

Citrus Black Spot

**Consolidated
Pest Risk Assessment
pertaining to the export of
fresh Citrus fruit from
the Republic of South Africa to the
European Union**

2000-2009

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***Citrus Black Spot:
Pest Risk Assessment document
for the review of
current phytosanitary regulations
pertaining to the export of
fresh Citrus fruit from
the Republic of South Africa to the EU***

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**Submitted to the Agriculture Commission of the
European Communities
by the South African National Department of Agriculture,
Directorate Plant Health and Quality,
May 2000**

***CITRUS BLACK SPOT: A DISCUSSION DOCUMENT FOR THE
REVIEW OF THE CURRENT PHYTOSANITARY REGULATIONS
PERTAINING TO THE EXPORT OF FRESH CITRUS FRUIT FROM
SOUTH AFRICA TO THE EU***

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1. INTRODUCTION

Citrus plantings in Southern Africa cover approximately 65 000 ha, primarily for export to some of the most sophisticated world markets demanding very high quality standards. Approximately 95% of the local citrus industry's income is derived from the annual export of approximately 900 000 tons of citrus. Citrus has been exported to Europe from Southern Africa since 1926 and the EU market presently receives approximately 65% of the citrus volume exported from this region. The local citrus industry supports approximately 500 000 people as employees and dependants in South Africa, Swaziland Mocambique and Zimbabwe.

Numerous pests and diseases impact on the profitability of the southern African citrus industry. The control measures for these organisms not only reduce the profitability of production but may also lead to contamination of the environment

and undermine the sustainability of production. It is, however, those organisms classified as quarantine pests by the industry's major trading partners that represent the most severe risks to the industry.

Citrus Black Spot (CBS), *Guignardia citricarpa* Kiely, is a foliar and fruit disease which causes unsightly lesions on the rind, spoiling the appeal of the fruit with Valencias, Navels, grapefruit and lemons being most sensitive. The disease occurs in subtropical regions with a summer rainfall in the following countries: Argentina, Australia, Brazil, China, Hong Kong, Indonesia, Japan, Kenya, Korea, Honduras, Jamaica, Trinidad, Nigeria, Mozambique, the Philippines, Peru, South Africa, Taiwan, Uruguay, Venezuela, Zimbabwe and USA (Sutton & Waterston, 1966).

CBS is classified by the EU as a quarantine pest. The recent imposition and subsequent implementation of intensified phytosanitary restrictions on citrus imported into the EU from countries in which CBS occurs (Directive 98/2/EC), is having a major impact on the southern African citrus industry. As a consequence the livelihoods of the approximately 0.5 million people reliant on this industry are seriously jeopardized.

CBS has been studied for many years by scientists in South Africa and other countries where it occurs. A review of the research results of these studies, together with consideration for both the impact of the current EU phytosanitary regulations on trade with countries exporting citrus to the EU and the implications

for countries heavily reliant on this trade, supports the need to review the current EU regulations pertaining to this issue. This document presents a consideration of the phytosanitary status of the organism within the framework of the guidelines: The International Standards for Phytosanitary Measures Part 1 - Import Regulations; Guidelines for Pest Risk Analysis, Published by the Secretariat of the International Plant Protection Convention, FAO, UN, Rome (1996).

2. **BACKGROUND INFORMATION**

2.1 **Epidemiology**

An understanding of the epidemiology of CBS requires particular consideration of the inoculum availability, the climatic conditions required for infection and the growth cycle of the tree, particularly fruit development. Ascospores are produced on dead leaves on the orchard floor, they represent the main source of inoculum and are not produced on fruit (Kiely, 1948; McOnie, 1964a & 1964b; Kotzé, 1981). The ascocarps develop within 50-180 days of leaves having dropped (Kotzé, 1963 & 1981). Abundance of ascospores depends on the frequency of wetting and drying as well as prevailing temperatures (Kiely, 1948; McOnie, 1967; Kellerman & Kotzé, 1977). The availability of moisture, once ascospores have matured, leads to swelling of the asci which press against the opening of the perithecium and forcibly eject the ascospores which become airborne (Kiely, 1948; McOnie, 1967; Kellerman & Kotzé, 1977;). The ascospores make contact with

young citrus fruit, germinate and infection takes place (Kiely, 1948; McOnie, 1967; Kellerman & Kotzé, 1977). Young fruit are only susceptible to ascospores during prolonged spells of rain during the first four months after fruit set and then become resistant, regardless of rain and the abundance of inoculum (Kellerman & Kotzé, 1977; Kotzé, 1981).

Once a fruit is infected, the fungus remains quiescent until the fruit becomes mature, in which case it may develop further to produce pycnidia (Kiely, 1948; Kotzé, 1963; McOnie, 1967). The termination of quiescence and subsequent expression of disease symptoms in the form of rind lesions in which pycnidia occur, coincides with the onset of colour development (Kiely, 1948 & Kotzé, 1963).

Pycnidiospores are produced within the pycnidia and, on maturity, are extruded in a gelatinous mass (McOnie, 1964b & 1967). Under suitable conditions, being moderate to high temperatures (Kiely, 1949; Wager, 1952; Kotzé, 1981) and the presence of moisture (Darnell-Smith, 1918), pycnidiospore formation and release takes place within 30 days of initiation of symptom expression (Kiely, 1948; Korf *et al.*, In prep.). Unlike the ascospores which are airborne, the sticky pycnidiospores rely on running water for dissemination (Kiely, 1948; McOnie, 1965). Pycnidiospores are short lived, with viability decreasing by over 60% within the first four days after release from pycnidia (Kiely, 1948). Pycnidiospores require specific stimuli to be released, such as water (Wager, 1949) and to germinate, such as orange peel or juice extract (Darnell-Smith, 1918).

Pycnidiospores do not infect mature fruit (Kiely, 1948; Wager, 1949). Kiely (1948) demonstrated that it was possible to infect young fruit with pycnidiospores during a window of susceptibility. However, this required the creation of specific artificial conditions (Kiely, 1948). Experiments evaluating the infection potential of pycnidiospores under more natural conditions produced results to the contrary (Kiely, 1949; Wager, 1949). Consequently, pycnidiospores are not considered to be an important source of inoculum in the disease cycle (Kiely, 1948; Kotzé, 1981 & 1988) either through downward movement of rain water or splashing upwards (Kiely, 1948; McOnie, 1965). Kiely (1948) concluded that CBS infected fruit did not constitute a risk of expanding the geographical distribution of the organism.

Mycelia are also not considered to constitute an infection risk (McOnie, 1965 & 1967). The primary route for further infection from fruit is apparently via pycnidiospore infection of leaves with subsequent ascospore production (Kiely, 1948).

The successful completion of the infection cycle is dependant on specific environmental conditions as reflected by the global distribution of the organism. Although CBS occurs in numerous countries with a sub-tropical, summer rainfall climate, it has never become established as a disease of citrus in any region with a Mediterranean climate, including the Western Cape Province of South Africa, South Australia, Western Australia, Chile, Spain, Greece, Italy, Israel, Turkey and California. The disease has failed to become established in Mediterranean climatic

regions despite the unrestricted movement of large quantities of citrus fruit from areas where CBS occurs into some of these Mediterranean regions for many years.

2.2 New information

Korf *et al.* (In prep), itemise research findings that have recently become available and may therefore not have been taken into consideration during the drafting of the current phytosanitary regulations.

- (i) Pycnidiospores were considered to carry no risk of transferring infection from ripe fruit to ripe fruit.
- (ii) In addition to the specific conditions previously known to be required for pycnidiospore germination, Korf *et al.* (In Prep.) showed that moderate temperatures of between 10°C and 35°C are required with 22°C being optimum. A pH range of between 3.5 and 4.5 was also required.
- (iii) Various standard packhouse treatments were effective in killing pycnidiospores present on infected fruit entering the packhouse.
- (iv) Fungicidal wax treatments suppressed the formation of pycnidiospores subsequent to packing.
- (v) Standard shipping temperatures for citrus exported from SA to EU suppressed pycnidiospore development.
- (vi) Within 30 days of fruit being held under optimal environmental conditions, after being exposed to induced colour development, pycnidiospore development had been completed.

- (vii) The survival of pycnidiospores on infected peel was less than ⁴~~3~~ hours when exposed to sunlight.

2.3 Specific risk mitigation considerations

The following points surrounding the biology of CBS, its control and fruit handling practices in southern Africa, as reflected in research results, have relevance to the mitigation of phytosanitary risk from CBS posed by citrus exported from southern Africa.

- (a) The management of CBS relies on an integrated programme and not an individual treatment with excessive use of chemical treatments being avoided in the interests of Integrated Crop Management, Resistance Management, Environmental Conservation and the pursuit of sustainability.
- (b) The timing of ascospore release in the different citrus producing regions is monitored with the use of spore traps (Smith, 1966).
- (c) Chemical treatments such as dithiocarbamates are used in a preventative program to reduce the inoculum (Kellerman & Kotzé, 1977).
- (d) Depending on spore counts, systemic chemicals such as benzimidazoles, which have both a curative and a preventative action, are used during periods of peak spore release (Kellerman & Kotzé, 1977).
- (e) Fruit is picked after colour break which implies that most infections will have expressed symptoms in the form of rind lesions, thus enabling infected fruit to be culled by the packers in the packhouse (Korf *et al.*, In Prep.).

- (f) Fruit is dipped in a tank containing orthophenyphenol which destroys any spores on the surface of the fruit (Korf *et al.*, In Prep.).
- (g) Fruit is thereafter waxed using post-harvest chemicals which suppress subsequent pycnidiospore development (Korf *et al.*, In Prep.).
- (h) Fruit is then shipped at temperatures which do not allow the development of the fungus (Wager, 1952; Korf *et al.*, In Prep.).
- (i) In the event of infected fruit entering any of the Mediterranean countries of Europe, considering that:

- A. (i) Pycnidiospores are not the primary source of infection in the disease cycle (Kotzé, 1988); (ii) pycnidiospores are produced over a short period (Korf *et al.* In prep.); (iii) at a specific developmental stage of infection (McOnie, 1967); (iv) under specific environmental conditions (Darnell-Smith, 1918; Wager, 1952); (v) requiring running water for dissemination (Kiely, 1948); (vi) and requiring exposure of the short-lived pycnidiospores to specific stimuli for germination (Kiely, 1948; Korf *et al.*, In Prep.); and (vii) as a general phytopathological principle, a minimum inoculum concentration is required for the existence of any reasonable chance of infection occurring, implying that one citrus fruit with one lesion is unlikely to provide sufficient inoculum;

the following sequence of extremely unlikely events would have to be combined to create any possibility of infection:

- B. A number of whole infected fruit, with infections synchronised at a

specific stage of lesion development, would have to be placed in a citrus tree and exposed to the specific environmental requirements within a short space of time.

- (j) In the unlikely event of such conditions occurring where the fungus may be able to temporarily survive, the prevailing long term climate would prevent the permanent establishment of the organism in any country with a Mediterranean climate. The unlikelihood of an infection being established in this way is further supported by failure of Wager (1949) to do so under ideal conditions in a suitable climatic region of South Africa.

3. PEST RISK ANALYSIS

3.1 Stage 1: Initiating the Pest Risk assessment process

3.1.1 PRA initiated by a pest

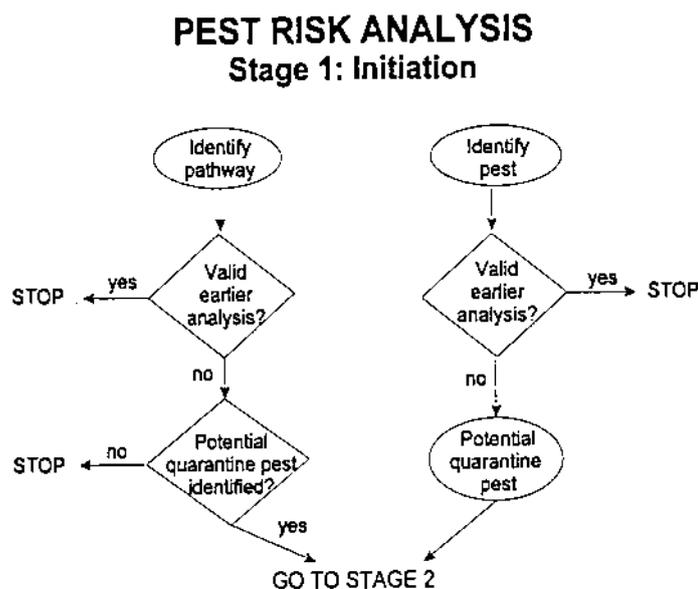


Figure 1

Since the issue at question relates to Citrus Black spot (CBS), the “PRA Initiated by a Pest” is considered appropriate. The guideline (IPPC, 1996) states that the need for a revised PRA “Initiated by a Pest” may arise due to, among others:

- a proposal is made by another country,
- a new treatment system, process, or new information impacts on an earlier decision,

both of which are relevant to this situation.

3.1.2 Review of Earlier PRA

The existence of EU phytosanitary regulations (Directive 9B/2/EC) restricting the

entry of citrus fruit infected with CBS, suggest that an earlier PRA was conducted. However, no documentation of such a PRA was available to the authors for review.

3.1.3 Conclusion for stage 1

In accordance with the guideline procedure (IPPC, 1996), CBS is thus identified as a “potential” quarantine pest at the end of stage 1 (Figure 1).

3.2 Stage 2: Pest Risk assessment

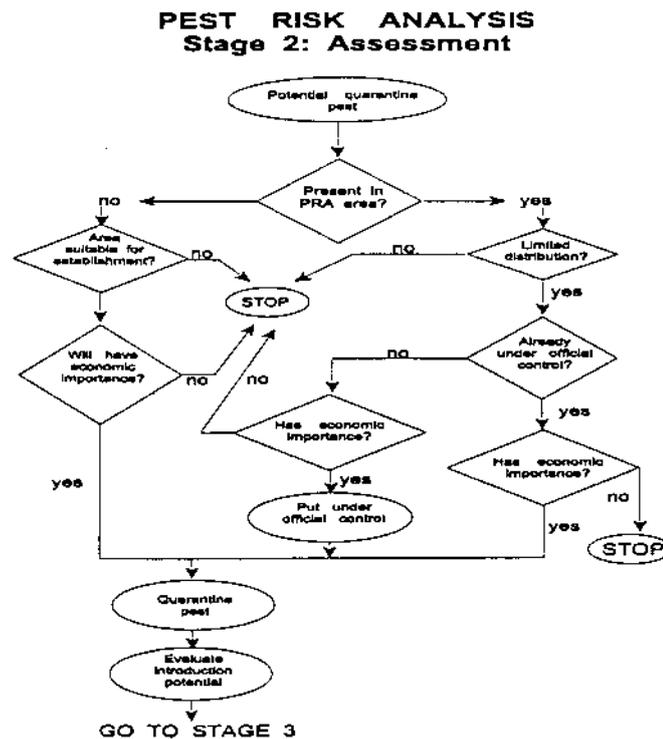


Figure 2

Stage 2 of the PRA examines whether the criteria for quarantine pest status are satisfied, namely:

“a pest of potential economic importance to the area endangered thereby and not yet present there, or present but not widely distributed and being officially controlled”;

area should be understood to mean:

“an official defined country, part of a country, or all or part of several countries”, being the European Union;

and “endangered area” should be understood to mean:

“an area where ecological factors favour the establishment of a pest whose presence in the area will result in economically important loss”, potentially being the southern European citrus producing countries;

as described in the Guideline (IPPC, 1996).

3.2.1 Geographical and regulatory criteria

There is no evidence of an established presence of CBS as a citrus pest in the PRA area. However, there are records of occurrence of the organism in Sicily and Spain although not in association with citrus (Sutton & Waterston, 1966).

3.2.2 Economic Importance Criteria

The Guideline (IPPC, 1996) states that for potential economic importance to be

expressed, a pest must become established and spread and thus the risk must be characterised through consideration of the following factors.

- A. Establishment potential. The Guideline states that biological information from areas of present occurrence should be used in comparing the PRA area with areas of present occurrence. The Guideline states that expert judgement should be used to assess the establishment potential. The Guideline states that if a pest has no potential for establishment in the PRA area, then it does not satisfy the definition of a quarantine pest and the PRA for the pest stops at this point. It is abundantly clear from the global distribution of the organism, which is explained by its epidemiology, that Mediterranean climatic regions are not suitable for establishment of the organism as a disease of citrus. This is strongly supported by the distribution of the organism in both Australia and southern Africa. In both regions the disease occurs in the summer rainfall northern parts, but despite no restrictions on the movement of fruit from northern to the southern regions, the disease has never established in these southern, Mediterranean climatic regions. Since citrus production in the EU occurs in southern European countries with a Mediterranean climate, the PRA should therefore be terminated at this stage.
- B. Spread potential after establishment. The same considerations as in assessing establishment potential apply.
- C. Potential economic importance. The potential economic importance, if the organism could establish in the PRA area, could be high. However, nowhere in the world has the organism become established as a citrus pest under a

Mediterranean climate. Both the establishment and spread potential of the organism in the PRA area is minimal, making the potential economic importance insignificant.

3.2.3 Introduction potential

The Guideline (IPPC, 1996) provides a checklist of factors to consider:

A. Entry

- A.1. Opportunity for contamination of commodities. The incidence of the organism on exported fruit is low due to the combination of pre-harvest controls and removal of infected fruit during packing.
- A.2. Survival of pest during transport. Packhouse treatments have been shown to be effective in killing pycnidiospores and shipping temperatures to be detrimental to the development of the organism (Korf *et al.*, In Prep.).
- A.3. Ease of detecting the disease at entry inspection. CBS infection develops into obvious rind lesions which are readily detectable. However, the presence of these lesions is not a reliable indication of the presence of live organism since most pycnidia develop and release pycnidiospores shortly after the onset of colour development, that is before picking. Isolation and incubation of the organism from lesions is therefore necessary to confirm the presence of live organism.
- A.4. Movement into the PRA area. Considerable quantities of citrus fruit from southern Africa may enter the PRA risk area.

B. Establishment

- B.1. Quantities of fruit. Considerable quantities of fruit may enter the PRA risk area.
- B.2. Quantities of organism. The quantities of live CBS organism are however low due to pre-harvest controls, senescence of lesions prior to packing, packhouse selection, packhouse treatments and shipping temperatures.
- B.3. Intended use of the commodity. The fruit entering the PRA risk area is primarily for consumption as fresh fruit. The likelihood of this fruit being placed inside a citrus tree in the PRA risk area is remote.
- B.4. Environmental conditions and availability of host in the PRA risk area. Citrus from southern Africa may enter the PRA risk area during the dry summer months (April - September). For the organism to potentially establish in the PRA risk area, several imported fruit (a) on which infections occur despite control and selection practices; (b) with lesions synchronized at the specific stage of development where pycnidiospores are present; (c) would have to be placed inside a citrus tree; (d) be exposed to running water; (e) the short-lived pycnidiospores would have to be exposed to specific germination inducing stimuli being citrus juices or citric acid; for the possibility of infection to occur and then the organism would have to behave as it never has before in any part of the world for the disease to become established in a Mediterranean climate.

3.3 Conclusion for Stage 2

In accordance with the Guideline (IPPC, 1996), the PRA should terminate at stage 2 (Figure 2). There are three points in stage two where this is indicated, namely in the sections entitled "Economic importance criteria: establishment potential" (3.2.2.A) and "Introduction potential" (3.2.2.B) and "PRA risk area suitability for establishment" (3.2.3.B). The PRA therefore indicates at this point that the organism is not suitable for classification as a "quarantine pest" and the implementation of phytosanitary measures is not justified.

3.4 Stage 3: Pest Risk Management

**PEST RISK ANALYSIS
Stage 3: Management
from Stage 2**

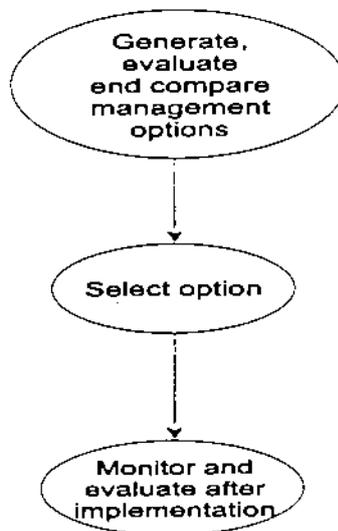


FIGURE 3

The Guideline (IPPC, 1996) states that pest risk management should be proportional to the risk identified in the PRA. Furthermore it states that the “Minimal Impact” principle should be supported: “Phytosanitary measures shall be consistent with the pest risk involved and should represent the least restrictive measures available which result in the minimum impediment to the international movement of people, commodities and conveyances”.

The PRA indicates that the organism in association with citrus exported to EU from southern Africa does not qualify for categorisation as a quarantine pest on fruit. Consideration of the low risk status and major impact of the present highly restrictive phytosanitary measures, indicates that the current restrictions are not technically justifiable in accordance with the “Minimal Impact” principle. The social and economic impact of recently intensified phytosanitary measures affecting citrus exported to the EU are severe for southern Africa because of CBS.

3.4.1 Risk Management options

The Guideline (IPPC, 1996) provides a checklist of options for consideration:

- (a) Inclusion in list of prohibited pests. Failure to classify CBS as a quarantine pest in Stage 2 of PRA makes this inappropriate.
- (b) Phytosanitary inspection and certification prior to export. The fact that on inspection after packing very few lesions encountered would contain viable spores, makes it inappropriate to reject fruit for export because of the

presence of rind lesions. Furthermore, CBS failed to be classified as a quarantine pest in the PRA. Accordingly, it would be appropriate for certification to be based on quality standards.

(c) Requirements before export, e.g. treatment, disease free area, growing season inspection, certification. None of these would be appropriate because of:

- (i) non-classification as a quarantine pest;
- (ii) treatment - both cost implications and environmental impact not being justified within "minimal impact" principle;
- (iii) pest free area - both non-classification as a quarantine pest, and severe social and economic impact on southern Africa;
- (iv) growing season inspection and certification - presence of the organism in the orchard is not a reliable indication of either presence of live organism on exported fruit, risk of introduction or risk of establishment.

(d) Inspection at entry. This is inappropriate since in this PRA the organism, in association with citrus fruit, fails to satisfy the international definition of a quarantine pest (3.3). Furthermore, the occurrence of fruit lesions does not necessarily indicate the presence of a viable inoculum, or indeed, any live organism since ascospores are the main source of inoculum, but do not occur on fruit, and pycnidiospores which may be associated with fruit lesions are unimportant as inoculum in the disease cycle (2.1, 2.2 & 3.2.3.A.3). There may also be no pycnidiospores associated with old fruit lesions (2.1 & 2.2).

(e) Treatment at point of entry. The same considerations as for (d) apply.

- (f) Detention on post-entry and post-entry restrictions. This is inappropriate due to failure to satisfy the requirements for classification as a quarantine pest.

3.5 Conclusion for Stage 3

In accordance with the Guideline procedures (IPPC, 1996) it is concluded that CBS does not qualify as a quarantine pest. The implementation of phytosanitary restrictions appropriate for quarantine pests are therefore not supported by this PRA.

4. **CONCLUSION**

Consideration of technical information on CBS, within the internationally accepted IPPC framework for Pest Risk Analysis (IPPC, 1996), indicates that CBS on citrus fruit exported from southern Africa to the European Union does not qualify for classification as a “quarantine pest”. Furthermore, consideration of the “Minimal Impact” principle for establishing “Pest Risk Management” actions, indicates that current restrictions are inappropriately restrictive.

The classification of CBS as a quarantine pest and the imposition of intensified phytosanitary restrictions on the import of citrus into the EU from countries where CBS occurs, has severe consequences for the southern African citrus industry. Strict enforcement of these restrictions jeopardises the livelihoods of approximately 0.5 million people in the region.

In accordance with this evaluation, it is concluded that the current EU phytosanitary restrictions relating to the import of citrus fruit from areas of southern Africa where CBS occurs, as encompassed by Commission Directive 98/2/EC, should be rescinded.

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EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL

Directorate E - Food Safety: plant health, animal health and welfare, international questions
E1 - Plant health

**Report of the Commission Working Group on evaluation of the Pest
Risk Assessment (PRA) prepared by South Africa
on Citrus Black Spot (CBS)**

Brussels, 24/10/2001

1. INTRODUCTION

After a first discussion took place during the meeting of the relevant Regulatory Committee for Plant Health of 19-20 March 2001, it was decided that a Commission Working Group (WG) should be established, in view of the technical evaluation of the PRA submitted by South Africa.

2. COMMENTS OF THE WG ON THE DOCUMENT PREPARED BY SOUTH AFRICA

The WG agreed to present its comments according to the structure of the document supplied by South Africa. The references in *Italics* below refer to the said document.

1. Introduction

p. 2: The description of the geographical distribution of CBS is out of date. In particular, CBS is not present in the USA, but it is present in Swaziland.

p. 2: Commission Directive 98/2/EC introduced some changes in the EU requirements as regards the import into the Community of Citrus fruits originating in third countries. It should also be stressed that prohibition of import of the said Citrus fruits in certain Member States was lifted.

2. Background information

2.1. Epidemiology

The WG considered that ascospores, pycnidiospores and mycelium could each be a means of spread of CBS. It agreed with the PRA provided by South Africa that ascospores are by far the most efficient means of dissemination of CBS where the disease is already well established (Kotzé, 1981). However, the WG stressed that spread of CBS via the pycnidiospores may be as important as the role of ascospores at the beginning of the establishment of the disease in an area (McOnie, 1964). Although the probability would be low, it is believed possible that a single conidium could initiate infection and disease development on individual trees and this could ultimately lead to the eventual establishment of the disease in a Citrus producing area, over a long period of

time (Smith, 1996). This may explain why the disease has usually been found to establish slowly in newly infected areas (5-30 years) (Kotzé, 1981).

The WG accepted that the route of establishment whereby infected fruit were placed in a Citrus tree in non-CBS regions was unlikely to occur. However, it wondered whether the conidia within pycnidia on infected fruit could initiate infection of fallen leaves (e.g. if infected fruit or peel was placed on an orchard floor) or whether conidia from infected fruit or peel could splash up onto low-hanging parts of the Citrus tree, infect and produce ascocarps and airborne ascospores, thus allowing the more efficient means of dissemination of CBS. This particular aspect appears not to be published in the scientific literature but may require scientific investigation if it does not. Similarly, the role of insects in spreading conidia requires consideration as an alternative pathway.

