed towards increased disease suitability in the fruit importing country and created an artificial climatological year for the endpoint importing country. Daily minimum and maximum temperatures were increased by 2 °C across the 10 years of data. In this way we build a climatological year with warmer weather equivalent to a global warming scenario as predicted by global climate models for southern Mediterranean areas (Trnka et al., 2011). However, the predicted reduction in precipitation was not considered in this study. We ran the model for the South Florida to Barcelona and Nelspruit to Valencia trade routes (Appendices JJ and KK).

2.3.3. What-if scenario: effect of failed disease control at origin country

A worst-case scenario would be a catastrophic failure of disease control resulting in a significant increase in *P. citricarpa* infections on citrus fruits. We artificially reduced disease control to 50% efficacy and thereby inflated the number of infected fruit entering into the pathway by approximately 5-fold. We ran the model for the South Florida to Barcelona and Nelspruit to Valencia trade routes (Appendices LL and MM).

2.3.4. What-if scenario: effect of an increase in dispersal distance

Perryman et al. (2014) reported findings from a *P. citricarpa* splash dispersal study in a wind tunnel. However, this study used uncharacteristically large droplets onto spore suspensions or artificially inoculated fruit with uncharacteristically large lesions, did not quantify the number of spores dispersed in the small droplets dispersed up to a distance of 8 m in the wind tunnel, and did not demonstrate pycnidiospore viability or infection following dispersal (Fourie et al., 2015). Whilst their findings were anomalous relative to studies and observations under natural conditions (Kiely, 1948; Wager, 1949; McOnie, 1965; Whiteside, 1967; Spósito et al., 2007, 2008, 2011), we address the hypothetical longer dispersal distance of small droplets to 8 m in wind-driven rain in the repack model as one of the *What-if* scenarios described below:

$$\mathrm{Dispersal}_{8\mathrm{m}} = \mathrm{Exp}\,(-0.67 * s), \quad \text{eq. 5}$$

where *s* is the distance from the inoculum source.

The estimated number of fruit discarded to this endpoint was adjusted by the calculated exposure risk probability, $R_{exp} = 0.000677$, assuming a random distribution of infected fruit over the surface of the drying area and considering that portion of the citrus residue within the 8-m dispersal gradient (eq. (5) above) that overlapped with commercial citrus trees. We adjusted the proportion of infected fruits or peels that would be discarded with lesions oriented upward and on the top of a drying pile by 50%. We also assumed that pycnidia on fruit surfaces and/or peels remain viable for approximately 2 weeks before decay or solar drying renders them nonviable, despite this period of viability for the pulp and peel component in the waste being highly unlikely, as previously explained. Probability of pycnidiospore germination and infection was estimated as described above (Appendix A – P13).

2.4. Examination of a plant material pathway

We assumed the trade is illicit and unregulated, and that tree production practices are nonstandard and therefore poor and/or no quality control is practiced relative to disease management in the production of such trees. Thus, all plant material entering the pathway was assumed to be infected, either symptomatic or asymptomatic; this is likely an overestimate. For each 1-month period, we assumed that 100 *P. citricarpa*-infected trees were illegally imported into the target country of concern.

The mode of transport should not influence the outcome and was not considered. We assumed that the timeframe for trans-shipment is sufficiently short that plant losses were nil. On arrival, we assumed the trees were immediately planted in either commercial plantations or in dooryards.

Trees could be either non-bearing and simply composed of infected foliage or could be old enough to be bearing and therefore have infected fruit and foliage. Thus, unlike the commercial fruit trade pathway, the illicit plant material pathway consider pycnidiospore as well as ascospore infection subsequent to planting of the infected tree. We examined suitability of year-round weather data for sequential stages of pycnidiospore or ascospore release, germination and infection considering introduction can occur during any of the 12 months of the year. We then tested if establishment and disease development can occur using the PIP_{index} and ‘years to infection’ calculation as described above. We examined the Addo to South Florida; Limeira to Addo; Nelspruit to Citrusdal; and South Florida to Barcelona routes (Appendix L, M, N and O, respectively).