In respect of the suggestion that CBS has never established under a Mediterranean climate, the WG noted a lack of sufficiently detailed meteorological data characterising such a climate. It also noted that "Mediterranean climate" is too broad a term for characterising the various climatic conditions of the Citrus producing areas in Europe. Moreover, CBS has already established in areas with various environmental conditions (cool, misty, dry, hot, semi-arid, subtropical, etc.) (Wager, 1952). Based on the literature, too much hope had been pinned in the past on climate as a limiting factor (Kotzé, 1981). Therefore, detailed data on the meteorological conditions (temperatures, humidity, rainfall, etc.) should be provided for the Citrus producing regions in South Africa and Europe in order to be able to make a valid comparison.

Additionally, it should be noted that irrigation is widely used in European Citrus producing areas, and this should be taken into account regarding the possible establishment and spread of CBS in those regions.

The WG also noted from various publications (e.g. Kotzé, 1981) that climate influences the rate of spread of CBS, but not necessarily the establishment of the disease itself. Climatic factors are not the sole reason for the impossibility of establishment of CBS. According to Kotzé (1981), the most important factors determining a CBS epidemic are summer rains and presence of lemon orchards. Numerous examples in South Africa show that the first disease outbreaks always occur in lemon orchards, where inoculum builds up gradually until all adjacent Citrus orchards become infected. Citrus orchards are commonly irrigated by sprinkler irrigation (its role in the spread of various fungal and bacterial diseases is equivalent to rain) and lemon trees are grown next to other types of Citrus trees. Additionally, lemon and other Citrus trees are grown in home gardens even in major towns. Moreover, as CBS has established in so many areas with marked climatic differences, there would appear to be no reason why it should not eventually establish in any area where Citrus is grown (Wager, 1952). Additionally, as CBS has a sexual stage, the risk of quick adaptation of this fungus to factors limiting establishment such as climate may be higher than a fungus without a sexual reproductive phase.

Finally, it should be stressed that some of the areas cited as being free from CBS have had or still have regulations prohibiting the import of Citrus fruits which may explain its absence from these areas.

2.2. New information

The publication referred to in this chapter (Korf *et al.*, in prep), has yet to be published. The WG agreed that it may be presumed that the measures listed have a suppressive effect on the development of CBS, but no eradication effect, in particular on the conidia

within fruiting bodies (pycnidia) present on the fruits and on the mycelium possibly present in the peel. The above authors also report that the form in which the pathogen remains on the fruit after treatment, viz. mycelium instead of conidia, is not regarded as infective. However, Kiely (1948) reported that latent mycelium can cause the appearance of lesions on fruit.

It should also be stressed that this non-eradication effect is consistent with the fact that the pathogen causing CBS has been readily isolated from Citrus fruits at the time of their import into the EU (Tab. 1).

As regards the survival of pycnidiospores, the WG noted that mature pycnidiospores may germinate even after 48 hours of incubation, even in light (Korf *et al.*, in prep.), whereas Wager (1949) reported that pycnidiospores taken from CBS lesions present on orange peel, that was slowly mummified and dried up, remained alive for up to four months. Moreover, the WG noted that most of the results of Korf *et al.* (in prep.) refer to mature pycnidiospores. However, it is well known that various crops of pycnidiospores may be produced by a pycnidium. Therefore more information is needed on the survival of the other crops of pycnidiospores (immature pycnidiospores).

The WG asked for additional information on the monitoring, both in Citrus orchards and in packinghouses, of the possible CBS strains resistant to applied fungicides (i.e. benzimidazoles). In the 1980s, CBS has developed resistance to important fungicides such as benzimidazoles, whereas benomyl was previously believed to be reliable (Kotzé, 1981). Strains of CBS tolerant to benzimidazoles have already been reported in South Africa (Herbert and Grech, 1985). It is therefore uncertain whether risk mitigation measures involving fungicides can permanently ensure full control of the disease.

2.3. Specific risk mitigation considerations

The WG stressed that as long as CBS is listed as a quarantine pest for the EU, the risk mitigation measures proposed in the document are not considered to be appropriate. This is especially valid as Smith (1996) stated that “one infection on a fruit can cause symptoms over a large area of that fruit” and that, referring to ascospore infection, “relatively low spore numbers were therefore sufficient to cause severe infections”. Kellerman and Kotzé (1977) showed that fruit infections remain latent for 3 to 12 months after blossom. However, the point was also made that there is no such thing as “zero risk” and that in phytosanitary terms the measures should be appropriate to what is considered to be the “acceptable level of risk”.

The WG pointed out that the proposed risk mitigation measures are targeting only one possible pathway, i.e. direct spread of CBS from an infected fruit to Citrus trees. More information and data are desirable, in particular on the possible spread of the disease from infected Citrus peel to Citrus trees in private gardens or orchards, considering that:

- a) pycnidiospores are produced both on infected fruit and on the leaf litter under the trees (Smith, 1996),
- b) on infected Citrus peels, even mummified and dry ones, pycnidiospores survive for up to four months (Wager, 1949), and
- c) in some European Citrus producing areas, Citrus trees are grown almost everywhere, even in major towns (i.e. in private gardens). The WG wondered whether it was possible that perithecia might be formed on infected fruits and fruit peel thrown away in the

vicinity of citrus trees, for instance in a garden where Citrus trees are also present (i.e., perithecia formed during rotting from the underlying mycelium present in the peel, and not from the pycnidia). In such a case, ascospores may be formed secondarily on the rotting fruit or fruit peel on the ground of a garden or orchard floor, just as it happens with rotting leaves, and then ascospores may be released and infection of trees may result from these secondary ascospores. Such an occurrence has never been reported in the scientific literature but may warrant a scientific investigation to determine whether it could ever happen under natural conditions

It would also be desirable to have additional data on the half-life of the fungicides used as post-harvest treatments. The treatments do not kill the fungus, since EU Member States were able to culture the fungus from infected fruits in the past two years, and since the fungus can be induced to produce sporulating pycnidia in five days under favourable climatic conditions.

3. Pest Risk Analysis

3.2.1. Geographical and regulatory criteria

The references to the presence of CBS in Sicily and Spain are erroneous since they do not refer to strains of CBS pathogenic to Citrus, the subject of the PRA.

3.2.2. Economic importance criteria

According to the content of this chapter, CBS cannot establish in the PRA area. However, and as explained above, the WG is of the opinion that it has not been completely proven that CBS cannot establish in the PRA area.

3.2.3. Introduction potential

A. Entry

As a general remark, the WG stressed that this subchapter *A. Entry* should have finished with a conclusion, in order to decide or not the continuance of the PRA.

- A.1.* Living stages of CBS have been intercepted on several occasions on fruits imported into the EU (Tab. 1).
- A.2.* It is stated that the usual post harvest activities have been shown to be effective in killing pycnidiospores, though in chapter 2.2., the same activities have been considered as being merely suppressive.
- A.3.* Experience has shown that living stages of CBS have been found on fruits imported into the EU. Moreover, it should be noted that the EU Member States have agreed to use a harmonised protocol aiming at the detection and confirmation of CBS on Citrus fruits after a 5-day incubation procedure that induces pycnidium formation in lesions initially devoid of pycnidia. This means that, under favourable conditions, pycnidia may be formed in the EU on fruits initially treated against CBS.

B. Establishment

- B.3.* Other possible pathways should also be considered.

B.4. The various climatic conditions prevailing in the EU Citrus producing areas cannot be only characterised as a dry summer, especially over such a long period from April to September. Warm, humid conditions are normal in spring and autumn in various Citrus producing regions in the EU. Additionally, irrigation is a key factor for modifying the micro-climatic environment in Citrus orchards. Moreover, susceptible Citrus fruits are present over a long period of the year, due to the diversity of climates combined with the variety of species and cultivars grown in Europe. Several European climates would fall in the range for establishment of CBS given by Lee and Huang (1973).

The WG did not understand the meaning of the last sentence of this paragraph B.4.

3.3. Conclusion for Stage 2

The WG pointed out that there is not sufficient information supporting the conclusion of this stage.

3.4.1. Risk management options

- (b) Experience has shown that living stages of CBS have been found on fruits imported into the EU.
- (c)(ii) Cost implications and environmental impact have not been documented.
- (c)(iii) Social and economic impacts have not been documented.
- (d) Experience has shown that living stages of CBS have been found on fruits imported into the EU. Moreover, it should be noted that the EU Member States have agreed to use a harmonised protocol aiming at the detection and confirmation of living *Guignardia citricarpa* on Citrus fruits

As regards the risk management options listed in the letter accompanying the PRA (Ref. 14/2/1 EU), the WG understood that South Africa wishes to apply in respect of CBS the following measures:

- All Citrus fruit exports from South Africa shall continue to comply with the relevant official quality standards (class 1) for CBS;
- The current plant protection spray programs for CBS in those areas where CBS occurs, shall remain applicable, and
- Acceptable post harvest treatments including fungicidal dips and wax treatment, shall be applied as appropriate.

The WG noted that the second measure, i.e. maintaining the spray programs, is in contradiction with the allegation that “*both cost implications and environmental impact [of treatment are]... not being justified*” (see 3.4.1.(c)(ii)).

The WG stressed that additional data is needed in order to support the third above mentioned measure, i.e. post harvest treatments, in particular in respect of the efficacy of these treatments against all the living stages of CBS possibly found on Citrus fruits exported.

3. CONCLUSIONS OF THE WG

The WG appreciated the quality of the PRA on CBS submitted by South Africa. It confined its discussions to the technical aspects of this PRA.

The WG acknowledged that the quarantine status of CBS for the EU depends on the existence of realistic pathways for entry and climatic conditions supporting potential establishment in the EU.

The WG believed that some important information and data are still missing, in particular on the various pathways for spread of CBS, and also on the meteorological conditions for establishment of CBS.

Tab. 1: Interceptions of CBS in the EU Member States – number of consignments

Country of origin	2001*	2000	1999	1998	1997	1996
Argentina	2		7			
Australia					1	
Benin			1			
Brazil	4	1	16			
Guinea		1	1			
Mozambique		1				
South Africa	15	12	22			
Swaziland		5	7			
Zimbabwe			1			
Total	21	20	55		1	

*Data as of 15/12/2001

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**Response from South Africa
on the Report (dated 24/10/2001) of the EC Working Group (WG)
relating to the WG's evaluation of the Pest Risk Assessment (PRA)
by South Africa on Citrus Black Spot (CBS)**

*Compiled by the Directorate Plant Health and Quality: Subdirectorate Plant Health
of the SA National Department of Agriculture,
in collaboration with appropriately representative academic specialists,
researchers, technical field specialists and industry role players*

June 2002

1. A. Introduction

Following receipt of the EC Working Group report by SA in December 2001, a workshop was convened by the Directorate Plant Health and Quality, SA National Department of Agriculture on 04 February 2002. Eminent academics, researchers and technical field specialists with expertise in the field of CBS were present. The EC WG Report was discussed in detail and a work plan formulated to obtain further inputs from the technical specialists in order to be able to respond to the EC WG report. The responses that follow are presented according to the comments from the EC WG and the corresponding structure of the PRA document.

B. Comments and responses

1. Introduction

p.2 We acknowledge the information that the WG provided on the currently known geographical distribution of CBS.

p.2 We acknowledge changes to EC legislation as referred to.

2. Background information

2.1 Epidemiology

- 2.1.1 Paragraph 1. We recognise that the WG's comments relate to the role of ascospores, pycnidiospores and mycelium in the context of establishing the organism, as opposed to the role of these structures in the spread of the disease where the organism already occurs.

The EC WG suggests that a single conidium could initiate infection and disease development on individual trees and ultimately lead to establishment. We would like to refer you to the attached report by Professor JM Kotzé, internationally recognised as a leading CBS expert. Kotzé puts into context the reference to Smith (1996), as cited by the EC WG Response. Further to Kotzé's comments, we wish to advise that the Smith (1996) publication is in the form of a contribution to congress proceedings, where the manuscript would have been edited for language and format, but would not have been subjected to scientific peer review. We therefore suggest that this publication should not be deemed to be authoritative.

McOnie (1964) is referred to by the EC WG, in support of the WG comments that pycnidiospores may be important in the initial establishment of the organism in an area. Our interpretation of this article is that there is no indication in his article to this effect. We interpret McOnie's article as having found no evidence of pycnidiospores playing a role in the level of fruit infection, by virtue of there being no differences in the level of infection on a vertical scale within the tree. McOnie further refers to Kiely (1948) as supporting this conclusion – Kiely's paper also supports the contention that pycnidiospores do not wash down and thus lead to a higher infection level on the lower regions – and Kotzé (1963) found significantly higher infection rates in the upper section of the tree. McOnie makes the comment that he does not consider that his particular study dismisses the "possibility" that

pycnidiospores could be important under “some circumstances”. In other words, he is offering no information to support such a claim and merely cautions that his particular study does not conclusively dismiss the possibility thereof.

We are not aware of any convincing evidence that pycnidiospores do play the role suggested by the WG and are of the opinion that the bulk of evidence suggests that pycnidiospores do not play such a role. We do not consider it feasible to prove beyond doubt that this cannot happen and can only go on the strength of available evidence, which strongly suggests that it does not constitute appreciable risk.

We consider the rate of development of the disease within an area, when the organism first becomes established, as being influenced by a myriad of possible factors. Therefore we are sceptical about speculation that the rate of initial disease development may be considered supportive of the suggestion that pycnidiospores play an important role in the establishment of the organism.

2.1.2 Paragraph 2. The WG raises the question of whether pycnidiospores could infect fallen leaves. This question is the subject of a current study at the University of Pretoria. Information currently available in the literature indicates that this is unlikely. Ascospores are only known to be released after a lengthy dormancy period in the leaf on the tree followed by a 50 to 180 day maturation period in fallen leaves that have become partially decomposed, under conditions of intermittent, but not excessive wetting (Kotzé, 1981).

The EC WG further raises the question of whether pycnidiospores could splash upwards from the orchard floor and infect leaves with subsequent ascospore production. Despite indications in the available literature that this cannot be considered an important potential infection pathway in the disease cycle, we concur that, in the unlikely event of infected peel with

short-lived pycnidiospores being present at the time, there may be a remote possibility of pycnidiospores being transported from infected peel on the ground to the lower reaches of the tree by splashing. However, the remoteness of this possibility, together with the specific stimuli required for subsequent spore germination and infection to take place, strongly suggests that this is highly unlikely.

Furthermore, evidence from pycnidiospore infectivity studies currently being conducted in South Africa, suggests that the susceptibility of leaves to infection declines with age. Kiely (1948) obtained similar results. Together with the fact that the majority of the younger leaves occur on the higher reaches of the tree, these considerations make the likelihood of this pathway seem remote. There is a further hurdle to this pathway, in that a long maturation period is required, firstly within the living leaves and then within fallen leaves under specific environmental conditions, before ascospores could be released (Kiely, 1948, 1949; McOnie, 1967; Kotzé, 1981).

Insects may be viewed as potential agents of transmission in the same light as splashing, although we must add that it is the opinion of our pathologists that the likelihood of this pathway being successful is considered even more remote than by splashing.

2.1.3 Paragraph 3. Meteorological data. A global climate matching study has been underway in South Africa for several months now. The results of this study will provide the detailed information requested by the EC WG.

2.1.4 Paragraph 4. Regarding the effects of irrigation, our researchers concur that irrigation may well be a factor in encouraging ascospore release in a situation where the organism is established. It should, however, be noted that there would be considerable differences between the effects of drip- and micro-jet irrigation. Furthermore, the potential release of ascospores in association with irrigation is unlikely to lead to infection in the absence of the

high humidity and leaf wetness associated with general rainfall, being conditions necessary for infection to take place (Kiely 1948). In addition, such release would have to overlap with the presence of leaves or fruit that are at a susceptible stage of development. Before viable ascospores can be present on fallen leaves, these leaves must have been exposed to specific suitable environmental conditions to enable successful ascocarp maturation (Kiely, 1948, 1949; McOnie, 1967; Kotzé, 1981).

2.1.5 Paragraphs 5 & 6. The EC WG makes the statement that “Climatic factors are not the sole reason for the impossibility of establishment of CBS”. Firstly, we wish to have it noted that the term “impossibility”, should be viewed with circumspection. It is our opinion that if such requirements were to be applied more generally, international trade in fresh produce would be significantly curtailed.

Secondly, we can offer no reasonable explanation other than climate as to why the W. Cape province winter rainfall area of SA has remained free from CBS. Although the movement of propagation material into the W. Cape is now regulated to prevent the spread *inter alia* of citrus greening disease, it can be safely assumed that some of the propagation material used in the early establishment of the W. Cape plantings, originated from CBS-infected regions. Furthermore, there has been no restriction on the movement of fruit from CBS-infected northern regions of SA into the W. Cape for as long as CBS has been present in SA. Lemons are grown commercially and are highly abundant in home gardens throughout SA, including the W. Cape. The W. Cape has been found to be free of CBS through an intensive survey conducted by the National Department of Agriculture, and both the USDA and EU have recognised this region as being CBS-free.

The EC WG group cites Wager (1952) in support of their suggestion that CBS could eventually establish in any area where citrus is grown. Using the newly developed molecular identification techniques not available in Wager’s time, local research has shown that the unspecified *Guignardia* sp

that Wager noted is in fact *Guignardia mangiferae*, a cosmopolitan endophyte of a wide range of host plants (Meyer *et al.*, 2001; Baayen *et al.* 2002). Earlier, McOnie (1964, 1965) demonstrated that Wager (1952) had mistakenly based his surveys and conclusions on the presence of two species of *Guignardia* not one, and that unlike *G. citricarpa*, the other species does not cause CBS.

We consider the suggestion that because CBS has a sexual stage it is more likely to adapt and thereby expand its distribution, as being a factor that should not rate highly in the determination of an organism's quarantine status. In our opinion, should this principle be applied to other pathogens it would broaden the debate on pest establishment unacceptably, to the potential detriment of trade.

Furthermore, roughly 20 million tons of citrus have been exported from southern Africa to the EU over the past 75 years. Although unrestricted movement of citrus, complying with the CBS risk management requirements of the relevant EU directive, into the southern member states is a fairly recent occurrence, we consider it reasonable to assume that considerable quantities of the imported fruit have found their way into these regions over the years. Moreover, it has been a long-standing and common practice to send large quantities of South African grapefruit into Italy under licence, prior to lifting of the protected zone status. One can therefore assume that the PRA risk area has already been exposed to CBS infected fruit for many years, without any evidence of the CBS organism having become established.

2.2 New information

2.2.1 Paragraph 1. Publication by Korf *et al.* cited as being in preparation: At the time of compiling the PRA document, this publication had been submitted for review. It was returned with the request that it be sub-divided into

separate papers. This has been done and the work has now been published.

Reference is made to latent mycelium leading to the appearance of lesions, and on that basis the WG questions the claimed non-infectivity of mycelium. We concur that latent mycelium may be present, but do not view this as an indication of infectivity. Latent mycelium present in the fruit or leaves is a product of an earlier infection by spores, but the mycelium itself is not infective, only the spores.

2.2.2 Paragraph 2. We concur that the measures listed are suppressive but do not result in eradication. It should be noted that the relevance of these treatments is that they are intended to be viewed as a hurdle, or as additional control points in a systems approach, rather than as a complete risk-mitigating step on their own.

2.2.3 Paragraph 3. Survival of pycnidiospores. The context in which the short survival of pycnidiospores has been presented in the PRA, is in terms of the survival of the spores once extruded from the pycnidia. It was also stated in the PRA that post-harvest treatments kill exposed pycnidiospores. It has not been claimed that such treatments can eradicate the organism as latent mycelium or immature pycnidia.

With regard to Wager (1949), exposure of fruit and especially peel under field conditions could deliver very different results from observations made on fruit held in a protected indoor environment on a laboratory table, as done by Wager. To this effect, Korf *et al.* (personal communication), showed that exposure of peel to sunlight resulted in very rapid death of spores and mycelia. The brevity of pycnidiospore viability is also widely referred to in the literature.

2.2.4 Paragraph 4. We take cognisance of the fact that fruit from South Africa with live CBS mycelium continues to be intercepted in the EU, and of the

PRA-based requirement of appropriately managing the concomitant risks identified on the basis of scientific evidence. However, fungicidal treatments cannot “permanently” ensure “full” control of the disease, as questioned in the EU WG Response. In general, apart from eradication not being feasible from an economic or practical perspective, we also consider complete control of a regulated organism, where compliance with a zero interception tolerance is required, to be unattainable.

Development of resistance to bezamidazoles has little bearing on these risks in light of alternative chemistry being available, such as the strobulerines, use of which acts against the development of resistant CBS strains.

2.3 Specific risk mitigation considerations

2.3.1 Paragraph 1. The EU WG states that the proposed risk mitigation measures are inadequate as long as the organism is classified as a quarantine pest. It is our contention that it is the very classification of CBS as a quarantine organism for EU imports of fresh citrus that is considered inappropriate in terms of the PRA. We suggest, therefore, that justification for current risk mitigation procedures, on the basis of a quarantine classification for the organism, is tenuous in view of this classification being challenged.

The WG refers to Smith (1996) in support of its position. We again refer to the attached report by Prof Kotzé, and to our earlier comments on this publication (see point 2.2.1, above). The inaccuracy of the statement, namely “one infection on a fruit can cause symptoms over a large area of that fruit”, was confirmed by our group of specialists. Field pathologists have indicated that it is common to find complete protection of only a portion of a fruit, where leaves had prevented full spray coverage by a contact fungicide through physical screening of the unprotected portion of the fruit.

The inaccuracy of Smith's comments regarding inoculum levels is further clarified in the attached report by Kotzé.

2.3.2 Paragraph 2. With regard to the pathway: citrus fruit to citrus trees. We view the pathway to be the same, whether it is for whole fruit or peel separated from the fruit regarding the postulated transmission from infected fruit to commercial citrus or to home garden trees.

- a) Reference is made to Smith (1996) regarding pycnidiospores on leaf litter under the trees. We do not see that this has a bearing on the risk of establishing an infection in the EU as only fruit are sent to Europe. If the WG is concerned that CBS infected fruit or peel could land in leaf litter under Citrus trees in the EU, the issue of declining susceptibility of leaves with increasing age is covered in point 2.1.2 (paragraph 3), above, and see (b), below.
- b) Comments regarding the survival of pycnidiospores under field conditions have been addressed under point 2.2.3 above.

Regarding the postulation that perithecia might form on infected fruit or fruit peel thrown away in the vicinity of citrus trees, we refer to the attached report from Prof Kotzé, in which he emphatically states that this does not occur.

2.3.3 Paragraph 3. With reference to post-harvest fungicides. Korf *et al.* (2001) demonstrated that the treatments kill exposed pycnidiospores present at the time of application. We acknowledge that the treatments do not have a residual effect and therefore we consider the half-life of these products to be irrelevant.

3. Pest Risk Analysis

Suggested modifications of the PRA text, aimed at addressing certain points raised by the EU WG, are provided below in underlined format.

3.2.1 Geographical and regulatory criteria.

We acknowledge this.

3.2.2 Economic importance criteria.

We acknowledge this and trust that the clarification of various points in this document, as well as further results of current research, will provide the WG with sufficient information to share our certainty that CBS will not establish in the PRA area.

3.2.3 Introduction potential

We acknowledge that subchapter A. Entry, should have ended with a conclusion. Based on the evidence provided there, the conclusion should have been that CBS can indeed enter the area under consideration.

A.1 We acknowledge that the organism may enter the PRA region. The PRA does not deny this, it merely states that the incidence is low.

A.2 The EU WG has queried the consistency of statements regarding the efficacy of post-harvest treatments. We would like to draw attention to the fact that the PRA and supportive information has not claimed a curative or residual effect of post-harvest chemicals. It is correct to state that such products kill pycnidiospores with reference to exposed spores present on the fruit at the time of treatment. This may be clarified by inclusion of the above underlined text in the PRA document. The second component of the post-harvest treatments refers to the cold chain, which has also been shown

to have a suppressive effect as claimed in the PRA. This may be clarified by replacing “detrimental” with “suppressive”.

A.3 We acknowledge that, under favourable conditions in the EU, pycnidia can form on fruits treated against CBS. This does not, however, as put forward in our argument, pose a threat to the EU Citrus Industry.

B. Establishment

B.3 Other pathways. This has been addressed under point 2.3.2 above. The text may be clarified by inclusion of “The likelihood of this fruit or its peel being placed inside or below a citrus tree in the PRA risk area is remote.”

B.4 Regarding the last sentence of this paragraph in the PRA document, it can be rephrased as follows to make its meaning more clear (words inserted for this purpose are underlined):

‘B 4. Environmental conditions and availability of host in the PRA risk area. Citrus from southern Africa may enter the PRA risk area during the dry summer months (April – September). For the organism to potentially establish in the PRA risk area, the following conditions must be in place: firstly, for the possibility of infection to occur,

- a) Several imported fruit,
 - i) On which infections occur despite control and selection practices, and
 - ii) With lesions that are synchronised at the specific stage of development where pycnidiospores are present,
 - iii) Would have to be placed inside a citrus tree, and
 - iv) Be exposed to running water, or
 - v) Be placed below a citrus tree, and

- vi) Be exposed to the presence of water in such a form that it may transfer pycnidiospores to susceptible parts of the tree by splashing, or
- vii) The infected peel of these fruits, be placed in or beneath a citrus tree bearing susceptible foliage or fruit, and be exposed to the same conditions as above.

Further,

- b) This would have to occur before the short-lived pycnidiospores have been rendered non-viable by exposure to environmental conditions,
and
- c) Viable spores, in combination with susceptible host plant material, would have to be exposed to specific germination inducing stimuli, these being citrus juices or citric acid, as well as conditions suitable for infection to take place,

And finally the organism would have to behave as it never has before in any part of the world for the disease to become established in a Mediterranean climate.

With regard to the EU WG's comments on the climatic issues, this item has been addressed under point 2.1.3 above, and the provision of a definitive climate matching study in the near future is expected to deal conclusively with this issue.