3. Results

3.1. Commercial fruit trade pathway

Commercial fruit trade as a potential pathway for *P. citricarpa* introduction and establishment was investigated using the probabilistic risk model on data from ten fruit trade routes (Table 2). All fruit trade pathways were found to be epidemiologically non-viable for introduction and establishment. In 30 of 34 cases, the “years to infection” calculation resulted in infinity, with the remaining values ranging from 2500 to 97,465 (Table 2). For the Spain endpoints (F1, F4 and F6), the repack branch in the model yielded infinite years to infection since fruit from the repack facility was not within the 1 m pycnidiospore dispersal distance (results not shown). Based on the Monte Carlo simulation, and the resulting calculated values, we can conclude that the probabilistic risk model rejected the null hypothesis *$H0_1$: Fruit are a pathway for* P. citricarpa *from the production area considered to establishment at the endpoint point.* The risk of transmission of *P. citricarpa* via fresh fruit is epidemiologically insignificant, and based on the calculated PIP_{index} values > 1.2 for Barcelona, Citrusdal, Seville, Andravida, Tulare and Valencia endpoints, establishment and CBS disease development would not occur (Table 3).

3.1.1. What-if scenario: effect of dramatic increases in fruit exports from origin countries

For the 10 × export volume increase scenario, most cases resulted in infinite "years to infection", with 9 of 34 cases with values ranging from 294 to 97,465 (Table 4). For the repacking branch specific for Spanish imports, "years to infection" values were all infinite since fruit from the repack facility was not within the 1 m pycnidiospore dispersal distance (results not shown).

These values indicate that even in the extremely unrealistic scenarios of fruit exports increasing ten-fold, the resulting risk of transmission of CBS via fresh fruit is epidemiologically insignificant, which supports rejection of the null hypothesis $H0_1$.

3.1.2. What-if scenario: effect of climate change at endpoint countries

For the South Florida to Barcelona retail and repack pathways the model predicted that no infection would take place. For Nelspruit to Valencia, the lowest "years to infection" value was 2375 for oranges in the retail sector (Table 4). These values are epidemiologically insignificant, which supports rejection of the null hypothesis $H0_1$.

3.1.3. What-if scenario: effect of failed disease control at origin country

The model predicted that no infection would take place for South Florida to Barcelona. For the Nelspruit to Valencia retail pathway, predicted "years to infection" values were 558, 4,629, 3484 and 33,322 for oranges, mandarins, grapefruit, and lemons, respectively. These values are epidemiologically insignificant, which supports rejection of the null hypothesis $H0_1$.

3.1.4. What-if scenario: effect of an increase in potential dispersal distance

All 10 commercial fruit distribution pathways were examined to determine the effect associated with the consumer retail market (Table 4). In the retail sector, the model predicted infinity for "years to infection" in most cases (23 cases), and values ranging from 213 to 100,000 in the other 11 cases (Table 4). For the Spain endpoints (F1, F4 and F6), the repack branch in the model yielded "years to infection" values of infinity (9 cases), 704, 5405 and 48,709. These values are epidemiologically insignificant, which supports rejection of the null hypothesis $H0_1$.

3.2. Illicit plant material pathways

The probabilistic risk model was validated against four illicit plant material pathways (Table 2). One was predicted to be viable, one marginally viable, and two were non-viable for *P. citricarpa* introduction and eventual establishment and disease development based on the predicted climatological suitability (Table 3). All pathways were determined to be epidemiologically compatible for infection, and the model predicted a "years to infection" value of 1. For the South Florida to Barcelona and Nelspruit to Citrusdal pathways the PIP_{index} values < 1.2 indicated incompatible climatological conditions for establishment and CBS disease development at these endpoint. For Limeira to Addo, the PIP_{index} of 1.27 indicates establishment and disease development can occur. Likewise, for Addo to South Florida, "years to infection" was predicted to be 1 and the PIP_{index} of 5.62 indicated compatible climatological conditions for establishment and disease development. Thus, the probabilistic risk model failed to reject the null hypothesis, *$H0_2$: plant material is a pathway for* P. citricarpa *introduction, establishment and CBS disease development in the disease free area of concern.*