3.3 Conclusion for Stage 2

We acknowledge the EU WG's view, but trust that clarification of various points as provided in this document, as well as further results of current research, will provide the WG with sufficient information to share our view that this conclusion is appropriate. We do further acknowledge that if the current research were to produce results that do not support this conclusion, we would adjust our view accordingly.

3.4.1 Risk management options

(b) We acknowledge this.

(c) (ii) Cost implications and environmental impact. The following section documents these implications as requested by the WG.

Attempting to control CBS at a level that will make it possible to comply with current EU phytosanitary regulations that set a zero tolerance for interception of the organism, results in a far more intensive spray programme than one that is adequate to produce cosmetically acceptable fruit. An example of this is that 4 applications of a dithiocarbamate would usually be adequate to produce cosmetically acceptable fruit, but 7 or 8 applications are required to be able to potentially export to the EU under the current phytosanitary requirements regarding CBS. Such intensified spray programmes, constitute an increase in the growers' spray input costs of approximately 20%.

The dramatically increased pesticide input has unavoidable environmental impact and increases the level of pesticide residues that consumers are exposed to. Likewise, farm workers are subjected to higher pesticide exposure during application of the product through handling, exposure to spray drift, residues in the orchards in which they have to work, and the general environment is exposed to increased pesticide load. Likewise, the prospects of sustainability are impaired by an increase in fungicidal resistance pressure on the organism.

Despite every effort to fully control CBS infections, numerous factors, including weather conditions, often result in only partial control being achieved. The grower is then forced to divert the crop from the lucrative European market to alternative markets. This is done on the basis of a level of infection which is often unnoticeable from a cosmetic quality perspective, but is unmanageable in light of the zero infestation tolerance in the EU. Since approximately 65% of SA's citrus exports go to the EU, diversion of 10% of the crop away from this market, results in an immediate oversupply in the alternative markets with consequent price collapse.

In attempting to comply with a zero interception tolerance in the EU market, orchards that are selected for export to the EU are exposed to dramatically intensified grading scrutiny in the pack houses. This results in all fruit that has any blemish being discarded and reduces packout percentages by up to 25%, with a concomitant reduction in profitability.

Since trees become more susceptible to CBS infection as they age, the longevity of plantings is being reduced in an attempt to minimise the CBS infection levels. Whereas Valencia orchards generally had a 30-year life span in earlier years, with many orchards remaining profitable for 50 years before needing to be replanted, such orchards are now pulled out and replanted after approximately 20 years. Given that a new citrus planting only starts producing reasonable yields after five years of age, with a breakeven return on establishment cost expected at close to ten years, this has a massive impact on profitability.

Considering that the southern African citrus industry derives approximately 90% of its income from export of fresh fruit, that the EU takes approximately 65% of the industry's export volumes, that approximately 80% of the industry's production is based in areas where CBS occurs, and that alternative markets are either saturated or unprofitable, the risk management options being necessitated in an attempt to comply with the EU's current phytosanitary measures threaten to destroy approximately 50% of the current southern African citrus industry. Whereas adoption of a quality standard for CBS infection levels on fresh citrus imported by the EU, as was applied until the late 1990s, would restore the SA citrus industry to a viable and sustainable level of potential profitability.

(c) (iii) Social and economic impacts. The following outlines these impacts, as requested by the WG.

Considering that the SA citrus industry is the country's biggest fresh produce export industry, directly supporting approximately 500 000 employees and

dependants, the well-being of the industry has a major impact on the socio-economic position of the country.

In light of the considerable economic implications of attempting to comply with current EU phytosanitary legislation pertaining to CBS, coupled with the SA citrus industry's critical reliance on the EU market, the social and economic impacts of the estimated 50% downsizing of the industry would be devastating to SA. The socio-economic impact would not only be severe for SA, but for the greater southern African region, considering that significant citrus production is based in surrounding countries and the well being of the greater southern African region is very sensitive to the socio-economic status of SA.

The alternative of implementing a cosmetic quality standard for CBS infection levels, would enable the southern African citrus industry to continue playing the role of a vitally important component of the country's economy.

- (d) EU harmonised protocol for detecting and confirming the presence of *Guignardia citricarpa*: We acknowledge this as a positive movement away from basing identification on symptoms alone. The question still remains, however, as to what constitutes an appropriate action level that will enable risk management procedures to be appropriate to the phytosanitary status of the organism as supported by a PRA based on scientific evidence.

The EU WG's concern regarding a perceived contradiction between the proposed measures and the cost implications of current measures is noted. We trust that the details provided under points 3.4.1. c. (ii) & (iii) will clarify that, although the nature of the spray products does not change, the intensity of the spray programmes (that is, the number of applications per season) is intimately linked with the level of tolerance for CBS infected fruit.

With regard to the EU WG's request for additional data on the efficacy of the post-harvest treatments, we do not believe that there is an opportunity to demonstrate any higher level of efficacy for these treatments than what has already been

demonstrated. We trust that this document will have clarified the issue of control potential of post-harvest treatments and that the WG will be in agreement with us on this point.

C. Concluding remarks

We would like to note that risk mitigation measures included in the PRA are intended to be seen as a sequence of links in a system, rather than as individually 'foolproof' steps. It remains our view that the PRA supports a revision of the current EU phytosanitary measures pertaining to the import of fresh citrus in terms of the IPPC's principles of minimum interference with international trade and of applying the least restrictive measures available – Article VII 2 (a) and (g) of the New Revised Text, respectively.

Most importantly, however, the manner in which the WG responded is appreciated as its sound phytosanitary base provides valuable and clear pointers for the way forward.

Comments by J.M. Kotzé
on
Report of EC WG on Evaluation of the CBS PRA
Prepared by SA

1. **Introduction:** The EC WG is to be thanked for their professional comments.
2. **Comments**

The EC WG believed that a single conidium could initiate infection, and could lead to the eventual establishment of the disease in a citrus producing area. Smith (1996) is quoted. This is an unfortunate misquote. We refer to J.H. Smith, 1996: Proc. Int. Soc. Citriculture 351-352. Mr. Smith reported on his experience as the person in charge of CBS control at Letaba Estates. He was not a research worker and never published supporting evidence for the basis of his lecture. For example, he speculated: "This is probably due to the fact that black spot infections are locally systemic and one infection on a fruit can cause symptoms over a large area of that fruit". This "observation" stands alone and is not supported by refereed published data. To conclude that a single conidium can cause an epidemic situation is simply out of context, but a statement which is taken seriously by the WG. This is a misunderstanding.

Consider another statement in Smith's article: He had a problem in getting correlation between severity of disease incidence and ascospore counts. The reason for this problem is that he used a single spore trap, standing in one position for years. The estates stretch widely and it is well known that ascospores have to be monitored at different heights and several localities. Inoculum directly relates to severity of an epidemic.

Epidemiologists agree that:

$$E = I_0 e^{rt}$$

Where E = Epidemic

I₀ = Inoculum

e = Constant

r = rate of increase

t = time

Referring to the WG's comments, it needs to be emphasized that CBS outbreaks occur when:

1. Infected nursery trees are planted in areas where the climatic conditions are conducive to the completion of the life cycle.

When the leaves from trees, ex infected nurseries, drop and the climate is suitable for the development of perithecia on the orchard floor and ripe spores are released, at a stage when the live leaves and fruits are susceptible, the disease will become established. From this point onwards, the economic losses may be negligible or severe,

depending on the subtropic nature of the climate. If the climate requirements are not suitable, like the Mediterranean climate in the Western Cape, the pathogen does not complete its life cycle, the organism does not become established and the epidemic fails completely.

2. New orchards are exposed to airborne ascospores from nearby infected orchards. When the climate is not conducive to the development of the ascostage the epidemic will fail. This situation does not exist in EU. There are no infected orchards and leaves are not exported to the EU, only fruit.

Infected citrus fruits, fresh or rotten, do not produce airborne ascospores. Fruits only produce pycnidia in CBS lesions. The pycnidia may be empty, or contain spores. The spores are not always viable and need certain conditions to germinate. Pycnidiospores are waterborne – not airborne.

The WG questions whether citrus peel or infected fruit could possibly infect fallen citrus leaves (including lemon) in EU orchards or house gardens. There is no evidence that an isolated tree, away from orchards, will pose a threat. Without ascospores, or the physical introduction of infected trees, there is no chance of the disease establishing.

Referring to Wager (1952) it is pointed out that he found ascospores of Guignardia spp under cool, misty, dry, hot, semi-arid and sub-tropical conditions. Wager and McOnie did not have the benefit of modern technology. Meyer et al (2001) as well as Baayen et al. (2002) proved beyond doubt that Wager confused Guignardia mangiferae, (the universally present endophyte of many plant species), with Guignardia citricarpa, the citrus pathogen. The latter is associated with CBS only and G. mangiferae occurs in Europe, but is not a pathogen on citrus.

With reference to insects (for that matter snails, rats, birds, etc.) we have not experienced such involvement. However, if there should be transmitters of such a nature, they may cause a lesion and may be able to deposit pycnidiospores in the wound. What happens after that, will be dependent on the presence of water (moisture) for a long period of time. If an infection should occur on that fruit or leaf, it cannot play any further role unless climatic factors are highly favourable. Transmitters alone without the climatic conditions to stimulate further development of the pathogen will be insignificant.

It was found by Kotzé (1963) that irrigation (but mostly the lack of irrigation) can influence CBS development where the disease already occurred. Irrigation can cause the release of ascospores. Without wetness however, infection can not take place. Frequent wetting of dead leaves may enhance decomposition of the leaves, preventing the development of perithecia and may stop the progress of the pathogen.

Referring to the WG's question whether fungicidal treatments can permanently ensure full control of the disease, the answer is "no". No control treatment tried so far is 100% effective.

Add to the PRA reference list:

1. Korf, H.J.G., Schutte, G.C. and Kotzé, J.M. 2001. The effect of packhouse procedures on the viability of *Phyllosticta citricarpa*, anamorph of the citrus black spot pathogen. *African Plant Protection* Vol. 7 (2): 103-109.
2. Kotzé, J.M., 1963. *Studies on the black spot disease of citrus caused by Guignardia citricarpa Kiely, with particular reference to its epiphytology and control at Letaba*. DSc thesis, University of Pretoria.
3. Meyer, L., Slippers, B., Korsten, L., Kotzé, J.M., Wingfield, M.J. 2001. Two distinct *Guignardia* species associated with citrus in South Africa. *South African Journal of Science* 97 (5-6): 191-194.
4. Baayen R. P., Bonants, P. J. M., Verkley, G., Carroll, G. C., van der Aa, H. de Weerd, A. M., van Brouwershaven, I. R., Schutte, G. C., Maccheroni, W. Jr., Glienke de Blanco, C. and Azevedo, J. L. 2002. Nonpathogenic Isolates of the Citrus Black Spot Fungus, *Guignardia citricarpa*, Identified as a Cosmopolitan Endophyte of Woody Plants, *G. mangiferae* (*Phyllosticta capitalensis*). *Phytopathology* 92 (5): 464-477.

PARTICIPANTS:
WORKSHOP HELD TO DISCUSS SA NPPO RESPONSE
TO EC WG REPORT ON PRA DOCUMENT,
HELD ON 4 FEBRUARY 2002 AT 10:00 IN ROOM 343, DIRK UYS
BUILDING, HAMILTON STREET, PRETORIA

Main organisations represented:

- National Department of Agriculture Directorate Plant Health & Quality (NDA DPHQ = SA's NPPO)
- Citrus Research International: co-ordination of southern African citrus research, with major alliance partners being CRI (Pty) Ltd, University of Pretoria, University of Stellenbosch, Institute of Tropical and Subtropical Crops (Agricultural Research Council), citrus consultants association and Fresh Produce Exporters Forum.
- University of Pretoria (CBS research team)
- South African Citrus Growers' Association (SA CGA)
- Fresh Produce Exporters' Forum (FPEF)

(The academics and technical field specialists that made inputs, collectively had 173 years of practical experience with CBS!)

Name of participant	Organisation represented	Position / Capacity (noted where relevant)
Mr M. Holtzhausen	NDA-DPHQ	Deputy Director: Plant Health (NPPO) - Chairperson
Ms M. Nell	NDA-DPHQ	Workshop secretariat
Ms A. Baxter	NDA-DPHQ	NPPO representative
Ms C. Hattingh	NDA-DPHQ	NPPO representative
Ms J. Moen	NDA-DPHQ	PRA group
Dr GC Schutte	CRI	CBS researcher, with 11 years of CBS research experience
Mr H.F. Engelbrecht	Consultant	Technical specialist
Mr D.C. Lötter	CRI	Citrus producer
Mr L.A. Grey	CGA	Citrus producer
Mr J. Chadwick	CGA	CEO
Prof J.M. Kotzé	Consultant	Academic specialist, with 45 years of CBS research experience

Mr S. Symington	FPEF	CEO
Dr C.R. Kellerman	Consultant	Technical field specialist, with 34 years of CBS research and control experience
Dr H.F. le Roux	CRI	Technical field specialist, with 19 years of citrus disease management experience
Dr S. H. Swart	Consultant	Technical field specialist, with 13 years of CBS research and control experience
Dr V. Hattingh	CRI	Technical field specialist, General Manager of citrus industry-wide research partnership.
Prof F.C. Wehner	Univ. of Pretoria	Academic specialist, with 12 years of citrus plant pathology research experience.
Mr H.J.G. Korf	BASF	CBS researcher, with 10 years of CBS research and control experience.

Additional Academic and Technical experts consulted

Name of participant	Organisation represented	Position / capacity
Dr J Moll	CRI	Chairman of Disease Management Research Programme, with 27 years of citrus pathology experience
Mr GP Smith	Consultant	Technical field specialist with 36 years of citrus disease control experience



EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL
Directorate E – Safety of the food chain
E1 – Biotechnology and Plant health

**REPORT OF THE COMMISSION WORKING GROUP ON EVALUATION OF THE
PEST RISK ASSESSMENT PREPARED BY SOUTH AFRICA
ON CITRUS BLACK SPOT (CAUSED BY *Guignardia citricarpa* KIELY)**

BRUSSELS, 15-16 JUNE 2006

1. INTRODUCTION

The Pest Risk Assessment (PRA) prepared by South Africa (SA) on citrus black spot (CBS) has been firstly analysed by the European Commission working group (WG) in October 2001; the report thereof was sent to the South African authorities in December 2001. SA responded by providing three documents – general response¹ in September 2002; additional data on mapping the potential distribution of CBS² in December 2003 and, finally, further data on research of potential transmission of CBS from fruit to leaf litter³ in July 2004. These documents were in the focus of the second meeting of the WG, which took place on 15-16 June 2006 in Brussels.

2. COMMENTS OF THE WG ON THE DOCUMENTS RECEIVED FROM SOUTH AFRICA IN 2002-2004

In procedural terms the WG agreed to follow the previous approach and to present its comments according to the structure of the general response¹ supplied by SA and the corresponding structure of the PRA document.

Any reference to or quotation of the SA documents is made in *Italics*.

¹ *Response from South Africa on the Report (dated 24/10/2001) of the EC Working Group (WG) relating to the WG's evaluation of the Pest Risk Assessment (PRA) by South Africa on Citrus Black Spot (CBS) – 2 September 2002 (EC ref.: A/510162 of 09/09/2002)*

² *Further Data Relating to the PRA for Citrus Black Spot and the Export of Fresh Citrus Fruit from South Africa to the EU – 8 September 2003 (EC ref.: A/510132 of 21/01/2004)*

³ *Further Data Relating to the PRA for Citrus Black Spot and the Export of Fresh Citrus Fruit from South Africa to the EU – Research Report on Potential Transmission from Fruit to Leaf litter – 19 July 2004 (EC ref.: A/510854 of 26/07/2004)*

B. Comments and responses

2. Background information

2.1. Epidemiology

2.1.1

The WG confirmed that its comments chiefly relate to the establishment of the disease in an area in which it was previously known not to occur. Although it is still believed possible that a single conidium, under favourable conditions, could initiate infection and disease development on individual trees and that this could ultimately lead to the eventual establishment of the disease in a citrus producing area, the WG accepted the related Kotzé's comments attached to the SA response, as this argument is considered to be rather academic/theoretic.

The WG concurred that in places where CBS is endemic, ascospores are generally more important than pycnidiospores. Ascospores are wind-borne and are more efficient in spreading the disease within the field than the water-splashed pycnidiospores.

However, the point to discuss is the likelihood of establishment of the disease after an entry into a disease-free area. If CBS infected material is accidentally placed in an orchard, the distance to fruits or leaves on the canopy would be very short and both types of spores will be equally important. After the first establishment, the economic impact of the disease would nevertheless depend on the ability of the pathogen to spread, and then, ascospores would play the main role.

The WG therefore did insist that introduction of CBS via the pycnidiospores might be as important as the role of ascospores at the beginning of the establishment of the disease in an area. The WG believes that pycnidiospores of CBS may play a role in the disease cycle similar to that in cycles of other pycnidiospore-producing fungi. Kotzé's remarks on CBS epidemiology that (i) infection may come from both pycnidiospores and ascospores and, once epidemic proportions have been reached, the importance of pycnidiospores is usually eclipsed by that of ascospores, and (ii) ascospores are produced on the dead leaves on the orchard floor and represent the main source of inoculum once CBS has reached the epidemic stage (Kotzé, 1981 and 1996) are confirmed by various publications. According to Garrán (1996), although the fungus was isolated from symptomatic fruit during the first years (1991-1994) of establishment of CBS in Tucumán, the most important citrus growing region in Argentina, attempts to detect the sexual stage by weekly samplings of dead leaves were unsuccessful. Experimental results of the spatial distribution and aggregation of symptomatic fruits within the tree also lead Spósito (2003) to conclude that besides the ascospores, the conidia (pycnidiospores) might be also important in the CBS epidemiology in Brazil.

The WG believes that the establishment and further development of CBS in a disease-free area depends not on a myriad of possible factors but, as it happens with other fungal diseases, on very specific ones, such as the availability of inoculum, the presence of host(s) at a susceptible stage of growth and the prevalence of favourable climatic conditions (temperature, high relative humidity or leaf wetness). At this point the WG also noted the results of Noronha (2002) according to which germination of *G. citricarpa* conidia and appressoria formation take place under relatively broad temperature/wetness conditions.

2.1.2

In respect of the information provided in the paper of Truter, Labuschagne and Korsten entitled: “Evaluating the colonisation potential of pycnidiospores from *G. citricarpa* infected citrus fruits to leaf litter”, the WG observed the following drawbacks:

- A. The part ‘*Materials and methods*’ as a whole lacks appropriate level of detail. More specifically:
- No information is given under *Materials and methods-Convion evaluation* on (i) the number of green, yellow and brown leaves sampled, (ii) the number of Valencia orange fruit with red, hard and virulent spots sampled, and (iv) the criteria used for selecting the experimental material (fruit, leaves) for isolating *G. citricarpa* and confirm its presence or absence by PCR.
 - More replications in Convion and field experiments would be desirable.
 - An accurate identification of the ‘*Phomopsis-like fungus fruiting bodies and pycnidiospores*’ should be provided.
 - Given the long period for ascocarps to develop on leaf litter [50-180 days according to Kotzé (1981)], the incubation period of 2 months (60 days) was considered to be short for providing conclusive evidence;
 - The use of tap water for spraying the grids in Convion evaluation was considered as inappropriate, since the pycnidiospores may be affected by the presence of chlorine and by the pH of tap water;
 - There is no evidence of an effective contact between pycnidiospores and leaves in any of both experiments. The use of a spore suspension with a known concentration and viability would be desirable.
 - No meteorological data and information on the cultural practices, especially irrigation (frequency, system used, etc) were provided in the field evaluation. Thus, there is no evidence whether the alternate wetting periods needed for ascocarp formation were accomplished.
- B. Furthermore, the WG noted that some points under “Discussion” need further clarification:
- Published scientific references are needed to support the statements of the third paragraph.
 - WG agreed with the statement that water-borne pycnidiospores are not as important as air-borne ascospores in the dissemination of the pathogen when disease reaches an epidemic. However, the WG noted that both spores may be equally important for the establishment of the disease in a new area (Kotzé, 1981).
 - No reference is given on the strict packinghouse procedures or the existing regulations that reduce the chance that fruit with CBS symptoms will be exported. Interceptions of CBS infected citrus fruit in the EU Member States show that either these procedures are not effective or that they are not followed by all packinghouses.
 - WG agrees that treatments in the packinghouse may reduce the survival of the exposed to the chemicals pycnidiospores, but not of those within the pycnidia.
- Finally, the WG was of a general view that major improvements would be necessary for the paper to provide fully convincing, scientifically sound evidence.

Furthermore, the WG suggested that the colonisation potential of pycnidiospores from *G. citricarpa* infected citrus fruits and/or peel to attached leaves and fruits may be different

from that to leaf litter. Although it was recognized that there is a gap in research in this respect, the possibility of the pycnidiospores being splashed upward from infected fruit and/or peel on orchard floor onto the lower reaches of trees was not at all regarded as remote. Figures 1 and 2 show the growth habit of citrus trees in some of the Mediterranean orchards.



Fig.1



Fig.2

Young leaves and fruits are distributed not only on the higher reaches of but also within the whole canopy of the tree. The possibility of splashing of pycnidiospores upwards and subsequent infection of young leaves and fruit by CBS seems therefore very likely, as it happens i.e. in the case of some *Phytophthora*-induced diseases in *Citrus*, where inoculum (sporangia and zoospores) can be splashed up from the soil surface to the canopy, even to the height of 1-1.5 m (Erwin and Ribeiro, 1996; Graham *et al.*, 1998 and 2000). While the WG could agree on the characteristic of pycnidiospores as 'short-lived if exposed' from pycnidia, it strongly opposed the way in which it is put into the context of the SA response. Pycnidia carry viable pycnidiospores for longer period, even up to 4 months (Wager, 1949), which is also proved by their detection in CBS infected fruits intercepted at import into the EU. In respect of the *specific stimuli* required for conidia germination, the WG repeated that lemon trees are omnipresent in the whole Mediterranean area, widely grown not only in production orchards, but in private gardens and public greenery as well. On account of the *specific stimuli*, no reference is given and furthermore the WG wondered whether its presence is actually necessary under field conditions, since Kiely (1948) demonstrated that pycnidiospores are pathogenic to sweet orange fruits by just spraying a suspension of inoculum in water. Thus, it seems that these stimuli are not a limiting factor for pycnidiospore infection.

The WG noted the SA comment on the role of insects as potential agents of transmission.

2.1.3 – 2.1.5

In respect of information provided by SA on modelling of the potential distribution of CBS, the WG considered also two later versions of received paper (Paul *et al.*, 2005; Paul, 2005). While the WG concurred in the principal role of wetness for development of the disease, it wondered if considering only the rainfall as source of wetness was appropriate. In the WG view it was rather leaf wetness, induced also by dews and/or sprinkler irrigation, and its correlation with temperature, which was viewed as the crucial climatic variable. The WG stressed that no data have validated so far the exclusive significance of rainfall or irrigation for the development of CBS.

The WG recognized the climate comparison as the most common approach to assess the potential of establishment of non-indigenous species in an area. It agreed with Baker *et*

al. (2005) that if the area of origin and the host plants are known, climate in the area of origin can be compared with climate in the endangered area and the potential distribution of non-indigenous species can be determined. It pointed out, however, that over reliance on climatic matching techniques, when the underlying data are insufficient, may lead to underestimation of the risk of establishment of the target organism. The WG agreed that there is a need for better understanding of the environment at the crop level in order to assemble more reliable PRA on organisms like *G. citricarpa*.

It was observed, that in climate matching studies accurate predictions can generally only be made by selecting climatic variables and their thresholds according to biological responses which have been determined previously. When model parameters and conclusions are inferred from the known distribution of a disease, as it was done in the work of Paul *et al.* (2005, also Paul, 2005), it is uncertain whether the geographical distribution of the pathogen is an expression of its climatic tolerance, or rather an expression of whether it has had time to spread and adapt. It was also noted, that risk analysis for non-indigenous diseases requires the availability of as much information as possible on the organism and its interactions with the environment. The interpretation of these relationships should be based on data from field research or controlled environment experiments. The predictive ability of empirical models, developed from field studies, is limited by the scope of data, and usually they are not considered suitable for geographical extrapolation (Teng and Yang, 1993). On the other hand, with assessments in containment facilities, it is possible to quantify the effect of a broad range of environmental conditions on each component of the disease cycle. However, epidemics are usually more complex than a linear combination of its monocyclic components and this kind of models sometimes fails to mimic the disease development under field conditions. Therefore, most risk analysts stress the use of both, field and controlled environment experiments, to produce reliable risk assessments (Yang *et al.*, 1991).

It was further noted, that another source of uncertainty in climate matching studies is the lack of knowledge about the environmental conditions of the area under study. Even in areas where climate is known, the environmental factors selected and the methods used for their measurement need to be relevant to the main biological processes of the species concerned. For many foliar pathogens, the infection phase is one of the most critical components for disease development and it is highly dependant on temperature and wetness duration on plant surfaces (Magarey *et al.*, 2005). Monthly cumulative rainfall and average temperature gathered from weather stations have been commonly used as criteria in the climate matching studies. Canopy microclimate may, however, greatly modify the environmental conditions compared with a turf surface where most of the standard weather station measurements are made (Magarey *et al.*, 2001). This difference is more considerable in semi-arid areas, where canopy microclimate can provide favourable conditions for disease development even though the macroclimate is unfavourable (Palti and Rotem, 1973). This might be the reason why CLIMEX failed to predict Karnal bunt (*Tilletia indica* Mitra) in several regions where the disease is endemic (Royer, 1990). Results of field studies, which have recently been obtained in Spain (Vicent, in prep.⁴), clearly show that long-term average rainfall data are not a good indicator of the citrus canopy wetness in any of the experimental locations; despite the

⁴ See also Annex: Vicent, A.: Relationship between Environmental Variables and the Risk of Establishment of *Guignardia citricarpa* in Spain (not published yet, but authorized by A. Vicent)

lack of rain, dew periods on summer nights occur at temperatures over 15°C and even over 20°C, thus creating conditions similar to those in Brazil (Vicent, in prep.). The citrus-producing orchards in Spain are mainly located in river/creek valleys, generally in coastal areas, in which the temperature and humidity during the growth of the fruits (May – September) are above 24°C and 70% (during evening and night), respectively. Also autumn and winter temperatures, which are normally 16 – 20 °C, with lowest above 8 – 10 °C, are considered as providing suitable microclimate for the fungus to remain alive and active throughout the whole year (J.J.Tuset, personal communication).

In Greece, a total of 120,000 acres are grown with *Citrus* (mainly lemon, orange and mandarines). The main citrus-producing areas are located near the coast in the Northernwestern Greece (Hipiros Prefecture, 20% of the total area), Southwestern, Southern, Eastern and Northern Peloponnese (Ahaia, Helia, Lakonia, Messinia, Argolida and Korinthia Prefectures, 65% of the total area) and in the island of Crete (Southern Greece, 10% of the total area). The climatic conditions are similar to those in the Spanish citrus-producing areas: the average temperatures during the critical period [from May (fruit set) till the end of October-beginning of November, depending on the citrus species (fruit colour change)] are 19-31°C and the relative humidity (RH) is higher than 65%. During the cold period (November-April), the average temperatures do not fall below 9°C for most of these areas.

Many fungal pathogens that are widespread in the Mediterranean basin, such as the citrus brown spot pathogen *Alternaria alternata* (Fr:Fr) Keissl., are able to complete their infection cycle within the available dew periods. Under these conditions, successful infection requires either rapid germination and penetration or ability of the germinating spores to survive dry periods and resume growth when rewet (Bashi and Rotem, 1974). Some fungal pathogens can even continue the germination process during the dry period if the relative humidity is high (Schuh, 1993). Optimal conditions for germination of ascospores of *G. citricarpa* are 24°C and 16 hours of wetting period, but germination can occur from 15 to 33 °C (Timossi *et al.*, 2003). For conidial germination and appressoria formation *G. citricarpa* needs 12 to 48 hours of wetness and 10 to 40 °C (Noronha, 2002). According to Kiely's (1948) results, daily wetting of fallen leaves for half an hour is enough for ascospore maturation. He stated that both rain or nightly dew can provide the wetting periods necessary for ascospore development. These results, as well as Kotzé's statement (1981) that ascocarp development on leaf litter depends on intermittent wetting and drying, have been supported by recent personal communication of P.Barkley with J.P. Thermo on the importance of alternate wetting and drying of dead leaves for securing ripe perithecia. The WG noted, though, that no data are currently available on the effect of short or interrupted wetting periods on the germination of *G. citricarpa* conidia and appressoria formation.

In contrast to Paul *et al.* (2005, and Paul, 2005) reports on unsuitability of the New Zealand's climate for the establishment of CBS, the WG noted the remark of Everett & Rees-George (2006) on the suitability of climatic conditions (mainly temperature and wetness) of New Zealand for the establishment and spread of *G. citricarpa*.

Furthermore, the statement by Paul *et al.* (2005) that *citrus is not cultivated neither in Napoli nor in Genova* (Campania, resp. Liguria regions in Italy), *as these are the only sites on the European mainland with EI value greater than four*, was considered by the WG as erroneous since, according to the Eurostat figures, the area of lemon orchards in these two Italian regions amounted almost to 7% of the total lemon orchards area in Italy in 2002 (see Table 1.). It was also observed that substantial area of these orchards, in

particular in Napoli region, is planted with trees older than 25 years and according to Kotzé (1981) the disease is more severe on older trees.

Table 1

Lemon tree orchards in Italy in 2002 (area in hectares)			
Age	Italy	Liguria	Campania
Total	17619,82	29,75	1166,39
25-39 years	6610,61	2,35	315,77
> 40 years	4305,72	0,35	451,80

Source: Eurostat, 2006

The WG also noted that the climatic data for Greece, used by Paul *et al.* (2005) in the CLIMEX model, were extracted from 20 meteorological stations (data are available at the web, <http://metart.fao.org>) the majority of which (15 stations) are not situated near the main citrus-producing areas. Moreover, although Kerkyra (Corfu), with an EI=5 according to the CLIMEX model, is an island, it is in close proximity to the mainland, with 1,300 acres grown with *Citrus*. In addition, there are citrus trees (most of them are lemon trees) grown as ornamentals in private or public gardens throughout the island.

The WG finally supported opinion of Worner (1988, secondary source Paul *et al.*, 2005), that output of the CLIMEX analysis should be interpreted with caution and that knowledge on competition from other species and human influences (such as effective control methods and irrigation) on the environment where CBS may occur should also be considered in decision-making processes.

In relation to the SA statement that *apart from climate there is no other reasonable explanation available as to why the West Cape province winter rainfall area of SA has remained free from CBS*, the WG wondered if studies on possible effect of low temperatures or dynamics in daily/annual wetness of canopy could shed more light on this situation. The WG also noted, in this respect, the current SA internal legal basis on CBS, in particular the Amendment No.R.1223 of the control measures laid down by the Agricultural Pets Act No.36 of 1983, by which restrictions are put in place regarding movement of the *Citrus* plants within SA. The WG wonders, if this protection of the Western-Cape Province could not be such an additional reasonable explanation. The territorial delimitation by the Amendment No.R.1223 of areas in SA from which the movements of *Citrus* plants are prohibited moreover suggests that the recent limits of occurrence of CBS reached further to the west, compared to Paul (2005). If this was the case, and CBS occurred in e.g. Calitzdorp, Laingsburg and Riversdale (see Fig.3), i.e. areas with similar climatic conditions to Ladismith or Heidelberg, we would conclude that the situation of CBS in SA proves that the disease could be a threat for regions with Mediterranean climates.

On the basis of Figure 39 : The current occurrence of Citrus Black Spot in South Africa (Paul I., 2003)

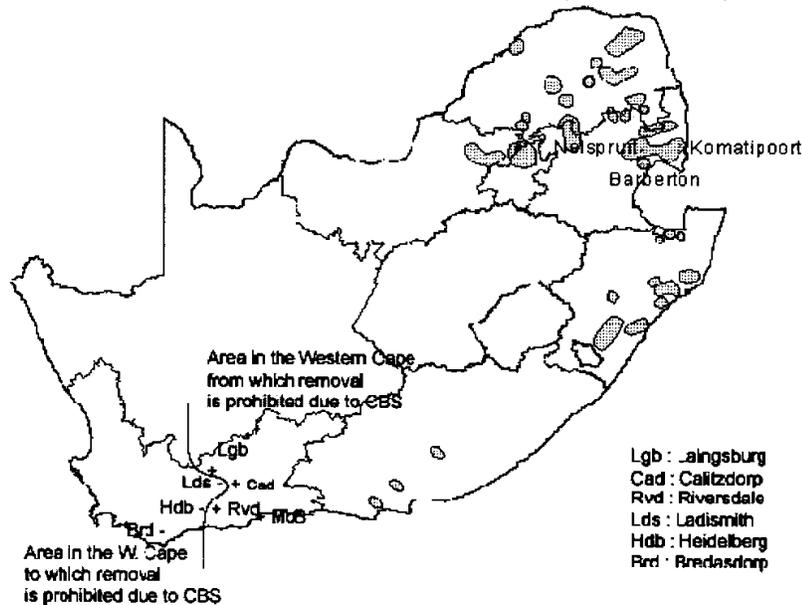


Fig. 3

The WG agreed with the SA comments regarding the reference to Wager (1952) and the later results of McOnie (1964, 1965), Meyer *et al.* (2001) and Baayen *et al.* (2002) as far as *G. citricarpa* and the cosmopolitan, endophyte, not pathogenic to *Citrus*, *G. mangiferae* are concerned.

WG also noted the SA response in relation to the possible role of the sexual stage of *G. citricarpa*. The WG insisted, however, that this factor should be considered as supplementary element in completing the pathogen's profile when the related risks are analysed, as it could have been because of its sexual stage that *G. citricarpa* developed resistance to benzimidazoles in the beginning of eighties (Kotzé, 1981) much faster than expected (Smith, 1996; Schutte *et al.*, 2003).

The comment, that 20 million tons of citrus fruits have been exported from SA to the EU over the past 75 years (i.e. since 1927) and the following assumption of considerable quantities of the imported fruit that have found their way into citrus producing regions in Europe over the years, thus exposing the PRA area to CBS infected fruit for many years, was considered by the WG as highly speculative and incorrect. It was only in 1993, with introduction of the EU internal market, when the Mediterranean EU Member States lifted their national boundaries and started to apply the harmonized phytosanitary requirements for trade and intra-EU movements of citrus fruits (Anonymous, 1992). Import of citrus fruit prior to 1993 was in fact prohibited, either on the basis of the national phytosanitary legislation before the accession of the respective countries to the EU (Greece in 1981; Spain⁵ and Portugal in 1986) or on the basis of the Council Directive No.77/93/EEC (Anonymous, 1976) afterwards.

⁵ The import of citrus fruits was banned in Spain in 1934.(O.M.14-08-1934.Gaceta de Madrid 228:1526).

The first import into Greece of citrus fruit from SA and Argentina was in 1999 (105 tones of lemons from SA and 5,531 tones of lemons and 30 tones of grapefruit from Argentina) according to the data provided by the Department of Rural Statistics, Directorate of Rural Policy and Documentation, Greek Ministry of Rural Development and Food.

Based on the propagule pressure theory described by Williamson (1999), the chance of invasion is a function of the number of individuals initially introduced in an area. The probability of CBS infected fruit to enter the EU area depends on the number of imports from a region where CBS is known to occur, the proportion of those imported goods that are contaminated and the proportion not intercepted by quarantine inspection. The WG therefore considered the time since 1993 as short for accepting the SA argument.

2.2. *New information*

The WG took cognisance of the publication of the paper by Korf *et al.* (2001). It also concurred *that presence of mycelium after post-harvest treatment of fruits is a product of an earlier infection and that the mycelium itself is not infective*. The WG nevertheless stressed that pycnidia and pycnidiospores could be produced by the latently present mycelium. As pycnidiospores are pathogenic (Kiely, 1948) they can cause an infection in a new area. Moreover, according to Agostini *et al.* (2006), *G. citricarpa* remains viable in fruit receiving a wide range of post-harvest treatments. Although the viability declined with time, the fungus was still recoverable until the fruit was completely decomposed by other organisms.

The WG also acknowledged the congruity of the SA response with the WG report of 2001 regarding the post-harvest treatments and packhouse procedures having only a suppressive and not an eradicated effect on the CBS pathogen, and the difference in survival between pycnidiospores extruded from pycnidia (exposed pycnidiospores) and those within maturing pycnidia. The WG wished to underline this difference also in relation to the length of presence of infective pycnidiospores within pycnidia on the citrus fruit peel and point out that Kiely (1948) and Wager (1949) reported on survival of pycnidiospores taken from CBS lesions.

The SA response concerning the field treatments was noted by the WG as it was that, based on Schutte *et al.* (2003) results, the strobilurin fungicide, azoxystrobin is now registered to be used in tank mixtures with a contact fungicide (i.e. mancozeb) for CBS control in South Africa, especially for the control of benomyl-resistant strains of the pathogen.

2.3. *Specific risk mitigation considerations*

The WG took note of the respective SA response, and of the report from Prof. Kotzé attached thereto concerning the non-occurrence of perithecia on infected fruit or fruit peel, in acknowledgement of his long-lasting experience with CBS. It was noted, though, that explicit data excluding such a possibility are not available.

Further scientifically sound evidence is needed on the period that pycnidiospores survive within the pycnidia formed on fruit lesions and the factors affecting this survival, as according to Wager (1949), pycnidiospores survive up to 4 months on infected citrus peels, even on mummified and dry ones.

3. Pest Risk Analysis

3.2.2. Economic importance criteria

As explained above, the WG kept its earlier opinion that it has not been completely proven that CBS cannot be established in the PRA area. On contrary, it was observed that the results of recent research warrant cautious approach.

3.2.3. Introduction potential

A. Entry

A.1. The following table demonstrates the incidence of interceptions of consignments of citrus fruits from SA infected by *G. citricarpa* at import into the EU Member States since 2003 (Tab.2).

Table 2: Import of citrus fruits and interceptions of consignments with CBS at import from South Africa into the EU Member States (in metric tons)

Country of origin: South Africa	2006 (I-VIII)		2005		2004		2003	
	Import (t)	Intercepted (t)	Import (t)	Intercepted (t)	Import (t)	Intercepted (t)	Import (t)	Intercepted (t)
CY	0	0	0	0	0	0	0	0
EL	1943	0	2720	0	13787	0	5292	0
ES	19376	0	65492	47	65924	38	54879	0
FR	7522	0	8935	0	15156	0	11017	0
IT	19885	0	50221	0	39990	0	38880	0
MT	345	0	543	0	658	0	1788	0
PT	561	0	2445	0	1208	0	678	0
Other EU MS	74686 ^x	769	396276	1942	261453	1519	351267	44
Total	n.a.	769 [*]	534051	1989 ^{**}	401995	1557 ^{***}	467608	44 ^{****}

Source: Eurostat, 2006; Europhyt, 2006

^x January – June (I-VI)

^{xx} January – October (I-X)

According to Europhyt notifications:

^{*} 14 consignments intercepted (I-X) (NL – 13, UK – 1)

^{**} 23 consignments intercepted (NL – 20, SI – 2, ES – 1)

^{***} 21 consignments intercepted (NL – 15, SI – 2, ES – 2, BE – 1, DE – 1)

^{****} 5 consignments intercepted (NL – 4, BE – 1)

- A.2. The WG took a note of the SA response.
- A.3. The WG disputed the SA statement that the fact that pycnidia may be formed in the EU on fruits imported from SA does not pose a threat to the EU citrus industry.

B. Establishment

- B.3. The WG viewed the possibility of the imported citrus fruit or its peel being placed below or nearby a citrus tree in the PRA risk area as probable, since, as stated above, citrus and especially lemon trees are omnipresent in the Mediterranean EU Member States.
- B.4. The WG wished to restate its previous comment on this point.⁶ In addition it also wished to refer to the above comments on the role of leaf wetness.

In the light of the above submitted comments, the WG did not consider the amended last sentence of par.B.4 as the correct depiction of the risk of CBS introduction from SA to the Mediterranean EU Member States.

The WG was of the opinion that the research results of Garrán (1996) and Spósito (2003) suggest that our understanding of *G. citricarpa* is non-exhaustive and incomplete, and that the fungus could behave differently from prior existing and available pieces of knowledge.

3.3. Conclusion for Stage 2

The WG took note of the SA standpoint. It believed, however, that above information would provide the SA with an incentive to adjust their view.

3.4.1. Risk management options

The WG acknowledged the additional information provided on points (c)(ii) and (c)(iii). Nevertheless, as *G. citricarpa* is regarded as quarantine pest for the EU and also listed as such, zero import tolerance cannot be withdrawn.

The WG wondered, if in relation to pesticide field treatments, azoxystrobin in mixture with contact fungicides such as mancozeb and mineral oil or another adjuvant could bring about more effective and environmental friendly, less costly and less intensity-demanding solution, as suggested by Schutte *et al.* (2003).

With regard to the post-harvest treatment, the WG accepted the SA response, as long as this treatment is seen as suppressive, not eradication management tool.

⁶ *The various climatic conditions prevailing in the EU citrus producing areas cannot be only characterised as a dry summer, especially over such a long period from April to September. Warm, humid conditions are normal in spring and autumn in various citrus producing regions in the EU. Additionally, irrigation is a key factor for modifying the micro-climatic environment in Citrus orchards. Moreover, susceptible Citrus fruits are present over a long period of the year, due to the diversity of climates combined with the variety of species and cultivars grown in Europe. Several European climates would fall in the range for establishment of CBS given by Lee and Huang (1973).*

3. CONCLUSIONS OF THE WG

In general, the WG highly appreciated the professional work done by SA, for which it expressed its words of thanks.

The WG regarded the spread of CBS by pycnidia on fruits and the establishment of CBS under Mediterranean climate as probable, both elements being supported by results of the recent research.

The WG questioned the approach followed by Paul *et al.* (2005) in using the CLIMEX model for mapping the potential geographical distribution of *G. citricarpa*, whereby the model parameters are inferred from the known distribution of the disease, rather than clearly related to the biology of the pathogen. As recent data from Spain show that long-term average rainfall figures are not a good indicator of the citrus canopy wetness, the WG recognizes a strong need for more site-specific climatic variables, particularly of leaf wetness in correlation with temperature, to be used and analysed.

The WG acknowledged that further research is desirable on more specific microclimatic matching studies between CBS-free areas and areas where it occurs, as well as on the effect of interrupted leaf wetness under variable temperature conditions on ascospore and pycnidia infection.

The WG concluded that the current EU vigilant approach is justified in order not to repeat some costly mistakes from the past, according to Kotzé (1981), when too much hope was pinned on climate as a limiting factor.

4. FOLLOW-UP OF THE WG

After informing the Standing Committee on Plant Health on the outcome of the WG meeting, the final report thereof is to be forwarded to the South African authorities.

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ANNEX**Relationship between Environmental Variables and the Risk of Establishment of *Guignardia citricarpa* in Spain**

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INTRODUCTION

Citrus black spot (CBS) caused by the fungus *Guignardia citricarpa* Kiely [anamorph *Phyllosticta citricarpa* (McAlpine) van der Aa] is a severe disease of fruit and foliage present in several citrus-growing areas in Africa, Asia, Australia and South America (Peres and Timmer, 2003). With the exception of sour orange (*Citrus aurantium* L.) and Tahiti limes [*C. latifolia* (Yu. Tanaka) Tanaka], almost all *Citrus* spp. are susceptible to the disease. *G. citricarpa* causes different types of symptoms on the fruit rind and reducing its value for the fresh market. Premature fruit drop due to black spot causes significant yield losses in Brazil, and probably in other citrus regions of the world (Reis *et al.*, 2006).

Exclusion of inoculum is by far the most efficient control measure in areas where a disease is not yet present. Thus, the primary method of protection established by many countries has been the development of quarantine protocols to limit entry of foreign diseases on imported agricultural goods. The European Union (EU) Council Directive 2000/29 considers *G. citricarpa* as a harmful quarantine organism. Consequently, a set of phytosanitary measures has been established against its introduction. Fruits for export should be produced in CBS free areas or from CBS free production sites confirmed by official surveys. When orchards are located in CBS free production sites, they must be subjected to appropriate fungicidal treatments. Finally, shipments should comply with a zero interception tolerance in the EU border inspections.

In order to avoid unnecessary barriers to international trade, the International Plant Protection Convention (IPPC) and the World Trade Organization (WTO) stipulate that a pest can only be given quarantine status and measures imposed if it is able to enter, establish and cause significant and unacceptable economic, environmental or social impacts in the area under consideration on the basis of scientific pest risk assessment (PRA) (Anonymous, 1993 and 1994).

Standardized guidelines for PRAs have been developed by the IPPC and have been adopted by the European and Mediterranean Plant Protection Organization (Anonymous, 1995 and 1997). Analytically, these schemes utilize qualitative or semi-quantitative approaches, such as scoring index systems. However, due to the economic impact associated with invasive species and their phytosanitary regulations, there is an increasing demand for PRA to be more quantitative. A quantitative PRA should model the components of the risk problem and describe quantitatively their inherent uncertainties (Rafoss, 2003). Nowadays, Monte-Carlo simulation is the standard approach for conducting quantitative risk analysis (Vose, 2000; Stansbury *et al.*, 2002). When the potential pathways of introduction are identified, it is possible to quantify the risks and probabilities associated with the pathogen entry and establishment. Parameters necessary to simulate the probability of entry, such as the proportion of commodities infected or proportion missed by quarantine can be obtained from the Plant Health Authorities. However, to estimate the probability of pathogen establishment and spread it is necessary to have a full knowledge of its biology and its interactions with the environment.

Climate comparison has been the most common approach to assess the potential for establishment of non-indigenous species. If the area of origin and the host plants are known, climates in the area

of origin can be compared with climates in the area under threat and the distribution of host plants can be determined (Baker *et al.*, 2005). With regard to *G. citricarpa*, a recent study conducted by South African scientists concluded that there is no risk of introducing CBS into the EU territory because the climate in the Mediterranean basin is unsuitable for its establishment (Paul *et al.*, 2005). Thus, phytosanitary regulations of the EU can be considered as protectionist and unjustified.

However, reliance to climate matching techniques only might lead to underestimation of the risk of establishment of the target organism in an area, especially when the underlying data are insufficient. Therefore, in order to assemble a more reliable PRA on *G. citricarpa* for the Mediterranean basin, a better understanding of the environment at crop level is needed. In the present work, macroclimatic and microclimatic environmental variables were obtained from several orchards located in the main Spanish citrus-growing areas and from meteorological databases. Needs for further studies on the epidemiology of CBS are also highlighted.

MATERIALS AND METHODS

Meteorological databases

Climographs were obtained for the CBS pathogen, *G. citricarpa*, based on thirty-year average monthly temperature and rainfall data from several weather stations. Cape Town (South Africa), Valencia and Huelva (Spain) were considered CBS-free areas and Nelspruit (South Africa) and São Paulo (Brazil) were considered locations where the disease is endemic. Climate data were obtained from the following sources: South African Weather Service (1961-1990) for Cape Town and Nelspruit, Instituto Nacional de Meteorologia, Brazil (1961-1990) for São Paulo and Instituto Nacional de Meteorologia, Spain (1971-2000) for Huelva and Valencia. Graphs were drawn with 6-month displacements of calendar months to ensure that climatic seasons in northern and southern hemispheres coincide. Additionally, climatic classifications were obtained for all locations following the Köppen climate system (Köppen, 1931).

Field data

The studies were carried out in three commercial “Fortune” mandarin (*C. reticulata* Blanco cv. Dancy x *C. reticulata* cv. Clementine) groves in Spain. Two of the orchards were located in Chiva (Valencia province) and the third in Gibrleon (Huelva province). In the first two orchards, the trees, grafted on “Carrizo” citrange rootstock (*Poncirus trifoliata* (L.) Raf. x *C. sinensis* (L.) Osbeck), were planted in 1995 on a 6 by 2.5 m spacing. In the third orchard, the trees, grafted on Volkamer lemon (*C. volkameriana* Ten. & Pasq.), were planted in 1994 on a 7 by 3 m spacing. All orchards were drip-irrigated and the rows had a north to the south direction. Crop management, fertilization and pest control followed recommended practices (Anonymous, 2004). Trees were pruned each year and canopy size remained constant during the period of study.

Temperature, wetness, and rainfall were monitored with an automated weather station (Watch Dog Plant Disease Station. Spectrum Technologies Inc., ILL, USA). Environmental monitors were located between two trees in the central row of each orchard. Data were collected at about 2 m above the soil surface. Electronic wetness sensors were placed with a northerly exposure and fixed at a 30-degree angle from the horizontal. Wetness threshold was adjusted at the beginning of the study using a paper string wetness recorder (Bazier, Jules Richard, France) and making field observations. Data were collected every 15 min from January 2003 to December 2005 and processed using the software Specware 6.01 (Spectrum Technologies Inc., ILL, USA).

RESULTS

Meteorological databases

The climographs showed clear differences between disease-free areas and areas where CBS is endemic (Fig. 1). According to the Köppen system, Cape Town (South Africa), Huelva and Valencia (Spain) fell into the Mediterranean type climate (Cs) with warm to hot, dry summers and mild, wet winters with rainfall occurring almost exclusively during the winter months. In contrast, Nelspruit (South Africa) and São Paulo (Brazil) were characterized by the presence of rainy summers and fell into the humid-subtropical category (Cw and Cf respectively).

Field data

Rainfall and temperature data recorded from all three orchards were similar to the thirty-year monthly averages showed above. During the three-year period of study, rainfall was virtually absent in the summer and climatic data followed the typical Mediterranean pattern (Fig. 2).

Wetness was, in general, more frequent in the winter six-month period (January-March and October-December) than in the summer six-month period (April-September). However, wetness was concurrent with temperatures over 20 °C only from June to October (Fig. 3). The minimum hours of wetness were recorded in Gibrleón orchard with temperatures between 15 and 25 °C. No hours of wetness were recorded in any of the experimental orchards with temperature above 25 °C.

DISCUSSION

The results of our study, based on the meteorological databases and the current geographical distribution of CBS, are in agreement with those of Paul *et al.* (2005). It seems that the characteristic warm and rainy summers of tropical and humid-subtropical citrus-growing areas provide environmental conditions conducive to CBS establishment and development. In contrast, Mediterranean climate, which is characterized by cold, wet winters and hot, dry summers, is apparently less favourable to CBS establishment and development.

However, in climate matching studies, accurate predictions can generally only be made by selecting climatic variables and their thresholds according to biological responses which have been determined previously. When model parameters and conclusions are inferred from the known distribution of a disease, as in the work of Paul *et al.* (2005) and in the present study (Fig. 1), it is uncertain whether the geographical distribution of the pathogen is an expression of its climatic tolerance, or whether it is only an expression of how far it has had time to spread or adapt.

Risk analysis for foreign diseases requires the availability of as much information as possible on the organism and its interactions with the environment. These relationships should be based on data from field research or controlled environment studies. The predictive ability of empirical models, developed from field studies, is limited by the scope of the data, and usually they are not considered suitable for geographical extrapolation (Teng and Yang, 1993). On the other hand, with assessments in containment facilities, it is possible to quantify the effect of a broad range of environmental conditions on each component of the disease cycle. However, epidemics are usually more complex than a linear combination of their monocyclic components and this kind of models sometimes fails to mimic disease development under field conditions. Therefore, many risk analysts stress the use of both, field research and experiments in controlled environment, to produce reliable risk assessments (Yang *et al.*, 1991).

Another source of uncertainty in climate matching studies is the lack of knowledge about the environmental conditions of the study area. Even in areas where climate is known, the environmental factors selected and the methods used for their measurement need to be relevant to the main biological processes of the species concerned. For many foliar pathogens, the infection phase is one of the most critical components of disease development and it is highly dependant on temperature and wetness duration on plant surfaces (Magarey *et al.*, 2005). Monthly cumulative rainfall and average temperature data collected from weather stations have been commonly used as criteria in climate matching studies. However, canopy microclimate may modify environmental conditions greatly compared with a turf surface where most standard weather station measurements are made (Magarey *et al.*, 2001). This difference is more accentuated in semi-arid areas, where canopy microclimate can provide favourable conditions for disease development even though the macroclimate is unfavourable (Palti and Rotem, 1973). Our field studies clearly show that long-term average rainfall data are not a good indicator of the citrus canopy wetness in any of the three locations. Despite the lack of rain in summer months, dew periods occur during the nights at temperatures over 15 °C or even over 20 °C.