4. Discussion

The probabilistic risk assessment model described here considers the relevant biological literature on *P. citricarpa* and was used to quantitatively examine the effect of each step in a distribution pathway on the overall risk of potential introductions by importation of commercial citrus fruit or whole citrus plants. The model confirmed that infected citrus plants were highly likely pathways for introduction of the pathogen into new areas (Marchionatto 1926; Doidge 1929; Kiely 1948; Wager 1953), but indicated that infected citrus fruit was an epidemiologically insignificant means for CBS spread, even when importing unrealistically high volumes of infected fruit to areas with disease-conducive climates.

A quantitative risk assessment for entry of *P. citricarpa* via the citrus fruit pathway from CBS-affected countries to Spain was conducted as part of the EU's PRA for *P. citricarpa* (EFSA 2014b). The authors of the PRA concluded that entry via the citrus fruit trade pathway was very unlikely with CBS regulations in place, but moderately likely without regulation and poor control measures at origin, modelling scenarios with fruit infection incidences of ±2, 16 and 72%. Our model assumed ±89.7% control of field infection, and based on the climatic conditions at origin resulted in CBS infection incidences in harvested fruit ranging from 5.6% to 8.0%. Additionally, we evaluated a control failure 'what-if' scenario (only 50% control), which resulted in CBS incidences ranging from 27.3% to 39% entering the pathway. This 'what-if' scenario is plausible, but not realistic for fresh citrus fruit exports given the high quality standards required. Nonetheless, even in this failed control what-if scenario, citrus fruit was not shown to be an epidemiologically significant pathway for *P. citricarpa*.

The EFSA model used "fruit exposed to air" in a citrus production area as the final step in the quantitative model (EFSA 2014b). Hereafter, the concurrence of infected fruit volumes exposed to air and suitable climatic conditions for infection was qualitatively assessed using overlays on annual and monthly scales (EFSA 2014b). The authors acknowledged the subsequent limitations for transfer to a suitable host, but did not quantitatively consider this in their model. Based on our probabilistic model, these final steps in the model are significant hurdles, which must be sequentially overcome in order to allow effective dispersal to a susceptible host and subsequent infection. Firstly, infected fruit must be disposed in very close proximity of a citrus trees. Effective water dispersal of *P. citricarpa* pycnidiospores is essentially a downward and short-range phenomenon (Kiely 1948; Wager 1949; Kotzé 1963; Whiteside 1967; Spósito et al., 2011; Perryman et al., 2014), as is generally the case for splash-dispersed fungal inoculum (Fitt et al. 1982, 1989; Huber et al. 1996, 2006; Madden, 1992, 1997; McCartney et al., 2006; Travadon et al., 2007). Secondly, *P. citricarpa* infections on packinghouse treated fruit or peel segments (Seberry et al., 1967; Korf et al., 2001; Agostini et al., 2006; Schutte et al., 2014; Schreuder et al., 2018) were shown to have a very low reproductive potential. Thirdly, rainfall followed by warm temperatures and long wetness periods are required for pycnidiospore dispersal and infection (Magarey et al., 2015). Consideration of these hurdles in a quantitative manner significantly reduce the number of fruit that might realistically result in entry and infection of *P. citricarpa*; dependent on climatic conditions of the endpoint destinations, these steps led to a 500- to 30,000-fold reduction in number of infected fruit.

Our model provides a tool for potential use by regulatory agencies to quantitatively assess the risk of introduction of CBS into new areas. As input values, we used actual fruit volumes and disease incidence predicted from the climate suitability for CBS in the exporting country/region. We analysed multiple commercial/potential fruit trade routes and demonstrated in all cases that the quantitative probability of introduction of *P. citricarpa* via fruit trade pathways is negligibly low, i.e., epidemiologically insignificant. The model also provided a means for us to test multiple 'what-if' scenarios that could affect a given pathway. These included raising levels of importation, testing the effects of global warming, failure of disease control at the source, and drastically increasing the hypothetical dispersal distance of pycnidiospores by water dispersal. Analyses of these scenarios supported the conclusion that fruit is not a viable pathway.