Many fungal pathogens that are widespread in the Mediterranean basin, such as the citrus brown spot pathogen *Alternaria alternata* (Fr:Fr) Keissl., are able to complete their infection cycle within the available dew periods. Under these conditions, successful infection requires either rapid germination and penetration or the ability of the germinating spores to survive dry periods and resume growth when rewetted (Bashi and Rotem, 1974). Some fungal pathogens can even continue the germination process during the dry period if the relative humidity is high (Schuh, 1993). Optimal conditions for germination of ascospores of *G. citricarpa* are 24 °C and 16 hours of wetting period, but germination can occur from 15 to 33 °C (Timossi *et al.*, 2003). For the germination of conidia and apleria formation, *G. citricarpa* needs 12 to 48 h of wetness and 10 to 40 °C (Noronha, 2002). However, no data are available on the effect of short or interrupted wetting periods on the germination and infection by *G. citricarpa*. Thus, without this information, it would be unwise to ignore in advance the likelihood of establishment of CBS in the Mediterranean citrus-growing regions.

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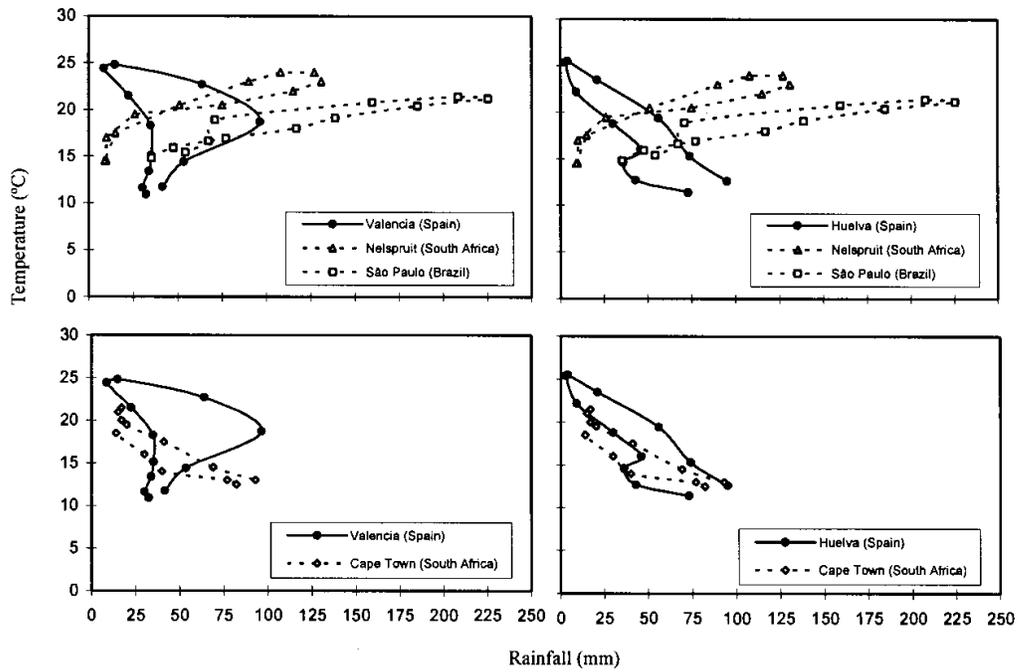


Fig. 1. Climographs for the citrus black spot fungus, *Guignardia citricarpa*, based on thirty-year average monthly temperature and rainfall data. Data for consecutive months from January to December (Northern Hemisphere) and July to June (Southern Hemisphere) are connected with a line.

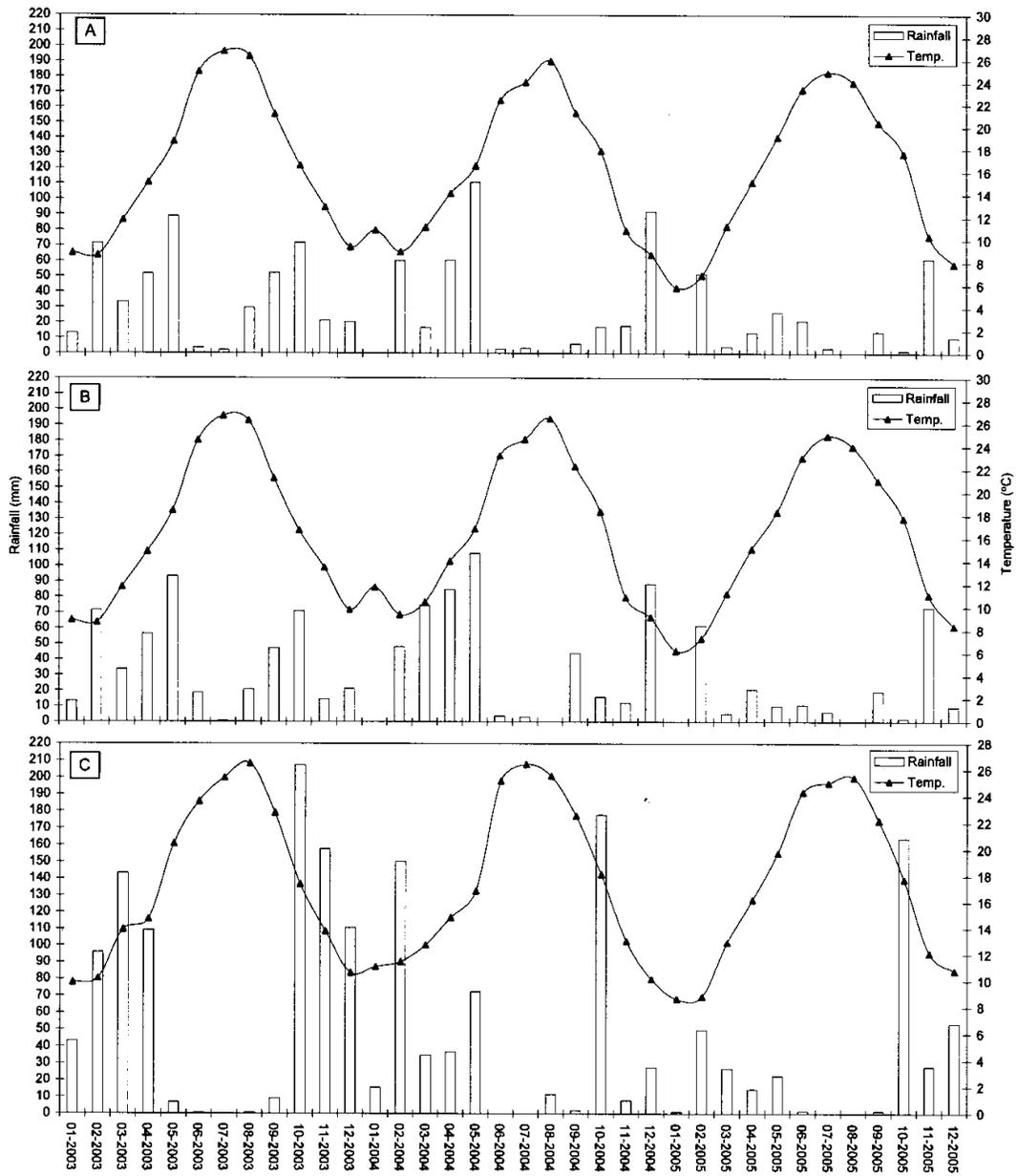


Fig. 2. Average monthly temperature (solid lines) and total monthly rainfall (vertical bars) in three citrus orchards from 2003 to 2005. A and B, Chiva (Valencia); C, Gibraleón (Huelva).

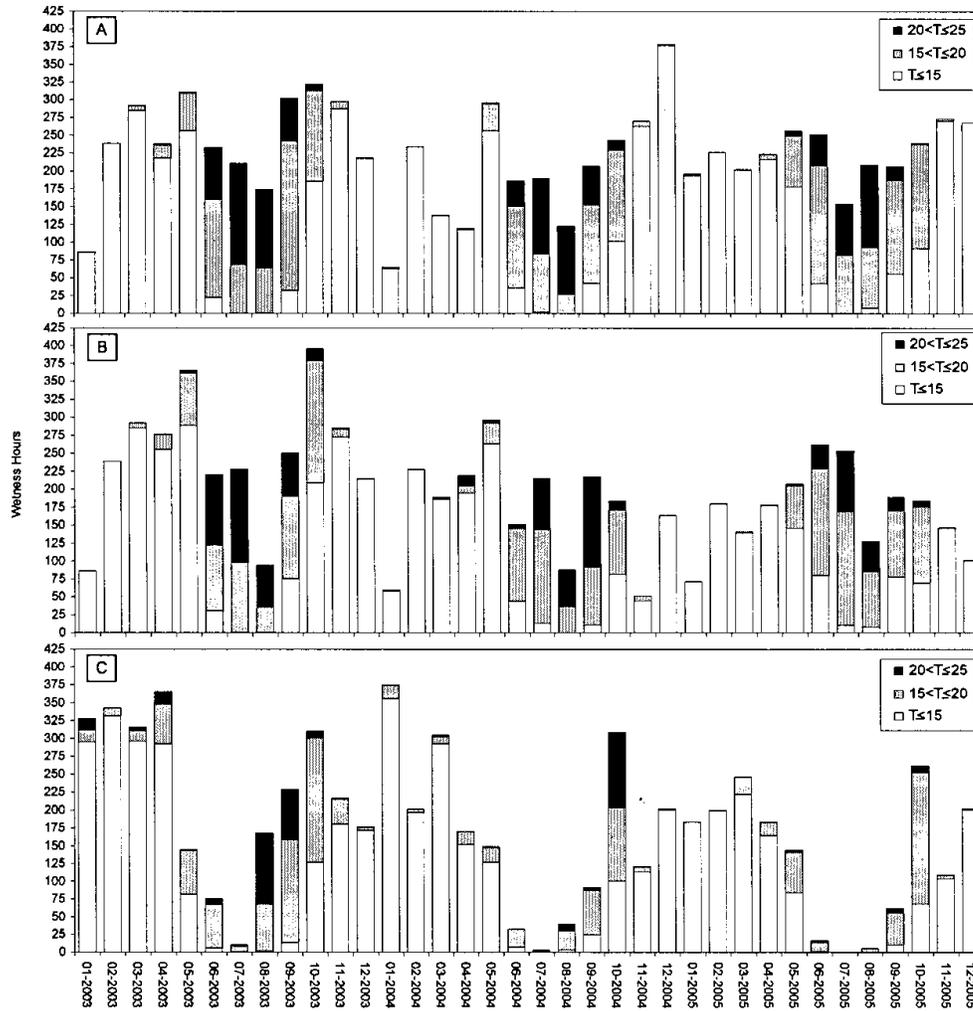


Fig. 3. Analysis of the frequency of wetness hours and temperature intervals for each month in three citrus orchards from 2003 to 2005. A and B, Chiva (Valencia); C, Gibraleón (Huelva). (Temperature in Celsius degrees (T): $T \leq 15$; $15 < T \leq 20$; $20 < T \leq 25$).

Report of the South African CBS Expert Working Group on evaluation of the Pest Risk Analysis for Citrus Black Spot (*Guignardia citricarpa*) on fresh citrus fruit from South Africa to the European Union

Pretoria, 01 June 2007

1. Introduction

This report constitutes a response from the South African CBS Working Group to the following communication from the EU: “Report of the commission working group on evaluation of the PRA prepared by SA on CBS, 15-16 June 2006 (report dated November 2006)”. It is further noted that the November 2006 report from the EU, made reference to the following prior exchange of related information:

- (1) The PRA - CBS on citrus fruit Exports from South Africa to the EU (PRA dated May 2000, covering letter dated 31 May 2000),
- (2) Response of the European Commission Working Group (dated 24 October 2001),
- (3) Response from South Africa (report dated June 2002, covering letter dated 28 August 2002),
- (4) Additional data on climate matching submitted to the EU by SA (covering letter dated 08 December 2003),
- (5) Additional data submitted to the EU by SA, in the form of a research report on the colonisation potential of CBS from fruit to leaf litter (covering letter dated 19 July 2004).

It is additionally noted that components of Item (4) above were covered by the publication of the scientific paper: “Paul, I., A.S. van Jaarsveld, L. Korsten & V. Hattingh. 2005. The potential global geographical distribution of Citrus Black Spot caused by *Guignardia citricarpa* (Kiely): likelihood of disease establishment in the European Union. *Crop Protection* 24: 297-308”. SA had previously advised the EU of this publication.

Likewise it should be noted that Item (5) above, has been taken up in the scientific publication: “Truter, M., P.M. Labuschagne, J.M. Kotze, L. Meyer & L. Korsten. 2007. Failure of *Phyllosticta citricarpa* pycnidiospores to infect Eureka lemon leaf litter. *Australasian Plant Pathology* 36: 87-93”. This article has not previously been sent to the EU by SA. For ease of reference, a copy of the publication is included (Annexure 1).

From a procedural perspective, the SA WG has refrained from repeating its previously presented position on aspects being discussed. It has limited its comments to direct response to matters raised in the EU WG’s response of November 2006. This response uses the italicised numbering as contained in the EU Report of November 2006.

2. Comments of the SA WG on the EU response dated November 2006

- B. Comments and responses*
- 2. Background information*

2.1 Epidemiology

2.1.1

2.1.1.1 Importance of pycnidiospores

The SA WG previously stated that pycnidiospores are unimportant in both the initial stages of potential disease establishment and the potential subsequent development of the disease. The EU WG concurred with SA that pycnidiospores are of lesser importance with regard to the subsequent development of the disease in an area where it is established. However, the EU WG contended that pycnidiospores may be as important as ascospores in initial stages of disease establishment.

The EU WG offered, as substantiation of its position, reference to reports of initial difficulty in detecting ascospores at the early stages of disease establishment and the results of epidemiological studies conducted in Brazil and Argentina.

The SA WG comments on this as follows:

- (a) The SA WG includes scientists with many years of practical experience with CBS under field conditions, who attest to the ease of failure to detect the presence of ascospores, especially in situations of low prevalence, unless sampling and monitoring is conducted in a knowledgeable fashion. The difficulty of a party to detect spores, especially when detailed information on how the monitoring was conducted has not been provided, cannot be used as a reliable basis for supporting a contention that ascospores are less important than pycnidiospores at an early stage of disease development.
- (b) Results of epidemiological studies conducted under the climatic conditions prevalent in Brazil and certain parts of Argentina cannot be extrapolated to an expectation of how the organism may respond in regions that have vastly different climatic conditions, such as Europe.

2.1.1.2 Spore germination versus disease development and establishment

The EU WG referred to work on *G. citricarpa* ascospore germination with reference to Noronha (2002), as justification for the EU WG's contention that germination can take place over a wider range of conditions than SA had previously contended.

The SA WG notes that in the general plant pathological sense, the term "establishment" refers to a situation where the organism is able to sustainably complete its life cycle (Agrios, 2005), which in turn is in accordance with the IPPC Glossary (FAO, 2006). The occurrence of an infection event does not necessarily imply that the conditions that permitted infection are also suitable for successful completion of the life cycle, especially of an organism with a complex life cycle such as *G. citricarpa* (Kotzé, 1981). Consequently, the prospect (likelihood) of spore germination cannot be equated to the risk of disease establishment, whereas the EU WG seems to have done so, leading to an inappropriate estimation of the risk of potential establishment.

2.1.1.3 Relevance to PRA

The SA WG contends that whereas scientists may indefinitely sustain such exchange of opinions about the finer details pertaining to potential infection events, the likelihood of an infection event must be considered to be at most remote. The SA WG notes that the EU WG itself has previously acknowledged that the probability of such an event must be low (Report of the Commission Working Group on evaluation of the PRA prepared by SA on CBS, 24 October 2001).

The SA WG contends that a remote prospect of an infection event (whether such a remote likelihood does in reality exist or not) cannot be equated to any material risk of disease development and even less so of establishment. The SA WG contends that the EU WG has provided nothing additional in its response of November 2006, the relevance of which has not been qualified above, to justify retention of a view on the relevance of pycnidiospores that is materially different from that previously stated by the SA WG (2000, 2002).

2.1.2

2.1.2.1 Comments on Truter report (2004)

The SA WG notes that all the shortcomings that the EU WG had identified in the research report, have been addressed in the subsequently edited and peer reviewed scientific publication (Truter *et al.*, 2007). The SA WG is consequently proceeding on the basis that the study is scientifically sound and that there is no prospect of this fruit to fallen leaf pathway occurring.

2.1.2.2 Splash dispersal of pycnidiospores from the orchard floor

2.1.2.2(a)

The EU WG questioned aspects pertaining to “specific stimuli”.

The SA WG notes that it had provided the references to “specific stimuli” in the 2000 PRA. The SA WG wishes to point out that it had not intended to suggest that “specific stimuli” may not occur naturally, as this would be incongruous with the completion of the organism’s life cycle under suitable conditions. Conversely, evidence of infection under suitable conditions that occur naturally, should not be used to infer that such suitable conditions do not include specific stimuli.

2.1.2.2(b)

The EU WG disagreed with the SA WG’s opinion about the likelihood of splash dispersal from the orchard floor.

The SA WG remains of the opinion that, if an infected fruit, with the fungus at the particular stage of development where ripe pycnidia are available for the extrusion of pycnidiospores, were to be placed directly below a citrus tree with low hanging, young and susceptible foliage or fruit, and there were to be exposure of the infected fruit to sufficient moisture to induce pycnidiospore extrusion, followed shortly thereafter by a direct impact from a water droplet, with the appropriate

physical properties to dislodge the pycnidiospores from the gelatinous mass in which they are extruded and propel them through the air and deposit them onto foliage or fruit that is in a susceptible stage, the prospect of splash dispersal would theoretically be possible, but unlikely. The SA WG disagrees with the EU WG's reference to *Phytophthora* dispersal (an organism with motile zoospores, where the conditions for infection and completion of the life cycle are easily satisfied) as justification for the assertion that the same would apply for dispersal of immotile *G. citricarpa* pycnidiospores oozing from pycnidia (on fruit lesions) in a gelatinous mass. The SA WG contends that even if splash dispersal of pycnidiospores were to occur more readily than it had stated, this again cannot be equated with the prospect of leading to an infection event, and certainly not with a "very high" probability thereof. The SA WG has further commented on this in 2.1.1.2 & 2.1.1.3 above.

2.1.3 – 2.1.5

(a) The EU WG commented that the modelling study of Paul *et al.* (2005) appeared to have utilised model parameter quantification without basing such selection on the biological relevance of the parameters to the particular organism.

The SA WG agrees that consideration of biological responses of the organism are important in the modelling process (Sutherst & Maywald, 1985), but objects to the claim that the modelling exercise was conducted without due consideration thereof. The authors have advised that all available data on the nature of the pathogen were considered during the modelling process and that perusal of the reference list of Paul *et al.* (2005) reflects this.

(b) The EU WG commented that the modelling exercise should have included data from experiments conducted under controlled environmental conditions and should have included micro-climatic considerations. The EU WG furthermore refers to Magarey *et al.* 2005 as justification for its contention relating to micro-climatic considerations.

The SA WG acknowledges that useful indications of biological responses can be derived from such studies, but cautions that the complexity of such interactions in the holistic environment of the organism's response to its environment, can frustrate the appropriate and meaningful use thereof in a modelling exercise. An understanding of the organism's biology, which has influenced the quantification of parameter values, has indeed been based on numerous preceding studies under measured or controlled environmental conditions, as reflected by the list of references quoted in Paul *et al.* 2005. However, it should be noted that such studies cannot generate specific parameter values that can be inserted into the modelling exercise, they can only influence the relative weighting of specific parameter values. Furthermore, the complexity of micro-climate precludes its effective use in modelling of this nature and whereas micro-climatic conditions are relevant in terms of the biology of the organism, macro-climatic conditions are indeed adequate to effectively model potential distribution. The SA WG considers reference to Magarey *et al.* 2005 to be out of context relative to distribution modelling as this publication relates to infection modelling.

The EU WG further refers to Palti & Rotem (1973) in support of its concerns about the effect of micro-climate as potentially overriding macro-climatic barriers.

The SA WG questions the relevance of this reference, particularly in light of drawing parallels between unrelated pathogens such as *Phytophthora* spp. in vegetables and *G. citricarpa* on citrus.

(c) The EU WG supports its concern about the level of reliance on the outcome of the CLIMEX modelling, by reference to the failure of a CLIMEX modelling exercise to predict the distribution potential of another organism.

The SA WG acknowledges that the outcome of a modelling exercise must not be considered in isolation, but wishes to point out that failures may be due to inappropriate use of the CLIMEX model (Sutherst, personal communication 2007) and that there have been numerous accurate modelling outputs (Sutherst, 2003). It should be noted that the CLIMEX software used in the quoted study (Royer, 1990) was a very early version and has been considerably upgraded since then and prior to its use by Paul *et al.* (2005). Royer (1990) reported (a workshop proceedings, not a publication in a refereed scientific journal) on modelling of Karnal bunt, a disease that is totally unrelated to CBS. In terms of its modelling, the climate envelope of Karnal bunt is not as restricted by certain climatic parameters as is the case with CBS.

Furthermore, the output of a CLIMEX modelling exercise is sensitive to the reliability of the existing range input data. In the case of Paul *et al.* (2005), a great deal of careful attention was paid to obtaining accurate and reliable input distribution data. The value of the model output is further strengthened by closely matching the geographical distribution of the species which has occurred in reality over a long period of time, including the failure of the organism to establish in regions (with climates similar to parts of the PRA risk area) despite protracted exposure to establishment challenge that is the same in aspects (infected fruit), and possibly more severe (infected propagation material) than the exposure of the PRA risk area. The reliability of the Paul *et al.* (2005) modelling output is further supported by having accurately predicted the potential occurrence of the organism in Ghana prior to its subsequent establishment in that area (Timmer, personal communication 2005).

(d) The EU WG provided a document by A. Vicent (2006), and refers to this article in support of the WG's concern that macro-climatic data used in modelling may lead to misleading results due to different micro-climatic conditions. In particular the EU WG refers to disparity between average rainfall data and leaf wetness data.

The SA WG considered the document by A. Vicent (2006). It notes that Vicent concurs with the SA WG regarding the similarity of the Western Cape and European Mediterranean region on a macro-climatic scale. The SA WG obtained data from micro-climatic weather stations situated in grapevine vineyards in the Western Cape Province, viz. Paarl and Ladysmith (weather data kindly provided by Dacom Plant Service, www.dacom.nl). Relevant data has been attached as Annexure 2. The graphs depicting monthly data for mean temperature, total rainfall and total leaf wetness hours (note that leaf wetness was calculated using temp, RH, wind as LW-sensors are notoriously inaccurate and unreliable (Magarey *et al.*, 2004)) clearly demonstrate the similarities between the Spanish and Western Cape climates on a micro-climatic scale. As in the Spanish citrus orchards, similar leaf wetness periods, as a result of dew periods, and moderate temperature conditions were recorded.

The SA WG also notes that Vicent (2006) refers to the ability of *Alternaria alternata* to complete its “infection cycle” within available micro-climatic conditions in the Mediterranean basin. It is then put forward that the occurrence of such conditions suggests that conditions conducive to *G. citricarpa* pycnidiospore germination do also occur and that therefore, the climate is conducive to CBS establishment. Apart from the shortcomings in extrapolating from the likelihood of spore germination to establishment, as raised by the SA WG in other parts of its response, the SA WG considers this line of argument to be flawed in the following two ways.

The SA WG considers it inappropriate to draw parallels between *A. alternata* and *G. citricarpa*. *Alternaria* is a common facultative parasite occurring across a wide range of climatic conditions, with multiple cycles per season under favourable conditions, producing thick-walled spores that are resistant to drying and other adverse conditions. This is in stark contrast to *G. citricarpa*, that is host-specific, monocyclic and has a distribution that is limited by climate. *Alternaria* spore germination is associated with toxin production that may result in necrosis of the plant tissue. Hence symptom expression is related to spore germination, not necessarily with an infection event.

The SA WG notes that Vicent’s observations in fact provide strong support for the SA WG’s position. *Alternaria* brown spot does indeed occur abundantly in the Western Cape, providing further biological evidence of the similarity between the climatic conditions in Western Cape and Mediterranean citrus producing areas. However, under these same conditions CBS does not occur in SA and one can therefore reasonably expect the same in the Mediterranean basin.

(e) The SA WG notes the information provided by the EU WG with regard to the distribution of citrus plantings within certain parts of Europe. However, the SA WG is confident with the output of the Paul *et al.* (2005) study. This confidence is supported by Paul (2005) having verified the conclusions of Paul *et al.* (2005) in a number of ways. Paul (personal communication, 2007) further advises that the CLIMEX modelling has been repeated with an updated version of the software and with the aid of an interpolated 0.5° gridded climate data set, developed by New *et al.* (1999), that provides a continuum between discreet weather data points. This modelling indicates that there are no sites within the European Union member countries that have an EI>4. Furthermore, Paul (2005) conducted modelling using a response surface model, using two different climate data sets, as a means of comparison with the outcome of the CLIMEX modelling, being two different conceptual approaches to modelling. The outcomes of both modelling approaches were very closely matched.

With regard to the EU WG’s comments on the suitability of the New Zealand climate, the SA WG wishes to point out that the Everett and Rees-George (2006) publication refers to a report on a molecular study, that focussed on diagnoses. Their comments regarding New Zealand’s climatic suitability were made in the discussion of their paper, were related to temperature and rainfall alone and cannot be considered to be more than speculative.

The SA WG agrees with the EU WG that climatic modelling should not be considered as a stand alone, but must be considered in the context of the broader PRA. However, the converse is also true, being that factors that reduce the risk of establishment cannot be disregarded in the PRA

simply because they do not each (in isolation) constitute a complete guarantee that a particular event can never occur. Likewise, it is unreasonable to hold forth that such risk mitigation considerations should not be considered until proven beyond all doubt, given the complexity of biological systems. The SA WG maintains that a compelling justification of the climate modelling output as reported by Paul *et al.* (2005) lies in the fact that the Western Cape has been challenged by CBS for many decades without resultant establishment.