Probabilities used in our model are mostly based on published scientific evidence. However, given the paucity of data and model design, we had to use probabilities for certain model steps that were clearly

Table 4
"*What-If* "scenarios of increased imports, global warming, diminished control and increased dispersal distance at origin explored by probabilistic risk model.

Commercial citrus fruit trade pathways			"Years to infection", Retail[A]				
Path -way	Export origin	Endpoint (import destination)	Orange	Mandarin	Grapefruit	Lemon	Appendix
Increase Fruit Exports 10×							
F1	South Florida, USA	Barcelona, Spain	∞	∞	∞	∞	P
F2	South Florida, USA	Addo, South Africa	∞	∞	∞	∞	Q
F3	Addo, South Africa	South Florida, USA	24,360	97,465	∞	∞	R
F4	Nelspruit, South Africa	Valencia, Spain	294	4,424	1,872	∞	S
F5	Nelspruit, South Africa	South Florida	32,478	∞	∞	∞	T
F6	Limeira, Sao Paulo, Brazil	Seville, Spain	2,710	NA	NA	NA	U
F7	Limeira, Sao Paulo, Brazil	Andravida, Greece	∞	NA	NA	NA	V
F8	Tucuman, Argentina	Tulare, California, USA	∞	∞	∞	∞	W
F9	Nelspruit, South Africa	Citrusdal, South Africa	97,465	∞	∞	∞	X
F10	Emerald, Queensland, Australia	Tulare, California, USA	∞	97,465	∞	∞	Y
Global warming 2 degree C increase in temperature at the endpoint							
F1	South Florida, USA	Barcelona, Spain	∞	∞	∞	∞	JJ
F4	Nelspruit, South Africa	Valencia, Spain	2,375	∞	19,485	∞	KK
Failed CBS control							
F1	South Florida, USA	Barcelona, Spain	∞	∞	∞	∞	LL
F4	Nelspruit, South Africa	Valencia, Spain	558	4,629	3,484	33,322	MM
Increase water dispersal distance from 1.0 m to 8 m							
-	**Retail branch**						
F1	South Florida, USA	Barcelona, Spain	∞	∞	∞	∞	A
F2	South Florida, USA	Addo, South Africa	∞	∞	∞	∞	B
F3	Addo, South Africa	South Florida, USA	10,000	20,000	∞	∞	C
F4	Nelspruit, South Africa	Valencia, Spain	213	2,865	1,623	100,000	D
F5	Nelspruit, South Africa	South Florida	97,465	48,709	∞	∞	E
F6	Limeira, Sao Paulo, Brazil	Seville, Spain	1,949	NA	NA	NA	F
F7	Limeira, Sao Paulo, Brazil	Andravida, Greece	∞	NA	NA	NA	G
F8	Tucuman, Argentina	Tulare, California, USA	∞	∞	∞	72,463	H
F9	Nelspruit, South Africa	Citrusdal, South Africa	∞	∞	97,465	∞	I
F10	Emerald, Queensland, Australia	Tulare, California, USA	∞	∞	∞	∞	J
-	**Repack branch**						
F1	South Florida, USA	Barcelona, Spain	∞	∞	∞	∞	A
F4	Nelspruit, South Africa	Valencia, Spain	704	48,709	∞	∞	D
F6	Limeira, Sao Paulo, Brazil	Seville, Spain	5,405	∞	∞	∞	F

[A] ∞, value is undefined, approaching infinity in limit.