(f) The EU WG commented on the absence of CBS in the Western Cape. These comments included queries regarding the potential role of SA regulations, pertaining to the movement of citrus propagation material, in the absence of CBS from the Western Cape. The EU WG also queried the exclusion of certain Western Cape Magisterial districts and the significance thereof.

The SA WG firstly points out that the regulations pertain to the movement of propagation material, not fruit, whereas the PRA in question pertains to the fruit pathway. As indicated by the SA WG in previous exchanges (see point 2.1.5 of the 2002 SA response), citrus propagation material was also free to move from CBS infected areas to the Western Cape for several decades (especially backyard lemon trees) before implementation of the regulations.

Regarding the Magisterial districts, the SA WG wishes to advise that the exclusion of a particular Magisterial district, such as Laingsburg, cannot be interpreted as indicating that CBS occurs in that district. Such districts were not included because citrus is not grown in these areas on a large scale, commercial basis, for export. Nonetheless, the SA WG can advise that CBS has never been reported from any part of the Western Cape Province.

(g) The EU WG again raised questions about the significance (in terms of adaption risk) of the sexual stage of *G. citricarpa*, and referred to fungicide resistance as potential justification for its ongoing concern.

The SA WG advises that the benzimidazole resistance in *G. citricarpa* is regulated by a single dominant gene (http://www.frac.info/frac/work/work_benz.htm). Furthermore, it has been demonstrated that a single isolate can develop resistance to carbendazim through protracted exposure to the chemical in the absence of reproduction (Koenraad *et al.*, 1992). Adaptation to climate could be expected to involve genetic change or mutation at several genes, which would most likely involve biological fitness penalties to the mutated individual. Moreover, the chance of environmental adaptation through mutation or genetic recombination is so slim that it would take a very high disease pressure (i.e. extremely high number of individuals to be subjected to environmental selection pressure or the mutagenic agent) over a long period of time to be successful. The chance of the aforementioned population density realising after an infection event by *G. citricarpa* in an unsuitable climate for disease establishment, is extremely unlikely. The SA WG believes that the argument that “the risk of quick adaptation of this fungus to factors limiting establishment such as climate” due to the sexual reproductive cycle of *G. citricarpa*, is highly academic and improbable.

(h) The EU WG’s comments on the volume of citrus product potentially having entered the European citrus producing regions is noted and the SA WG acknowledges that these volumes are not quantified. However, it does not agree with the EU WG’s position, which implies that no such

movement took place prior to the implementation of the EU internal market. As indicated in the 2002 SA response, prior to the implementation of EU internal market arrangements, there had been a practice whereby SA citrus had routinely been imported into Italy under permit. The SA WG would also like to correct the dates that it previously provided in terms of the duration of the SA citrus exports to Europe. SA has been actively exporting citrus fruit to Europe since 1907.

(i) The EU WG offers the theory of a proportional association between inoculum and risk of establishment as support for its contention that the EU citrus producing countries have not previously been exposed to high risk. The SA WG would like to note that this line of argument seems to be at odds with the EU WG's earlier position regarding the relevance of low volume or low incidence of infected host in terms of risk of establishment.

2.2 *New information*

The EU WG put forward that if mycelium is latently present it can develop to the formation of pycnidia and pycnidiospores and can lead to infection in new areas.

However, in the absence of any studies that indicate that this is a likely occurrence under field conditions, and given the comments provided previously regarding extrapolation from occurrence of the organism to establishment, the SA WG is sure that the EU WG will agree that connecting potential presence of latent mycelium to infection in new areas, must be considered to be speculative and without sufficient evidence to justify acceptance.

2.3 *Specific risk mitigation considerations*

The EU WG makes the point that there is no explicit data excluding the possibility of perithecia on fruit.

Several researchers have studied *G. citricarpa* in a number of countries for more than 100 years and the occurrence of perithecia on fruit has never been reported. This seems to the SA WG, to be adequate assurance that it does not occur. Conversely, pursuit of proof beyond all doubt that a particular event may never occur is also not logically achievable.

3. *Pest Risk Analysis*

3.2.2 *Economic importance criteria*

The EU WG has indicated (EU WG response 2001, EU WG response 2006) that it disagrees with the conclusion of Stage 2 of the PRA (2000) because "it has not been completely proven that CBS cannot establish in the PRA area".

The SA WG notes that, in accordance with basic principles of scientific philosophy, the EU WG is setting an unattainable criterion as a pre-requisite for the potential of a particular conclusion to the 2000 PRA, namely that it must be completely proven that an event cannot occur. Accordingly, the EU WG seems to preclude any possibility of satisfying the criteria for termination of the PRA at this stage. It seems that this would not be in keeping with the IPPC guidelines in relation to PRAs,

whereby conclusions should be science based. Precluding the opportunity to meet the criteria through scientific investigation would accordingly be in conflict with this principle.

In the 2006 response, the EU WG adds that it is of the opinion that recent research warrants a cautious approach.

The SA WG provided specific responses to the points of concern as raised by the EU WG in 2001, and subsequently provided additional research data. The SA WG herewith again provides specific responses to points of continued concern as raised by the EU WG in its response of 2006. The SA WG is convinced that this information, evaluated collectively, does not support the “cautious approach” conclusion.

The SA WG is confident that, if the EU WG were to remain unwilling to agree to the original PRA’s (2000) conclusion to Stage 2, it will at least agree that, whereas it is not practicable to completely prove that an event cannot occur, objective consideration of the information provided, supports as a reasonable conclusion, that the risk of *G. citricarpa* potentially establishing in Europe through the pathway of fresh citrus fruit exports from SA, is very low. Likewise, the subsequent and associated risk of spread and development to epidemic levels must also be considered very low. Consequently, although the organism could still be classified as a quarantine organism for the EU (in association with pathways other than fresh citrus fruit), the original conclusion of Stage 2 in the 2000 PRA would remain relevant in terms of the fresh citrus fruit pathway. Accordingly, the associated restrictions should balance this very low level of establishment risk against the restriction to trade, in accordance with the IPPC principle of “minimum impact”.

3.2.3 Introduction potential

A. Entry

A.1.

The SA WG notes the interception data provided by the EU WG in its 2006 response. The SA WG cautions that the presentation of such data can be misleading in the context of the volume of infected product that occurs in the pathway. The reason for this is that an entire consignment of fruit may be rejected on the basis of a single lesion, on a single fruit, being found during the inspection of such a consignment.

A.3.

The SA WG concurs that it is not appropriate to include a conclusion in the text of Point A.3 and therefore agrees that the text “This does not, however, as put forward in our argument, pose a threat to the EU Citrus Industry”, should not be included under this point.

B. Establishment

B.3.

The SA WG cannot agree with the use of the terms “nearby” and “probable”. Given the information and discussions exchanged in this regard, the SA WG trusts that the EU WG will

agree that unless the fruit is in the immediate vicinity of a tree, such as within the canopy or directly under the tree, it is of no relevance in the context of this point. The SA WG therefore suggests replacing “nearby” with “below” as put forward in the SA response of 2002. Use of the term “probable” may be deemed to imply that this is a reasonably common incident. The SA WG trusts that the EU WG will agree that this is surely not appropriate. The SA WG therefore proposes replacing “probable” with “low”.

B.4.

The EU WG refers to its previous comments (made in its response of 2001). The SA WG notes that it had commented on associated points and accordingly amended the text of the PRA in its response of 2002, and further provided results of relevant subsequent studies. By referring back to its comments of 2001, the EU WG seems not to have taken cognisance of this information. Furthermore, the SA WG, in the earlier part of this response, have further addressed these issues, in particular the relevance of reference to micro-climatic conditions. The SA WG trusts that this provides the EU WG with sufficient assurances that the amended (2002) text of point B.4 is appropriate.

The SA WG considered the continued reference to Lee and Huang (1973) in relation to point B.4 as being out of context, in that it relates to conditions suitable for ascospore development, discharge and infection, as opposed to the crucial aspects of the disease cycle required for establishment, such as perithecium maturation and the importance of cold stress, as demonstrated by Paul *et al.* (2005).

The EU WG specifically objects to the last sentence of the amended point B.4 (“And finally the organism would have to behave as it never has before in any part of the world for the disease to become established in a Mediterranean climate.”). The SA WG does not consider there to be any basis for objecting to this sentence, unless the EU WG can offer some reliable evidence of the organism having previously established in such a region.

The EU WG refers to Garrán (1996) and Spósito (2003). The SA WG, has at earlier points within this response, discussed the relevance of various aspects of these studies. Furthermore, in the context that the EU WG refers to these publications in point B.4, the SA WG notes that the work of Garrán (1996) is not novel, as it principally covers the same work conducted by Brodrick (1969).

3.3 Conclusion for Stage 2

The SA WG refers to its comments in point 3.2.2 above.

3.4.1 Risk management options

The SA WG appreciates the comments of the EU WG regarding in field fungicide control strategies and can attest to such programmes having been implemented, but notes that these programmes continue to be associated with heavy cost and environmental load.

The EU WG (2006) stated that “Nevertheless, as *G. citricarpa* is regarded as quarantine pest for the EU and also listed as such, zero import tolerance cannot be withdrawn”. The SA WG is concerned that, with regard to the fresh citrus fruit pathway, this seems to suggest a disregard for the “Minimum Impact” principle of the IPPC. In keeping with this principle, a zero import tolerance, is at odds with both the very low risk of establishment and the consequent failure of the PRA to classify *G. citricarpa* as a quarantine organism in association with the fresh citrus fruit pathway. However, the SA WG takes note of the EU WG’s concerns and proposes that the following Risk Management options may provide an appropriate balance of concerns:

1. Fresh citrus may only be exported from SA to EU from production sites that are registered with the SA Department of Agriculture for supply to the EU market; and
2. All production sites that occur within areas that have not officially been declared as free of CBS by the SA Department of Agriculture, shall implement effective CBS Good Agricultural Practices in accordance with criteria as set by the SA Department of Agriculture; and
3. All SA citrus presented for export to the EU shall be subjected to an official quality inspection, using an inspection sample of no less than 2% of each lot; and
4. All SA citrus exported from SA to EU must comply with the SA Minimum Quality Standards.

4. *Conclusions of the EU WG – November 2006*

Paragraph 1. The SA WG reciprocally appreciates the exchange of information to date and thanks the EU WG.

Paragraph 2. The SA WG has previously expressed its disagreement with the use of the term “probable” in this regard, has suggested changes and has provided information in this response to the EU WG document of 2006 in support of its position on this point.

Paragraph 3. The SA WG has addressed the points raised by the EU WG on the study of Paul *et al.* (2005) at earlier points within this document. The SA WG has likewise commented on the points raised by the EU WG regarding micro-climatic conditions (including leaf wetness) at an earlier stage in this document, having specifically noted that some of the information provided by the EU WG in fact supports the SA WG’s position on the matter.

Paragraph 4. The SA WG addresses the point of further research in its concluding comments below.

Paragraph 5. The SA WG trusts this exchange of information will provide the EU WG with adequate science-based assurance that it can adjust its position. The SA WG (including the quoted author) considers reference to Kotzé (1981) in this regard as being out of appropriate context.

5. Conclusions of the SA WG

The EU WG seems to commonly refer to the concepts of inoculum dispersal, germination and infection as if the likelihood of one is equivalent to the likelihood of disease establishment. The SA WG strongly opposes this, as it is not in keeping with basic plant pathological principles.

The SA WG has, from the outset, presented its assessment of the risk in the form of a series of sequential hurdles (with progressively decreasing feasibility of cumulative realisation), that in combination preclude any realistic risk of the organism becoming established in Europe through the SA fresh citrus fruit pathway.

Whereas the SA WG acknowledges and appreciates the scientific professionalism of the EU WG in its attention to detail of the various individual hurdles, and again concurs that not one of them in isolation represents an absolute guarantee that it cannot be overcome, the SA WG trusts that the EU WG must surely be in agreement that the sum of these hurdles represents an unlikely situation.

The SA WG is confident that the EU WG must share its frustration with perpetuation of this interaction process. The SA WG is of the opinion that the two groups can continue with the current exchange of information, on the finer details pertaining to each individual technical point in isolation, without any realistic prospect of resolution. However, the SA WG is of the opinion that the extensive exchange of information over the past seven years, has provided adequate scientific basis to conclude that, in accordance with the IPPC principle of “Minimum Impact”, the current level of EU regulation is unduly restrictive and in need of amelioration.

The SA WG appeals on the EU WG to reciprocate accordingly, but failing this suggests that the parties have reached a point where it would be appropriate to subject the collective exchange of information to date to third party review, in particular, with regard to the merits of the SA WG’s statement in the last sentence of the paragraph above.

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ANNEXURE 1

Truter, M., P.M. Labuschagne, J.M. Kotze, L. Meyer & L. Korsten. 2007. Failure of *Phyllosticta citricarpa* pycnidiospores to infect Eureka lemon leaf litter. Australasian Plant Pathology 36: 87-93.

ANNEXURE 2

Weather data obtained from microclimatic weather stations in the Western Cape.

APPENDIX 1

Members of the SA CBS Expert Working Group.

Failure of *Phyllosticta citricarpa* pycnidiospores to infect Eureka lemon leaf litter

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Abstract. Pycnidiospores of *Phyllosticta citricarpa* from pure cultures, symptomatic citrus black spot Valencia orange fruit and peelings were evaluated for their potential to infect and colonise citrus black spot-free Eureka lemon leaf litter in a controlled environment and in the field in different production regions of South Africa. Leaf litter, consisting of freshly detached green and old brown leaves that were exposed to viable pycnidiospores under controlled conditions or in the field underneath citrus trees, were not infected and colonised by *P. citricarpa*. Ascospores, conforming to *Guignardia citricarpa*, the pathogen, or *G. mangiferae*, a cosmopolitan endophyte, were collected with a Kotzé Inoculum Monitor from leaves placed in the field only at Tzaneen and Burgersfort. Distinguishing between these two species on ascospore morphology alone is not possible. A diagnostic polymerase chain reaction conducted on representative leaf material from all the treatments revealed the presence of only *G. mangiferae* on 12.5% of the treatments. This study demonstrated the failure of *P. citricarpa* pycnidiospores to infect citrus leaf litter under controlled and field conditions. Symptomatic citrus black spot fruit or peel lying on the ground underneath citrus trees, therefore, cannot lead to infection and colonisation of freshly detached leaves or natural leaf litter or represent a source of inoculum in citrus orchards for these leaves.

Additional keywords: inoculum load, spore trap.

Introduction

Citrus black spot (CBS) is caused by *Guignardia citricarpa* (anamorph *Phyllosticta citricarpa*) and the superficial cosmetic fruit spots are unacceptable in the global fresh fruit trade. Symptoms can develop on more than 90% of the fruit produced from unsprayed orchards, ranging from one up to a thousand spots per fruit (Calavan 1960). Three kinds of symptoms are widely recognised: hard spot, freckle and virulent spot (Cobb 1897; Kiely 1948). Two other symptoms, speckled blotch and cracked spot, occur predominantly in South Africa (Kotzé 1963; McOnie 1963; Brodrick 1969) and Brazil (De Goes *et al.* 2000), respectively. Of these symptoms, hard spot and virulent spot may contain pycnidia within the lesions, although freckle spot may turn into virulent spot and speckled blotch may turn into hard spot as the season progresses (Kotzé 1981).

Black spot is an economically important disease of citrus in summer rainfall regions of South Africa and various other subtropical countries. Although the disease has spread to most of the summer rainfall areas in South Africa since its first reported occurrence in 1929 (Doidge 1929), it has not been able to establish in predominantly winter rainfall areas. These areas have official CBS-free status and consist of the citrus production regions of Northern Cape and Western Cape (European Union 1998; Mabiletsa 2003). Confirmation of this distribution pattern in South Africa was recently illustrated by Paul *et al.* (2005) using global modelling of weather patterns

to map CBS occurrence. The global distribution of CBS is restricted by specific climatic parameters and cold-stress with temperatures below 11°C indicated to be the main restrictive parameter (Paul *et al.* 2005).

Environmental conditions required for successful infection of susceptible citrus material include the presence of adequate moisture and relatively high temperatures, ranging between 18 and 30°C for at least 15 h (Kotzé 1963; McOnie 1967). These conditions usually prevail in the summer rainfall areas of South Africa from late spring to autumn. The critical infection period is usually from October until January, as fruit susceptibility and the main ascospore release period coincide (Kotzé 1981, 1996). The critical infection period may start and end a month earlier or later depending on prevailing rainfall and mean temperature.

Fruit remains susceptible to infection from fruit set up to 5 months later, whereas leaves remain susceptible from development up to 10 months of age (Kiely 1948, 1950; Kotzé 1963; McOnie 1964*b*; Truter *et al.* 2004*b*). Two types of spores produced by the pathogen can infect susceptible citrus material (Kiely 1948; McOnie 1965; Whiteside 1967; Kotzé 1996). The airborne ascospores from perithecia are only produced on leaf litter and are the main source of inoculum and dissemination of the disease (Kiely 1948; McOnie 1964*b*, 1965; Kotzé 1981; Korf 1998). Pycnidiospores of the anamorph are produced in pycnidia on symptomatic fruit, leaf litter and with the highly

susceptible cultivar, Eureka lemon, on petioles and small twigs (Kiely 1948; McOnie 1964*b*; Whiteside 1967). In general, the waterborne pycnidiospores are regarded as unimportant in the dissemination of the disease, mainly due to the limited spread of the pathogen by means of water and the short viability period of the pycnidiospores (Kiely 1948; McOnie 1964*b*; Korf 1998).

Ascospores and pycnidiospores required moisture for production and discharge. In the presence of adequate moisture, ascospores are forcibly released from perithecia to a height of ~12 mm to be dispersed by air currents, whereas masses of gelatinous pycnidiospores ooze from pycnidia to be dispersed by water (Kiely 1948; Kotzé 1963; McOnie 1964*a*, 1964*b*). Viable ascospores and pycnidiospores landing on young attached citrus fruit and leaves will usually lead to successful infection under favourable environmental conditions (Kiely 1948; Kotzé 1963; McOnie 1964*b*, 1965; Whiteside 1967).

Following successful infections, the pathogen remains latent in the fruit and leaves for several months as a small knot of mycelium between the cuticle and epidermis. The latent period in fruit usually lasts until fruit maturity, although several factors regarding the host and environment can influence symptom expression. Leaf infections can stay latent for up to 36 months before leaf fall and under favourable conditions, production of pycnidiospores and ascospores on the leaf litter occurs (Kiely 1948; Whiteside 1965; McOnie 1967; Kotzé 1996). Alternate wetting and drying of leaves and temperature fluctuations provide optimal conditions for maturation of perithecia.

Pycnidiospores on symptomatic fruit or peel as an inoculum source for leaf litter in a citrus orchard has not yet been described and raises the concern that it could lead to infections if symptomatic fruit or peelings are discarded in a citrus orchard. The concern that symptomatic fruit may introduce the pathogen into CBS-free areas has led to more restrictive requirements for market access and trade. The premise of this approach was that only attached green leaves can be infected and will eventually add to the inoculum load produced on leaf litter. Therefore, the aim of this investigation was to determine whether pycnidiospores from an active growing culture and from CBS-symptomatic fruit or peelings could infect and colonise both freshly detached CBS-free green leaves and natural leaf litter from Eureka lemon under controlled and field conditions.

Materials and methods

Pycnidiospores from three different sources were used as inoculum in separate experiments: pure culture, infected fruit and peelings of infected fruit.

Experiment 1: pure culture

A *P. citricarpa* isolate (GC-m155), originally obtained from naturally infected Valencia fruit from Burgersfort (Mpumalanga province), was subcultured onto 2% potato dextrose agar (PDA) (Biolab, Merck) and incubated for 21 days under continuous fluorescent light at 25°C. Pycnidiospores produced were harvested by repeatedly rolling a sterile cotton swab over the culture and rinsing the spores from the swab in 15 mL of sterile tap water. Rolling and rinsing were continued until spores from the whole culture were harvested. The spore suspension was filtered through four layers of sterile gauze

to remove mycelial fragments. The concentration of the spore suspension was determined with a haemocytometer and the final concentration adjusted to 10⁴ spores/mL with sterile tap water. The spore suspension was kept at 15°C until used (within 4–6 h). A dilution series from the final spore suspension was plated on PDA and incubated at 25°C. Colony forming units per mL of the pycnidiospore suspension were determined by counting the developing *G. citricarpa* colonies on these PDA plates after 7 days.

Mature green leaves were picked from twenty-five 5-year-old CBS-free Eureka lemon trees. The trees were originally obtained from Stargrow nursery in the CBS-free citrus production region, Western Cape, and maintained in a greenhouse at the University of Pretoria for the duration of the study. Detached leaves were secured between two circular plastic grid sheets (350-mm diameter, 10-mm mesh size) with cable ties. Each grid set contained between 20 and 25 leaves. Ten prepared leaf grids were sprayed with the spore suspension on both sides until run-off and were then individually enclosed in plastic bags to maintain high moisture content conducive for pycnidiospore germination and infection. Ten control leaf grids were prepared and processed as described but were sprayed with sterile tap water instead. All leaf grids were removed from the plastic bags after 48 h at 25°C. Five of the control and pathogen inoculated leaf grids were further incubated in a growth chamber at 25°C, 90% relative humidity (R.H.) and a 14 : 10 h light : dark cycle, whereas the remaining grid sets were placed underneath citrus trees in Pretoria (Gauteng province). Prevailing minimum and maximum temperature and total rainfall were recorded in all the field experiments for the duration of each trial. All leaf grids were moistened on both sides three times a week with a fine mist of tap water until runoff. The leaf grids were removed from the growth chamber after 8 weeks, before the onset of leaf degradation, whereas the field exposed leaf grids were removed after 12 weeks. Leaf degradation within the growth chamber was enhanced by the constant high humidity of 90% R.H. The leaves were prepared for polymerase chain reaction (PCR) and ascospore capturing with the Kotzé Inoculum Monitor (KIM) within a week from collection. The experiment was conducted during May to July and repeated during September to November 2003.

The same procedures as described for the mature green leaves were followed using leaf litter collected from an orchard in Paarl (Western Cape province). Each grid set contained about 30 g of dry Eureka lemon leaf litter and five grid sets per treatment in the growth chamber and in the field, were used from May to July and repeated from September to November 2003.

Experiment 2: infected fruit

Another similar experiment was conducted using CBS symptomatic fruit as a natural pycnidiospore inoculum source instead of spraying leaves and litter with a pycnidiospore suspension. Valencia oranges with at least 20 red or hard spot symptoms per fruit were collected from a CBS affected orchard in Nelspruit (Mpumalanga). Fruit were submerged in tap water for 30 min, removed and incubated in a moist chamber at 25°C for 24 h to stimulate release of mature pycnidiospores and production of new viable pycnidiospores (Kiely 1948). Lesions

on selected infected fruit were microscopically examined to confirm the presence of pycnidia and pycnidiospores before being used. Isolations were made from selected CBS lesions as described by Meyer *et al.* (2006), deviating only by plating tissue onto 2% PDA supplemented with 50 mg/L rifampicin to confirm the viability and identity of the pathogen present. Identities of retrieved cultures were confirmed by PCR. Disease-free Valencia orange fruit from Citrusdal (Western Cape) were used as control. The fruit were visually inspected to confirm CBS-free status and rinsed with sterile tap water to ensure that they contained no traces of inoculum before being used.

Mature green CBS-free leaves were picked from forty 15-year-old Eureka lemon trees in Paarl. Leaves (20–25) were secured between two circular plastic grid sheets with cable ties as described for the first experiment. Three black spot infected fruit were placed in a plastic mesh and secured directly on top of each prepared leaf grid. Disease-free fruit were similarly prepared representing the control treatment. This time three fruit and leaf grids were used for each set of exposure conditions. Three incubation temperature conditions (20, 25 and 30°C) were selected and samples were incubated in different growth chambers at 90% R.H. with a 14 : 10 h light : dark cycle. The fruit–leaf grids were sprayed on both sides with a fine mist of tap water until run-off three times a week. Grid sets were also placed on the ground underneath citrus trees in CBS affected regions, Pretoria (Gauteng province), Tzaneen (Limpopo province), Burgersfort (Mpumalanga province) and Brits (North-West province), and CBS-free regions, Bellville, Constantia and Stellenbosch (Western Cape province). None of the citrus orchard blocks selected had received any chemical sprays against CBS for at least 5 years before commencement and did not receive any for the duration of the study. The fruit and leaf grids in the field were moistened by hand weekly on both sides until run-off. The grids in the growth chamber and field were collected after 8 and 12 weeks, respectively. Fruit with plastic mesh were removed from the grids and the leaves prepared for PCR and ascospore capturing. The removed fruit were microscopically examined for the presence of fruiting bodies and segments of the peel selected for PCR to confirm the presence of *G. citricarpa*. The experiment was conducted between May and July and repeated between September and November 2003. Localities for the field treatments were selected to include areas with summer rainfall with moderate to high levels of CBS and a CBS-free area with winter rainfall in Western Cape Province.

The same procedures described for the fruit and mature green leaves were again followed, this time using leaf litter instead of mature green leaves. The leaf litter was collected underneath the same Eureka lemon trees in Paarl as the green leaves. The leaf litter was secured between two plastic grid sheets with cable ties and treated the same as before. Three fruit and leaf litter grids per treatment were used between May and July and repeated between September and November 2003.

Experiment 3: peelings of infected fruit

Naturally infected Valencia oranges from Nelspruit with at least 20 red or hard spot symptoms per fruit were rinsed with sterile tap water and air-dried on paper towel. Ten randomly selected

fruit were kept separate for microscopic examination, whereas the remaining fruit were peeled. Lesions on selected infected fruit were microscopically examined to confirm the presence of pycnidia and pycnidiospores. Isolations were made from selected CBS lesions as described previously. The identities of retrieved cultures were confirmed by PCR. Disease-free Valencia orange fruit from Citrusdal were treated similarly and were included as controls.

Mature green CBS-free leaves were picked from forty 15-year-old Eureka lemon trees in Paarl. Leaves were secured between two circular plastic grid sheets with cable ties as described for the first and second experiments. The peel from four infected fruit were placed in a plastic mesh and secured directly on top of each prepared leaf grid. Peel from disease-free fruit was treated in the same way. The peel and leaf grids were incubated at 25°C in a growth chamber at 90% R.H. with a 14 : 10 h light : dark cycle. Peel and leaf grids were also placed on the ground underneath citrus trees in Pretoria. All grids were sprayed on both sides with a fine mist of tap water until run-off three times a week. The peel and leaf grids were removed from the growth chamber and field after 8 and 12 weeks, respectively. Peelings and the plastic mesh were removed from the grids and the leaves prepared for PCR and ascospore capturing. The removed peelings were microscopically examined for the presence of fruiting bodies, and segments were selected for PCR to confirm the presence of *G. citricarpa*. Five peel and leaf grid sets were prepared for each exposure condition and used from January until March 2004.

In the last experiment, the transfer of natural pycnidiospore inoculum from CBS infected fruit peelings to leaf litter was investigated. Natural leaf litter was collected under CBS-free Eureka lemon trees in Paarl. The leaf litter was secured between two plastic grid sheets with cable ties, about 30 g per grid, and treated as described for the peel and green leaf grids. Five peel and leaf grids per exposure condition were used between January until March 2004.

Polymerase chain reaction

Twenty leaf pieces (8-mm diameter) were selected from all the above treatments before being prepared for the ascospore capturing and incubated in moist chambers for 14 days at 28°C to induce development of fungal fruiting structures. The leaf pieces were microscopically examined for the presence of *G. citricarpa*-like pycnidia or perithecia. PCRs were conducted to confirm the presence of *G. citricarpa* or *G. mangiferae* with the primers CITRIC1 and CAMEL2 in conjunction with ITS4 primer as described by Meyer *et al.* (2006). DNA was extracted from 100 mg of selected leaf material from each treatment by grinding in liquid nitrogen and using the DNeasy Plant Mini Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions.

Ascospore capturing

The grids were submerged in water at 40°C for 5 min to induce ascospore release, followed by drainage for 10 min to remove excess water. Each grid pair with leaves was placed in the KIM, previously known as the Kotzé-Quest Inoculum Monitor (Truter *et al.* 2004a), and a microscope slide coated with a thin layer of vaseline was used to collect spores. Grids were

processed separately using one microscope slide for each grid. After the 2-h KIM operation at room temperature, the slide was removed, stained with lactofuchsin and examined with a compound microscope at $\times 400$ magnification. Each slide was divided into three 5-mm sections along the width of the slide. *G. citricarpa*-like ascospores were counted along four lanes, covering the width of the microscope field within the centre longitudinal 5-mm transect. These lanes ran across the length of the microscope slide from the starting point to where the trapping process stopped.

Results

Harvesting of spores with a swab was superior to other methods tested, including the method described by Korf (1998), being less time consuming and resulting in better spore yield. Sufficient numbers of pycnidiospores were produced in culture on a single 2% PDA plate (90 mm diameter) in 21 days to prepare a spore suspension of 10^4 spores/mL with which to inoculate all the treatments. More than 80% of the pycnidiospores in the final spore suspensions prepared in May and September germinated, leaving 3.6×10^4 and 5.2×10^4 colony forming units per mL for infection, respectively. Black spot-infected Valencia orange fruit yielded pycnidiospores in 78% of all the selected hard spot lesions that were examined microscopically. Fungal isolates retrieved from the selected lesion pieces yielded 64% *G. citricarpa*, confirmed by PCR, 35% *Colletotrichum gloeosporioides*, confirmed by morphological characteristics and 1% unidentified fungi.

Microscopic examination of selected leaves from all the treatments after the treatment period, revealed the presence of pycnidia and perithecia, but morphological characteristics of these fruiting bodies could not be confirmed to be those of *Guignardia* spp. Other fungi fruiting on the leaf material that were identified included *Alternaria alternata*, *Aspergillus* sp., *Cladosporium* spp., *C. gloeosporioides*, *Phoma* spp. and *Sphaerellopsis filum*. PCR tests conducted on the selected leaf pieces were negative for *G. citricarpa* for all treatments (Table 1). Seven samples tested positive for the endophyte *G. mangiferae* with PCR. After the first detection of *G. mangiferae*, additional leaf samples were collected from the same orchard where the leaves were originally collected to verify the natural occurrence of the endophyte. Of the 25 samples randomly collected from the same trees in this orchard, all 10 green leaf samples tested negative whereas two of the leaf litter samples tested positive for *G. mangiferae*.

In the experiments using symptomatic CBS fruit as an inoculum source, both infected and non-infected fruit as well as peelings were observed to have severe superficial microbial growth after the incubation period. Most of the fruit were mummified at this stage and all the peelings were dry and brittle. No pycnidia and pycnidiospores could be discerned by microscopic examination in the CBS lesions of the infected fruit or peel after the treatment period. Also, no evidence was found that ascospores were able to develop on the fruit or peel of infected and non-infected fruit after the treatment. Polymerase chain reaction tests conducted on selected fruit and peel segments of the used infected and non-infected fruit were negative for both *G. citricarpa* and *G. mangiferae*.

Ascospores, resembling those of *G. citricarpa* or *G. mangiferae* were captured with the KIM from four treatments: (i) detached green leaves placed in Tzaneen with and (ii) without infected fruit, (iii) detached green leaves exposed to infected fruit and (iv) leaf litter exposed to clean fruit placed in Burgersfort. In each of the four treatments, ascospores were captured from only one grid pair. Because PCR on selected leaf material from these grids tested positive for *G. mangiferae* and no *G. citricarpa* could be found on any of the leaf pieces used for PCR confirmation, ascospores captured, therefore, represented *G. mangiferae* and not the pathogen.

Discussion

This study demonstrated that viable pycnidiospores from a culture, symptomatic fruit or peel were not able to infect and colonise 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 favourable environmental conditions, *G. citricarpa* did not colonise any of the leaves. As Eureka lemon is the most susceptible cultivar to CBS, we can deduce that the same results will be achieved on other susceptible cultivars.

In a concurrent study, leaves on Eureka lemon trees were spray-inoculated with a pycnidiospore suspension from the same pathogen isolate as the present study (Truter *et al.* 2004b). The leaves were inoculated at different ages, ranging from 1 to 14 months, to determine the susceptibility period of green leaves. Latent infections established in 1- to 10-month-old leaves, demonstrated the effectiveness of the inoculation technique as well as the conduciveness of the controlled environment to infection. Favourable infection conditions were also present in the field as the mean maximum temperatures were above 18°C during both trial periods in all the localities. Infection conditions in the field were furthermore not dependant on rainfall as all grid pairs were wetted weekly. The presence of favourable infection conditions in the field was accentuated by abundant black spot symptoms on fruit in the orchards in the summer rainfall production areas during the trial as these blocks received no chemical treatment for CBS control.

Leaf inoculations with pycnidiospores from infected fruit and ascospores from leaf litter have only been reported for attached young green leaves (Kiely 1948; Wager 1952; McOnie 1967) and no reports were found on leaf litter inoculations. Wager (1952) placed symptomatic black spot fruit in a wire basket and hung it in a citrus tree in a CBS-free orchard to determine if the infected fruit can act as an inoculum source. Symptoms developed after several months on the fruit and similar to the current study leaf infections remained latent. Leaf infections usually remain latent, although symptoms can be produced on very old leaves or with trees under stress on younger leaves.

Another critical element for successful infection is the presence of ample viable inoculum. The inoculum load applied to the CBS-free leaves was quantified by determining the colony forming units per mL of the pycnidiospore suspension and by microscopic examination of the fruit lesions. Pycnidiospores produced on fruit were described as short-lived, with pycnidiospores older than 3–14 days failing to germinate, depending on the technique used (Wager 1949; Kiely 1948;

Table 1. Presence of *Guignardia citricarpa* or *Guignardia mangiferae* on black spot free Eureka lemon leaves after exposure to pycnidiospores under controlled conditions (growth chambers) and in the field

For the detection of *Guignardia* spp. on citrus leaves, values are the mean ascospore count per replicate with Kotzé Inoculum Monitor (five replicates for pure culture and three replicates for symptomatic fruit, each repeated twice, and five replicates for peelings of symptomatic fruit). —, negative for *Guignardia citricarpa* and *Guignardia mangiferae*. GM, positive for *G. mangiferae*

Treatment	Prevailing temperature (°C) ^A	No. of ascospores		Leaf litter collected from orchard floor		Freshly detached mature green leaves		PCR results	
		Freshly detached mature green leaves	Leaf litter collected from orchard floor	Treated	Control	Treated	Control	Freshly detached mature green leaves	Leaf litter collected from orchard floor
Pure culture									
Growth chamber	25	0	0	0	0	—	—	—	—
Field: Pretoria (Gauteng)	5.8–20.0; 13.2–26.9	0	0	0	0	GM	—	—	—
Symptomatic fruit									
Growth chamber	20	0	0	0	0	—	—	—	—
Growth chamber	25	0	0	0	0	—	—	—	—
Growth chamber	30	0	0	0	0	—	—	—	—
Field									
Pretoria (Gauteng)	5.8–20.0; 13.2–26.9	0	0	0	0	—	—	—	—
Tzaneen (Limpopo)	12.6–22.0; 15.7–26.2	75	142	0	0	GM	GM	—	—
Brits (North-West)	4.7–21.9; 13.1–29.5	0	0	0	0	—	—	—	—
Burgersfort (Mpumalanga)	6.6–17.5; 10.8–22.1	35	0	0	104	GM	—	—	GM
Bellville (Western Cape)	7.8–19.6; 11.5–22.3	0	0	0	0	—	—	—	—
Constancia (Western Cape)	7.8–19.6; 11.5–22.3	0	0	0	0	—	—	—	—
Stellenbosch (Western Cape)	7.3–20.9; 12.4–24.2	0	0	0	0	—	—	—	—
Peelings of symptomatic fruit									
Growth chamber	25	0	0	0	0	—	—	—	—
Field: Pretoria (Gauteng)	15.4–25.7	0	0	0	0	—	—	—	—

^A Leaf exposure to pycnidiospores from pure culture and symptomatic fruit was carried out from May to July (first temperature range) and repeated from September to November 2003 (second temperature range), whereas leaf exposure to peelings of symptomatic fruit was carried out from January to March 2004.

Korf 1998). Despite the short viability period of pycnidiospores, symptomatic CBS fruit can be a source of viable pycnidiospore inoculum for several months as the sporogenous layers in pycnidia are regenerative and numerous crops of pycnidiospores can be produced following regular wetting of the fruit (Kiely 1948; Wager 1952).

In a recent study, the viability of *G. citricarpa* was evaluated in peel and fruit under different temperature and humidity combinations over time (Agostini *et al.* 2006). The viability was determined by isolation of the pathogen from the fruit tissue but unfortunately, no attention was given to the vitality of pycnidiospores. Despite inconsistent results obtained from fruit isolations, the pathogen remained viable over 40 days as long as the lesion was intact on the peel or fruit, irrespective of the storage conditions. The isolation frequency was reported to decline with storage time, and is in agreement with previous work (Kiely 1948; Wager 1952; McOnie 1967). Although the pathogen remains viable over the period tested, similarly to Agostini *et al.* (2006), we do not consider commercial fruit to be a high risk for introduction of the pathogen into new areas, as the presence of susceptible tissue in close proximity to the source is required.

Of the three detection methods used on leaf litter, fruit and peelings, PCR with the species selective primers was the most sensitive method that distinguished between *G. citricarpa* and *G. mangiferae*. Furthermore, *G. mangiferae* was detected from leaf litter from which no ascospores were captured, indicating that the leaf litter was not devoid of *Guignardia* spp. The endophyte, *G. mangiferae*, occurs worldwide on citrus and other woody plants and is of no phytosanitary concern (Meyer *et al.* 2001; Baayen *et al.* 2002; Meyer *et al.* 2006). Our detection of *G. mangiferae* from leaves collected in Paarl is in accordance with the reported occurrence of the endophyte from CBS-free regions of Western Cape and other areas in South Africa (McOnie 1965). Dual infections by *G. citricarpa* and *G. mangiferae* have also been reported on citrus leaves and fruit (McOnie 1964b, 1964c; Sutton and Waterson 1966; Baayen *et al.* 2002; Meyer *et al.* 2006).

This is the first report on the artificial inoculation of leaf litter with pycnidiospores of *G. citricarpa*. The study evidently showed that *G. citricarpa* artificially inoculated or through natural inoculum exposure could not infect freshly detached mature green leaves or natural leaf litter. The detached leaves, either fresh or old, were not susceptible to pycnidiospore infection and that the inoculum produced on the leaf litter depends on the level of infection of young leaves while attached to the tree (Kiely 1948; Wager 1952; Kotzé 1963; McOnie 1964b, 1965; Whiteside 1967). There is no evidence that viable pycnidiospores produced on infected fruit could infect freshly detached mature green leaves and natural leaf litter and in practice lead to the production of inoculum in an orchard. Pycnidiospores produced on infected fruit or on leaf litter do not contribute to production of perithecia with ascospores on leaf litter and, therefore, do not increase inoculum levels in an orchard. There is no evidence that infected fruit lying on the ground in a CBS-free orchard will be able to infect detached leaves and contribute to the spread of the disease. Infected citrus fruit or peel poses no danger for the establishment of the

pathogen in CBS-free orchards when exposed to detached leaves only.

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Annexure 2. Microclimatic weather station data.

Weather data obtained from microclimatic weather stations situated in grapevine vineyards in the Western Cape province, viz. Paarl (Fig. 1) and Ladysmith (Fig. 2) (weather data kindly provided by Dacom Plant Service, www.dacom.nl). The graphs depict monthly data for mean temperature, total rainfall and total leaf wetness hours (note that leaf wetness is calculated using temp, RH, wind as LW-sensors are notoriously inaccurate and unreliable).

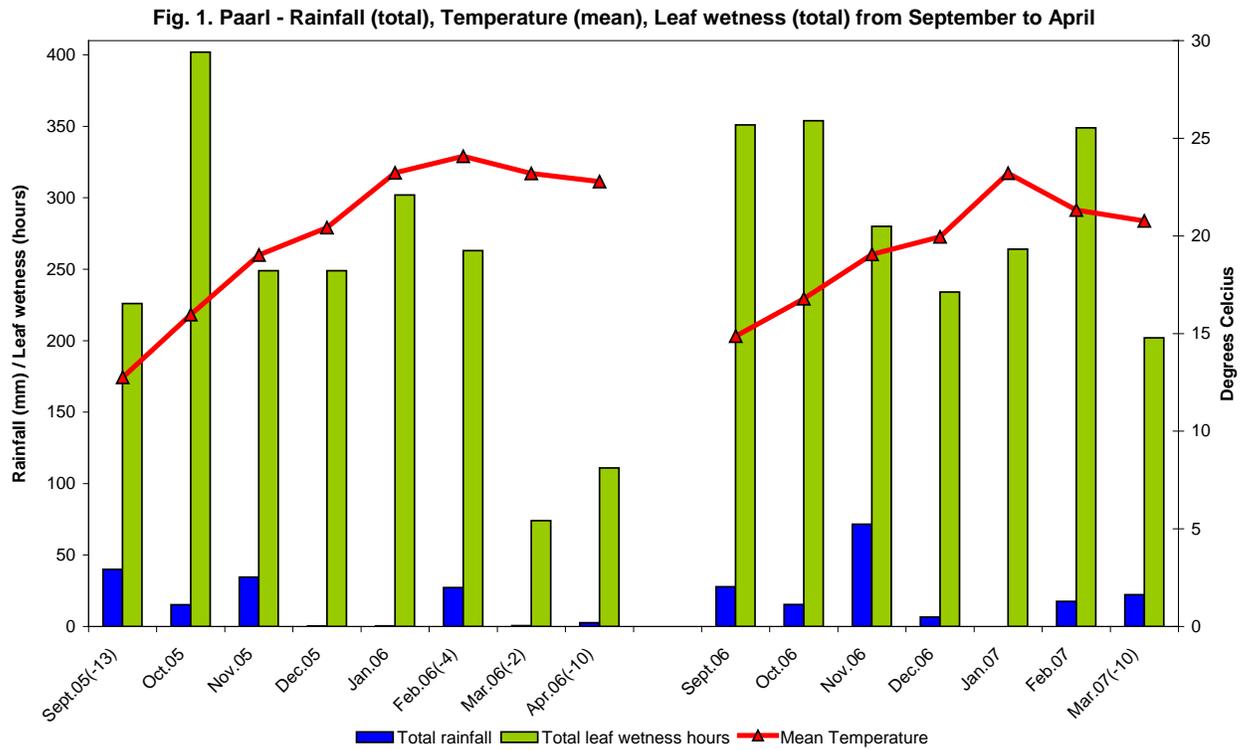
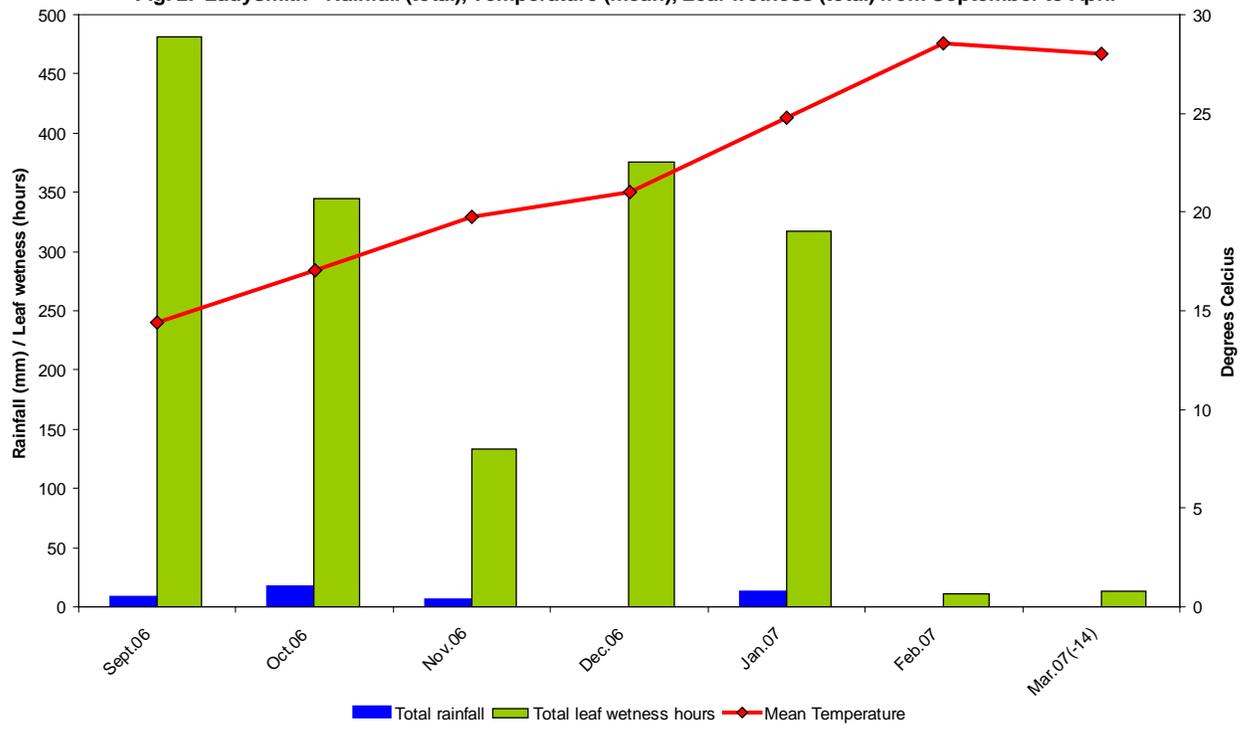


Fig. 2. Ladysmith - Rainfall (total), Temperature (mean), Leaf wetness (total) from September to April



Appendix 1. Members of the SA CBS Expert Working Group

A South African Citrus Black Spot Expert Working Group was convened to consider and formulate a response to the “Report of the Commission Working Group on evaluation of the Pest Risk Assessment prepared by South Africa on Citrus Black Spot (caused by *Guignardia citricarpa* Kiely), dated November 2006”. The SA WG met in Pretoria on 1 June 2007 to discuss its response. The table below lists the members of the SA WG.

Member's name	Affiliation	Relevant expertise
Mr M Holtzhausen	Directorate Agricultural Products Inspection Services, Department of Agriculture, South Africa	NPPO representative & plant pathologist.
Ms A Baxter	Directorate Plant Health, Department of Agriculture, South Africa	NPPO representative & plant pathologist.
Mr M Silimela	Directorate Plant Health, Department of Agriculture, South Africa	NPPO representative & plant health official.
Prof JM Kotzé	Consultant – Citrus Research International, Kokanje, South Africa	Plant pathologist & CBS expert.
Prof L Korsten	Department of Microbiology and Plant Pathology, University of Pretoria, Pretoria, South Africa	Plant pathologist & CBS expert.
Dr GC Schutte	Citrus Research International, Nelspruit, South Africa	Plant pathologist & CBS expert.
Dr HF Le Roux	Citrus Research International, Nelspruit, South Africa	Plant pathologist & field citrus disease control specialist.
Dr SH Swart*	QMS AgriScience Laboratories, Letsitele, South Africa	Plant pathologist & CBS expert.
Dr M Truter	Biosystematics: Mycology, Plant Protection Research Institute, Agricultural Research Council, Pretoria	Plant pathologist & CBS expert.
Dr I Paul	Small Grain Institute, Agricultural Research Council, Stellenbosch, South Africa	Plant pathologist & CBS expert.
Dr PH Fourie	Citrus Research International, University of Stellenbosch, South Africa	Plant pathologist.
Mr C Kellerman	SASCCON, Nelspruit, South Africa	Plant pathologist & CBS expert.

Ms E Carstens	Citrus Research International, University of Stellenbosch, South Africa	Plant pathologist & phytosanitary specialist.
Prof V Hattingh	Citrus Research International, University of Stellenbosch, South Africa	Plant pathologist & phytosanitary specialist.

*Not present at meeting of 01 June 2007 (inputs made through correspondence)

Pest risk assessment and additional evidence provided by South Africa on *Guignardia citricarpa* Kiely, citrus black spot fungus – CBS¹

Scientific Opinion of the Panel on Plant Health

(Question No EFSA-Q-2008-299)

Adopted on 17 December 2008

PANEL MEMBERS

Richard Baker, David Caffier, James William Choiseul, Patrick De Clercq, Erzsébet Dormannsné-Simon, Bärbel Gerowitt, Olia Evtimova Karadjova, Gábor Lövei, Alfons Oude Lansink, David Makowski, Charles Manceau, Luisa Manici, Dionyssios Perdikis, Angelo Porta Puglia, Jan Schans, Gritta Schrader, Robert Steffek, Anita Strömberg, Kari Tiilikkala, Johan Coert van Lenteren and Irene Vloutoglou.

SUMMARY

Following a request from the European Commission, the Panel on Plant Health was asked to deliver a scientific opinion on a document titled “Citrus Black Spot: Pest Risk Assessment document for the review of current phytosanitary regulations pertaining to the export of fresh citrus fruit from the Republic of South Africa to the EU”, received in June 2000 from the national plant protection organisation of South Africa. In particular, the Panel was requested, pursuant to Article 29(1) and Article 22(5) of Regulation (EC) No 178/2002, to provide a scientific opinion on the pest risk assessment and additional supporting evidence provided by South Africa, with regard to the suitability of the European Union (EU) citrus fruit producing areas, the likelihood of an introduction, leading to an establishment, and the appropriateness of the level of protection under the existing management options. The Panel was also asked to identify whether effective options, alternative to those already present in Directive 2000/29/EC, could be suggested to prevent introduction of citrus black spot into the Community.

This document presents the opinion of the Panel on Plant Health on the pest risk assessment conducted by South Africa on *Guignardia citricarpa* with the EU citrus growing areas as endangered area.

Citrus black spot (CBS), caused by the fungus *Guignardia citricarpa* Kiely, is a leaf-spotting and fruit-blemishing disease affecting *Citrus*, *Poncirus*, *Fortunella* spp. and their hybrids. Except for sour orange and Tahiti limes, all commercially grown citrus species and cultivars are affected by the disease. Lemon is particularly susceptible and thus, in an unaffected area, the disease usually first appears on this species. CBS is present in citrus-producing areas in

¹ For citation purposes: Scientific Opinion of the Panel on Plant Health on a request from the European Commission on *Guignardia citricarpa* Kiely. *The EFSA Journal* (2008) 925, 1-108.

Asia, Oceania, Africa and South America, but it has never established in Europe, North America, Central America and the Caribbean region. Due to the external blemishes, CBS symptomatic citrus fruit is unsuitable for the fresh market. Severe infections may cause premature fruit drop, especially in years favourable for disease development and when fruit is held on the trees past peak maturity. In addition, asymptomatic fruit at harvest may still develop symptoms during transport or in storage. The pathogen is classified as a harmful organism for the European Community in Annex II part A Section I of the Council Directive 2000/29/EC.

The Panel examined in detail the risk assessment provided, and considered the accuracy and quality of the information provided and methods applied for pest risk assessment purposes. The review was based on the principles of the International Standard on Phytosanitary Measures ISPM No. 11: Pest risk analysis for quarantine pests including analysis of environmental risks and living modified organisms (2004) and the International Standard on Phytosanitary Measures ISPM No. 14: The use of integrated measures in a systems approach for pest risk management (2002), by the International Plant Protection Convention (IPPC) (FAO, 2007).

The Panel, has studied the pest risk assessment and additional supporting evidence supplied by South Africa, as well as new information collected by the Panel, and concludes the following.

1. With regard to the suitability of the EU citrus fruit producing areas for establishment of CBS in terms of their climatic conditions:

Based on (a) the evaluation of the application of CLIMEX (Paul *et al.*, 2005), (b) the limitations of CLIMEX in predicting the potential distribution of pathogens such as *G. citricarpa*, (c) the relative climatic similarities between locations where CBS occurs in Eastern Cape Province and some locations where citrus is grown in the EU and (d) the results of the application of a generic infection model for foliar fungal pathogens, the Panel cannot agree with the conclusion by Paul *et al.* (2005) that the climate of the EU is unsuitable for the establishment of *G. citricarpa*.