overestimations. These included a 2-week viability of discarded citrus fruit and pulp as substrate to support pycnidiospore production and dispersal. This period might be conceivable for intact fruit disposed on a compost pile in the shade of a citrus tree, but certainly not for peel segments and pulp exposed to the low humidity and high temperature conditions of open-air drying facilities (Lanfranchi, 2012). The hypothesized 8 m dispersal distance (Perryman et al., 2014) was considered for the repack branch in a what-if scenario, which would otherwise have resulted in a dead-end, since it was not conceivable that such facilities are located within the 1-m dispersal distance from citrus trees otherwise used in the model. Other known overestimations include the following: failure to consider that Korf et al. (2001) was unable to recover viable pycnidiospores for lesions exposed to packhouse treatments; continuous presence of mature pycnidia on infected fruit was assumed; disregard for the reduced dispersal potential of obliquely oriented fruit surfaces in a cull pile; an unrealistically long assumed 2 week pycnidial viability in fruit/peel on fruit waste; the model used a 1 m lateral waterborne dispersal of pycnidiospores whereas Sposito et al. (2011) showed a maximum of 0.4 m; the pulp from one fruit was assumed to have a viable pycnidial load equivalent to one whole fruit in processing waste whereas fruit pulp has never been shown to be a substrate for pycnidiospore production; all processing waste drying facilities in Spain were assumed to be adjacent to citrus plantings. Despite these overestimations, the repack and processing branch in the fruit pathway, which was regarded by the EU PRA (EFSA 2014b) as the highest risk, was demonstrated to be epidemiologically insignificant. Nonetheless, the practice of waste disposal in sites immediately adjacent to commercial citrus plantings should not occur where general good agricultural practice and EPPO standards are followed, which would eliminate any theoretical residual risk associated with the fruit pathway (EPPO 2008).

For establishment and disease development once putatively introduced, we consider climatological suitability for infection and subsequent polycyclic recurrence leading to establishment at the endpoint(s) before disease development can be possible under suitable conditions. We used the PIP_{index} (the yearly sum of the monthly average ascospore and pycnidiospore infection risk index values associated with each location) as a predictive indicator of the probability of establishment and disease development. This climate suitability measure for the various locations correlated well with results from published studies (Yonow et al., 2013; Magarey et al., 2015).

Guarnaccia et al. (2017) recently detected *P. citricarpa* in fallen leaves under 20-60 yr-old trees in Italy, Portugal and Malta, proving that introduction has occurred in these areas. However, there was no indication of the presence of CBS disease or spread thereof. This indicated that these areas are likely to have climatic conditions that are marginally suitable for the fungus to survive but insufficiently suitable for CBS disease to occur. A new species *Phyllosticta paracitricarpa* was also found in Greece, which would previously have been identified as *P. citricarpa* (Guarnaccia et al., 2017). They suggested that multiple introductions have occurred over time into the Mediterranean, most likely via the infected plant material pathway, leading to infection and establishment, yet climatological suitability is unfavorable for disease.

The risk model discussed here corroborates this finding with the example of the PIP_{index} for Andravida in Greece of 1.15, which does not exceed the threshold ($PIP_{index} \geq 1.2$), shown empirically as necessary for establishment. Moreover, other CBS models have indicated that conditions for successful infections do occur under EU climate conditions, as well as other known CBS-absent areas with winter rainfall Mediterranean type climates, but that these were significantly fewer than those for warm summer rainfall areas where CBS disease occurs; importantly, these infections mostly occurred outside the period of fruit susceptibility, which would explain the absence of CBS fruit symptoms (EFSA, 2014a; Magarey et al., 2015; Magarey et al., unpublished findings). Likewise, Yonow et al. (2013) demonstrated highly constrained areas in EU with marginal suitability. Thus, even though establishment can and has now been documented to occur in some areas of southern Europe with marginally suitable climate, apparently conditions are still less than sufficiently suitable for disease development. Our results similarly demonstrated that relatively low levels of *P. citricarpa* infections are predicted to occur in Andravida (Greece), Barcelona, Seville, Valencia (Spain), Citrusdal (South Africa) and Tulare (California, USA), but that CBS disease development is unlikely to occur.

The climate in Florida is highly suitable for CBS and the disease was reported in Florida in 2010 (Schubert et al., 2012). Most researchers agree that because of the slow development of the disease, in part due to the presence of only one mating type and thus no sexual stage (Wang et al., 2016; Carstens et al., 2017), it may have been introduced 10–15 years prior to discovery. We know that the importation of fruit from countries where CBS occurs is rare into Florida, and even rarer into Southwest Florida where the disease was first detected. However, it is not uncommon for surveyors to find citrus trees in dooryards and exotic plant nurseries with obvious origins outside the United States. The first author of this manuscript and colleagues noted this on numerous occasions when surveying for citrus canker, citrus huanglongbing, and other diseases within the state of Florida during eradication campaigns and delimiting surveys for these diseases (Gottwald and Graham, personal experience). Therefore, the model prediction of 1 year to establishment from illicit plant material pathway is supported by practical experience, although symptom expression will likely require multiple years post-introduction. To the contrary, fruit was not shown to be a pathway to Florida, even to this highly suitable climate for CBS.

5. Conclusions

The CBS probabilistic pathway risk model developed here identified many steps in the citrus fruit supply chain that affect the risk probability of infected fruit moving to the endpoints in the pathway. Through the risk reduction measures and pathway effects on viability of infections, infected fruit numbers declined to zero prior to most pathway endpoints. By following all of these steps through the model, we can see the decreasing probability (as demonstrated by decreasing numbers of infected fruit) associated with each step in the pathway. At pathway endpoints, conditions for effective pycnidiospore dispersal from infected fruit and subsequent infection must be met for introduction and establishment to occur. This culminates in an extremely low (negligible) to zero probability of introduction via commercial fruit trade pathways. This is clearly demonstrated by utilizing the model's illustrative metric of "years to infection". When compared to the illicit plant material pathways which produced a "years to infection" value of 1, we clearly see why commercial fruit trade is a non-viable pathway, whereas illicit plant material is a highly viable pathway for disease introduction into new climatologically suitable regions. This model gives credence to the belief of most plant pathologists with CBS expertise that it is illicit plant material that has moved this disease into new climatologically suitable areas around the world. This model also clearly demonstrates that commercial fruit trade is not an epidemiologically viable pathway for CBS spread into previously CBS free citrus growing regions.

Funding

This work was supported by United States Department of Agriculture, Farm and Foreign Agricultural Services, Foreign Agricultural Service, Technical Assistance for Specialty Crops Agreement #2011-10 awarded through the Indian River Citrus League of Florida, US.

CRediT authorship contribution statement

T.R. Gottwald: Conceptualization, Methodology, Software, Validation, Formal analysis, Writing - original draft, Writing - review & editing, Visualization, Supervision, Project administration, Funding acquisition. **E.L. Taylor:** Conceptualization, Methodology, Software, Validation, Formal analysis, Writing - review & editing, Visualization, Data curation. **L. Amorim:** Conceptualization, Methodology, Validation, Writing - review & editing. **A. Bergamin-Filho:** Conceptualization, Methodology, Validation, Writing - review & editing. **R.B. Bassanezi:** Conceptualization, Methodology, Validation, Writing - review & editing. **G.J. Silva:** Conceptualization, Validation, Writing - review & editing. **G. Fogliata:** Conceptualization, Methodology, Validation, Writing - review & editing. **P.H. Fourie:** Conceptualization, Methodology, Validation, Formal analysis, Writing - review & editing. **J.H. Graham:** Conceptualization, Methodology, Validation, Writing - review & editing. **V. Hattingh:** Conceptualization, Methodology, Validation, Formal analysis, Writing - review & editing. **A.B. Kriss:** Conceptualization, Methodology, Validation, Formal analysis, Validation, Writing - review & editing. **W. Luo:** Conceptualization, Methodology, Software, Validation, Formal analysis, Writing - review & editing, Visualization. **R.D. Magarey:** Conceptualization, Methodology, Validation, Writing - review & editing. **G.C. Schutte:** Conceptualization, Methodology, Validation, Writing - review & editing. **M.B. Spósito:** Conceptualization, Methodology, Validation, Writing - review & editing.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to extend our appreciation to Doug Bournique and Karen Smith of the Indian Rive Citrus League for administrative support of the TASC (Technical Assistance for Specialty Crops) grant funding this project.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cropro.2020.105521.

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