2. With regard to the likelihood of an introduction, leading to an establishment, of CBS to the EU citrus fruit producing areas on CBS infected citrus fruit:

The Panel considers that *G. citricarpa* is associated with the citrus fruit in South Africa, is able to survive transport, storage and existing pest management procedures, especially in the form of quiescent infections and inconspicuous symptoms, and may be transferred to suitable hosts by means of splash dispersal from citrus black spot infected citrus fruit and peel. The Panel therefore concludes that *G. citricarpa* may enter the PRA area with infected citrus fruit. The Panel has also determined that, given the widespread distribution of susceptible hosts within the PRA area, cultural and climatic factors will not prevent the establishment of the pathogen.

The Panel concludes therefore that entry of *G. citricarpa*, leading to establishment in the EU citrus fruit production areas, on CBS infected citrus fruit is possible. The South African documents do not provide sufficient evidence to demonstrate that the importation of citrus fruit from infested areas is a very unlikely pathway for the introduction of *G. citricarpa* into these areas.

3. With regard to the appropriateness of the level of protection under the existing risk management options listed in Annex IV, Part A, Section I, point 16.4 of Council Directive 2000/29/EC:

Theoretically, the four existing risk management options are effective and in line with the IPPC principle of 'equivalence', but practical limitations may reduce their effectiveness. Option 16.4 (a) (pest free country) would be effective, but does not apply to South Africa. Option 16.4 (b) (pest free area) is effective in principle, but requires intensive continuous monitoring to maintain an accurate delimitation of this area. Information on such a monitoring programme was not provided to the Panel, so the effectiveness of this option could not be evaluated specifically for citrus fruit originating from South Africa. The Panel considered option 16.4 (c) (no symptoms in the field of production) to be not fully effective: the effectiveness depends on the intensity of the field inspection procedures in the field of production. Specification of the methods and level of accuracy of these inspection procedures is not required according to 2000/29/EC, and they are not specified by South Africa. The Panel considered option 16.4 (d) (appropriate field treatments) insufficiently effective, since no mitigating measure in the field, or combination of field treatments has been shown to fully prevent or eliminate fruit infections.

Observing the frequent interceptions of consignments of citrus fruit infested with *G. citricarpa*, originating from South Africa, the Panel concludes that the existing risk management options are not sufficient to prevent the entry of *G. citricarpa*.

The Panel observes that the existing measures apply to the whole territory of the European Community, where the movement of consignments of citrus fruit is not restricted. The Panel concludes that phytosanitary inspections and interceptions at all points of entry to the Community are appropriate in order to protect the citrus fruit growing areas. Therefore, the existing measures are in line with the IPPC principle of minimal impact.

Uncertainties associated with estimating the effectiveness of risk management options, which do not affect the Panel conclusions, may arise from lack of information on the sampling procedures that are part of various management options, and from insufficient data on the effectiveness of mitigation methods.

4. With regard to the identification of effective options, alternative to those already present in Directive 2000/29/EC, to prevent introduction of citrus black spot into the Community:

The Panel observes that post-harvest treatments of fruit are currently not listed as risk management options in Annex IV, Part A, Section I, point 16.4 of Council Directive 2000/29/EC. The combination of pre-harvest (field) treatments with post harvest treatments would further reduce, but not eliminate the risk of introduction. The Panel suggests including effective post-harvest treatments in option 16.4 (d). It is noted that, despite routine application of post-harvest treatment of citrus fruit by South Africa, frequent interceptions of infested consignments occur at the Community points of entry. The Panel suggests an investigation of the exact causes for infested consignments arrival at the EU border despite applied mitigation measures in South Africa.

For South Africa, as the country of origin, the Panel suggests that methods to accelerate citrus black spot symptoms development, combined with a standardised sampling scheme, could be applied in a pre-entry quarantine system to improve the detection of infested consignments before shipping.

For the European Community, the Panel suggests that demarcation of endangered and non-endangered areas could be combined with distinctive measures regarding end use and distribution of citrus fruit, that are less trade-restrictive.

Key words: pest risk assessment, *Guignardia citricarpa* Kiely, citrus black spot, *Citrus* spp., citrus, citrus fruit, European Community, European Union, South Africa.

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BACKGROUND AS PROVIDED BY THE EUROPEAN COMMISSION²

The current Community plant health regime is established by Council Directive 2000/29/EC on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community (OJ L 169, 10.7.2000, p.1, as last amended by Commission Directive 2007/41/EC – OJ L 169, 29.6.2007, p. 51).

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

Annex II, Part A, section I (c), point 11 of the above Directive includes *Guignardia citricarpa* Kiely (all strains pathogenic to Citrus) as a harmful organisms whose introduction into and spread within the Community shall be banned on plants of *Citrus* L., *Fortunella* Swingle, *Poncirus* L., and their hybrids, other than seeds. Annex IV, Part A, section I, point 16.4 identifies the specific import requirements for fruits of *Citrus*, *Fortunella*, *Poncirus* and their hybrids, other than fruits of *Citrus aurantium* L., originating in third countries.

The Commission (SANCO E.1) received in June 2000 from the national plant protection organisation of South Africa a document titled “Citrus Black Spot: Pest Risk Assessment document for the review of current phytosanitary regulations pertaining to the export of fresh citrus fruit from the Republic of South Africa to the EU”. The conclusions of the assessment question the Community's level of protection against *Guignardia citricarpa* Kiely on citrus fruits and the scientific justification of its phytosanitary measures. According to the document, the citrus growing areas in the Community do not have a climate suitable for an establishment of *Guignardia citricarpa* Kiely. Moreover, the introduction of *Guignardia citricarpa* Kiely leading to its establishment through the import of citrus fruit into so far uninfected areas is considered to be a very unlikely pathway.

The assessment prepared by South Africa was firstly analysed by the Commission expert working group (thereinafter referred as the EC-WG) in October 2001; the report thereof was sent to the South African authorities in December 2001. South African authorities responded by providing three additional documents – general response in September 2002; additional data on mapping the potential distribution of CBS in December 2003 and, finally, further data on research of potential transmission of CBS from fruit to leaf litter in July 2004. These documents were in the focus of the second meeting of the EC-WG, which took place on 15-16 June 2006 in Brussels, the report of which was sent to South Africa in November 2006. The European Commission received the latest response from South Africa, supported by recent scientific papers, in September 2007.

In that response, South African authorities insist on their position that the combination of sequential hurdles (with, in their view, progressively decreasing feasibility of cumulative effect) precludes any realistic risk of *Guignardia citricarpa* Kiely becoming established in Europe, and that the sum of these hurdles represents an unlikely situation. South Africa also expressed its view that the exchange of information so far between the EC and the South African authorities has provided adequate scientific basis to support their position and to conclude that, in accordance with the IPPC principle of "minimum impact", the current level of EU regulation is unduly restrictive and needs revision.

² Submitted by the European Commission, ref. SANCO E1/RV/al D(2008)510189

The Commission submitted that latest response from South Africa for discussion in the Standing Committee on Plant Health in December 2007. The Committee concluded to request a scientific opinion from EFSA before considering further steps in this matter.

TERMS OF REFERENCE AS PROVIDED BY THE EUROPEAN COMMISSION

EFSA is requested, pursuant to Article 29(1) and Article 22(5) of Regulation (EC) No 178/2002, to provide a scientific opinion on the pest risk assessment and additional supporting evidence provided by South Africa, with regard to the following issues:

- the suitability of the EU citrus fruit producing areas for establishment of CBS in terms of their climatic conditions;
- the likelihood of an introduction, leading to an establishment, of CBS to these areas on CBS infected citrus fruits;
- the appropriateness of the level of protection under the existing management options listed in Annex IV, Part A, Section I, point 16.4 of Council Directive 2000/29/EC.

EFSA is also requested to identify whether effective options, alternative to those already present in Directive 2000/29/EC, could be suggested to prevent introduction of citrus black spot into the Community.

The following documents should be included in the review:

1. Hattingh *et al.* (2000) Citrus Black Spot: Pest Risk Assessment document for the review of current phytosanitary regulations pertaining to the export of fresh citrus fruit from the Republic of South Africa to the EU. May 2000 ([Annex 1](#)).
2. Report of the Commission Working Group (EC-WG) on evaluation of the Pest Risk Assessment (PRA) prepared by South Africa on Citrus Black Spot (CBS). October 2001 ([Annex 2](#)).
3. Response from South Africa on the Report (dated 24/10/2001) of the EC Working Group relating to the WG's evaluation of the Pest Risk Assessment (PRA) by South Africa on Citrus Black Spot (CBS). September 2002 ([Annex 3](#)).
4. Further Data Relating to the PRA for Citrus Black Spot and the Export of Fresh Citrus Fruit from South Africa to the EU. September 2003 ([Annex 4](#)).
5. Further Data Relating to the PRA for Citrus Black Spot and the Export of Fresh Citrus Fruit from South Africa to the EU – Research Report on Potential Transmission from Fruit to Leaf litter. July 2004 ([Annex 5](#)).
6. Report of the Commission Working Group (EC-WG) on evaluation of the Pest Risk Assessment prepared by South Africa on Citrus Black Spot (caused by *Guignardia citricarpa* Kiely). June 2006 ([Annex 6](#)).
7. Report of the South African CBS Expert Working Group (hereinafter referred as the SA-WG) on evaluation of the Pest Risk Analysis for Citrus Black Spot (*Guignardia citricarpa*) on fresh citrus fruit from South Africa to the European Union. June 2007 ([Annex 7](#)).

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ASSESSMENT

1. Introduction

This document presents the opinion of the Panel on Plant Health (hereinafter referred as the Panel) on the pest risk assessment conducted by South Africa on *Guignardia citricarpa* Kiely with the EU citrus growing areas considered as endangered area. The documents evaluated are presented in detail in the Background and the Terms of reference as provided by the European Commission.

1.1. Evaluation procedure

The Panel examined in detail the documents provided, and considered the accuracy and quality of the information provided and methods applied for pest risk assessment purposes. The review was based on the principles of the International Standard on Phytosanitary Measures ISPM No. 11: “Pest risk analysis for quarantine pests including analysis of environmental risks and living modified organisms” (2004) and the International Standard on Phytosanitary Measures ISPM No. 14: “The use of integrated measures in a systems approach for pest risk management” (2002), by the International Plant Protection Convention (FAO, 2007).

The evaluation, after an introduction to the biology of *G. citricarpa* Kiely and the epidemiology of citrus black spot, is presented according to the three main issues in the Term of Reference from the EU Commission:

- the suitability of the EU citrus fruit producing areas for establishment of CBS in terms of their climatic conditions;
- the likelihood of an introduction, leading to an establishment, of CBS to these areas on CBS infected citrus fruits;
- the appropriateness of the level of protection under the existing management options listed in Annex IV, Part A, Section I, point 16.4 of Council Directive 2000/29/EC; the identification of effective options, alternative to those already present in Directive 2000/29/EC, which could be suggested to prevent introduction of citrus black spot into the Community.

1.2. Introduction to the pathogen *Guignardia citricarpa* Kiely

Citrus black spot (CBS), caused by the fungus *Guignardia citricarpa* Kiely (anamorph *Phyllosticta citricarpa* (McAlpine) van der Aa, is a leaf-spotting and fruit-blemishing disease affecting *Citrus*, *Poncirus*, *Fortunella* spp. and their hybrids. Except for sour orange (*Citrus aurantium* L.) and its hybrids (Kotzé, 2000) and Tahiti limes (*C. latifolia* Tan.), all commercially grown citrus species and cultivars are affected by the disease (Aguilar-Vildoso *et al.*, 2002; Kotzé, 2000;). Lemon (*C. limon* L.) is particularly susceptible and, thus, in an unaffected area, the disease usually first appears on lemons (Kotzé, 2000).

CBS affects fruit, leaves and twigs of citrus, however, only fruit symptoms are readily noted. Fruit is susceptible to infection for up to 4–6 months after petal fall, (Baldassari *et al.*, 2006; Kotzé, 1981, 2000; McOnie, 1964b; Reis *et al.*, 2003) and there is a long incubation period which may last for 2-12 months (Kellerman and Kotzé, 1977; McOnie, 1967). Lemon leaves remain susceptible from development up to 10 months of age (Truter *et al.*, 2004; 2007) but,

with the exception of lemons, leaves rarely show symptoms during their life span (up to approximately 3 years) (Kiely, 1948; McOnie, 1967).

Until recently, citrus pathogenic and non-pathogenic 'types' of *G. citricarpa* were thought to exist (McOnie, 1964a). However recent studies have demonstrated that the non-pathogenic type is the ubiquitous endophytic species *Guignardia mangiferae* A.J. Roy (Baayen *et al.*, 2002; Baldassari *et al.*, 2008).

The symptoms of the disease were first recorded in Australia in 1895 (Kiely, 1948). The disease is now present in citrus-producing areas in Asia, Oceania, Africa and South America. The pathogen is not established in Europe, North America, Central America and the Caribbean region (CAB International 2007), although interceptions have occurred.

Up to six different CBS symptom types have been observed, depending on the temperature and fruit maturity (Kotzé, 2000). Hard spot is the most typical symptom of CBS. It consists of shallow lesions, 3-10 mm in diameter, with a grey to tan centre and a dark brown to black rim. Hard spot usually appears when fruit starts maturing, even before colour break and on the side of the fruit most exposed to the sun. Usually, but not always, pycnidia develop in the centre of the lesions (Kotzé, 1981; 2000). False melanose, or speckled blotch, usually appears on green fruit as small (< 1 mm in diam.), raised, dark brown to black lesions, which are devoid of pycnidia and may coalesce as the season progresses (CABI, 2006). False melanose lesions may sometimes form a 'tear-streak' pattern when water with a high concentration of picnidiospores flows over the fruit (Spósito *et al.*, 2008). Freckle spot are gray, tan, reddish or colorless spots (1-3 mm diam.), slightly depressed at the center and lacking a halo. Pycnidia may develop incidentally (EPPO, 2003). Individual freckle spots may coalesce to form virulent spot type lesions, especially during storage (Kotzé, 1981; 2000). Virulent spot are sunken irregular lesions which occur on heavily infected fruit towards the end of the season. Under conditions of high humidity, numerous pycnidia may eventually develop within these spots. It is the most damaging form of CBS, because, unlike the other types of symptoms, it extends deeply into the mesocarp, occasionally involving the entire thickness of the rind, causing premature fruit drop and serious post-harvest losses (Kotzé, 1981). Lacy spot consists of superficial, small, yellow lesions with a dark yellow to brown centre and no defined margins. The lesions appear on green fruit, and may cover a large part of its surface but no pycnidia are formed. Cracked spot consists of superficial, slightly raised, variable in size, dark brown to black lesions with a cracked surface and irregular margins and also in this case no pycnidia are present. Both lacy spot and cracked spot symptoms have only been reported from South America (Aguilar-Vildoso *et al.*, 2002; Goes, 2001; Goes *et al.*, 2000).

Pre-harvest symptom development on fruit depends on the environmental conditions (high temperatures, drought and high light intensities favour symptom development), the age and physiological condition of the host (the disease is more severe on older trees, whereas vigorous, young trees may not show symptoms at all, even under epidemic conditions) and rind maturity (the more mature the fruit becomes, the better the chances for symptoms to appear) (Brodrick, 1975; Brodrick and Rabie, 1970; Kellerman and Kotzé, 1977; Kiely, 1969; Kotzé, 1981 and 2000).

Post-harvest development of symptoms is greatly influenced by the temperature and light intensity in the packinghouse, during transit to the market or in storage (Brodrick and Rabie, 1970; Kotzé, 1981; Wager, 1952).

The epidemiology of CBS is dependent upon the availability of inoculum during the period of host susceptibility, the climate (warm, wet or humid conditions favour infection) and the age of

the host tissue in relation to its susceptibility to infection (Kotzé, 2000). In addition, the flowering and fruiting pattern (e.g. overlapping mature and new fruit, number of leaf flushes, etc.) of the cultivated citrus species/varieties may influence the epidemiology of the disease (Ortiz *et al.*, 2004; Reis, 2002; Spósito *et al.* 2008).

Two types of infective propagules are involved in CBS epidemiology: ascospores produced within pseudothecia on leaf litter and dead twigs on the orchard floor (Kotzé, 1981; McOnie, 1964b; McOnie, 1965) and pycnidiospores (conidia) produced within pycnidia on fruit, leaves, twigs and abundantly on leaf litter prior to pseudothecia development (Kiely, 1948; Kotzé, 1963; 1981; 2000). Once the disease reaches epidemic proportions, airborne ascospores are the main inoculum source (Huang and Chang, 1972; Lee and Huang, 1973; Kotzé, 1981; McOnie, 1964b). However Whiteside (1967) found that pycnidiospores were the most important inoculum source at the initial stages of the disease in Rhodesia. The significant role of pycnidiospores in CBS epidemiology was further indicated by several recent studies in Brazil (Ortiz *et al.*, 2004; Spósito *et al.*, 2008).

Pseudothecia develop within 40-180 days after leaf drop, depending on the frequency of wetting and drying as well as on the prevailing temperatures (Kotzé, 1981). Maturation of ascospores occurs practically simultaneously in early summer on infected leaves abscised during late autumn, winter and early spring (Kotzé, 1963; Lee and Huang, 1973; McOnie, 1964c). Severe epidemics have been reported also in areas where ascospores were produced only on leaves shed in spring and early summer, because leaves abscised in winter had completely decomposed (Lee and Huang, 1973). The optimum temperature for pseudothecia formation is 21-28 °C and no pseudothecia are produced below 7 °C or above 35 °C (Lee and Huang, 1973). When mature asci within pseudothecia are moistened with water (rain, irrigation, heavy dew, etc.), ascospores are ejected up to a height of 1.2 cm from pseudothecia and are carried by air currents throughout the canopy and over longer distances (Kiely, 1948 and 1949; Kotzé, 1988; McOnie, 1964b; Huang and Chang, 1972; Wager, 1949). The effect of irrigation on pseudothecia maturation is less important in those areas characterized by high rainfall and sandy soils (McOnie, 1964c). Environmental conditions required for ascospore germination varied from 15-29.5 °C and 15-38 hours of wetness (Kotzé, 1963).

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

Control of CBS is based on cultural practices and fungicide sprays (Kotzé, 2000; Spósito *et al.*, 2008). Cultural methods include the use of disease free nursery stock, mulching of the orchard floor and removal of fallen leaves and late-hanging fruit. Chemical control involves the use of protective and curative fungicides. Chemical applications have to be carefully timed to coincide with the critical infection period. Nevertheless, according to Kotzé (1981), once the disease becomes established in an area, it is impossible to eradicate it.

CBS has significant economic impact mainly due to the external blemishes that make citrus fruit unsuitable for the fresh market. Severe infections may cause premature fruit drop (Kotzé,

2000; Spósito, 2003). Some losses due to fruit drop occur in years favourable for disease development and when fruit is held on the trees past peak maturity (CABI, 2006). In addition, asymptomatic fruit at harvest may still develop symptoms during transport or in storage (Agostini *et al.*, 2006; Kotzé, 1963). *G. citricarpa* is classified as an harmful organism for the European Union in Annex II part A Section I of the Council Directive 2000/29/EC (harmful organisms whose introduction into, and spread within, all EU Member States (hereinafter referred as EU-MS) shall be banned if they are present on certain plants or plant products).

2. Climatic suitability of the EU citrus fruit producing areas for establishment of *Guignardia citricarpa* Kiely

2.1. The South African assessment of climatic suitability

The South African assessment of the EU's climatic suitability for the establishment of *G. citricarpa* Kiely, referred to in Annex 4 (see Terms of reference) and other documents, is based on the use of CLIMEX software. An unpublished climate envelope approach was also briefly described but discounted by the authors because it was undertaken prior to a detailed survey of CBS in South Africa. The results of the CLIMEX analysis are discussed in detail below.

2.1.1. Introduction to CLIMEX

CLIMEX (Sutherst and Maywald, 1985; Sutherst *et al.*, 1999 and 2007) essentially provides two climatic risk mapping techniques. The first employs an algorithm which summarises the similarities in monthly mean, minimum and maximum temperatures, rainfall and rainfall pattern, relative humidity and soil moisture in a “match index” that can be selected and weighted according to the perceived influence of a particular variable and limited to relevant times of the year.

The second “compare locations” technique, predicts an organism's potential distribution based on (a) the climatic conditions in its current distribution and (b), if available, climatic responses obtained by research. A growth index, which represents the suitability of the location for growth and development, is calculated according to how close temperatures, moistures and day lengths are to a pest's known maxima, minima and optima. In the unfavourable periods, a stress index is estimated according to the degree to which the climate is too wet, dry, hot, or cold. The overall suitability of the location is represented by the ecoclimatic index (EI), formed by the product of these two indices. Responses to temperature, moisture and other factors are estimated by trial and error to try to reflect the known distribution of the pest, assuming that, in the centre of its range, the growth index will be at its maximum and the stress indices at minimum, while at the edges of its range, the opposite will occur with the EI greater than zero. Once CLIMEX has satisfactorily mirrored its current distribution, EIs can be calculated from meteorological data in the PRA area and mapped. Great care must be taken in using CLIMEX, principally because climate is not the only factor that influences distribution.

2.1.2. Summary of the CLIMEX analysis undertaken by South Africa

The CLIMEX analysis summarised in the documents prepared by the South African authorities is described by Ida Paul in Crop Protection (Paul *et al.*, 2005) and her thesis (Paul, 2006). CLIMEX version 1.1 (Sutherst *et al.*, 1999) was employed. This version is based on thirty year 1931-1960 monthly means from large numbers of weather stations worldwide with data taken from the UK Meteorological Office (1972). In Paul (2006), the Climatic Research Unit 1961-1990 mean monthly global climatic dataset interpolated to 0.5 degrees latitude and longitude was utilised (New *et al.*, 1999). The species parameter values used in this study are shown in Table 1.

Paul (2006) conducted both CLIMEX “match climates” (Chapter 4) and “compare locations” (Chapter 5) techniques but only the latter were included in Paul *et al.* (2005) and referred to in the documents prepared by the South African authorities.

Table 1. CLIMEX species parameters values used for *G. citricarpa* (Paul *et al.* 2005).

DYMEX - Model Parameters	
CLIMEX - Compare Locations (1 species)	
<small>Fri Sep 05 10:03:27 2008</small>	
Page 1	
<u>Climate Change Scenario</u>	
No Scenario is applied.	
<u>Soil Moisture</u>	
Soil Moisture Capacity	100
Evapotranspiration coefficient	0.8
<u>Guignardia citricarpa (Citrus Black Spot)</u>	
Temperature Index	
Limiting low temperature	17
Lower optimal temperature	24.5
Upper optimal temperature	32
Limiting high temperature	40
Moisture Index	
Limiting low moisture	0.18
Lower optimal moisture	0.45
Upper optimal moisture	0.85
Limiting high moisture	1
Light Index - not used.	
Diapause Index - not used.	
Cold Stress	
Cold Stress Temperature Threshold	11
Cold Stress Temperature Rate	-0.0001
Cold Stress Degree-day Threshold	6
Cold Stress Degree-day Rate	-0.00025
Cold Stress Temperature Threshold (Average)	-20
Cold Stress Temperature Rate (Average)	0
Heat Stress	
Heat Stress Temperature Threshold	40
Heat Stress Temperature Rate	0.001
Heat Stress Degree-day Threshold	25
Heat Stress Degree-day Rate	0.001
Dry Stress - not used.	
Wet Stress	
Wet Stress Threshold	1.45
Wet Stress Rate	0.0001
Cold-Dry Stress - not used.	
Cold-Wet Stress - not used.	
Hot-Dry Stress - not used.	
Hot-Wet Stress - not used.	
Cold Stress DD Threshold Temperature	17
Degree-days per Generation	0

Employing the match climates technique, Paul (2006), found that, out of 127 South African weather stations, only those in areas where CBS has been found showed a great than 60% climatic similarity with 16 stations close to known *G. citricarpa* locations. The match climates technique produced a similar picture for *G. citricarpa* locations in Australia. None of the 285 locations in Europe had a match index greater than 60%.

Using the “compare locations” technique, eighteen parameter values for temperature, moisture and stress indices providing the best fit for the distribution in South Africa and in Australia were selected. Particular attention was paid to areas around Nelspruit in South Africa, where the disease has been known to occur in serious epidemic proportions. Maps of the ecoclimatic index were prepared for South Africa, Australia and Europe (Paul *et al.*, 2005; Paul, 2006). All locations in the south-western part of Western Cape Province (South Africa) and inland New South Wales where *G. citricarpa* is absent had an EI less than or equal to 4 due to cold stress, suggesting that this represents the limit of climatic suitability for establishment. Five European locations had an EI greater than 4 but only four of these (on Sicily, Corfu and the Canary Islands) are in typical citrus growing areas. The 1961-1990 dataset produced only one point in the Mediterranean area with an EI greater than 4 in southern Turkey.

Paul *et al.* (2005) concluded that various places where *G. citricarpa* is currently absent are suitable for establishment, e.g. Florida and Texas, but stated that “*the risk of CBS introduction and establishment in the EU as a result of commercial trade in fresh citrus fruit, even from CBS-infected areas, appears negligible*”.

2.1.3. Limitations of the CLIMEX analysis

2.1.3.1. General limitations of CLIMEX for predicting pathogen distribution

Although, as Paul *et al.* (2005) point out, CLIMEX has been used to predict the potential distribution of several pathogens, its application is generally more complex than when used for insects. Three factors provide particular complexity:

- the relationship between pathogen infection and host phenology. All pest risk maps have to take into account the spatial presence of suitable hosts but, for many pathogens, temporal availability is also critical since infection may only occur if climatic conditions are suitable at specific host phenology stages. CLIMEX takes the whole year’s climatic data into account so cannot readily be constrained to analyse just the period of suitable host phenology;
- discrepancies between the pathogen and host’s climatic responses. The pathogen’s climatic responses may be much greater than the range suitable for the host;
- the importance of complex variables, such as leaf wetness, that are not taken into account by CLIMEX and may act at a much shorter time scale (hours) than that utilised by CLIMEX (weeks for the moisture index).

For the first factor, *G. citricarpa* and crop phenology, citrus has several flushes of leaves per year and leaves are susceptible for up to 10 months. Leaf tissues will be therefore continuously available for infection. However fruits are susceptible to infection for only several months after flowering, so the climatic conditions during this period alone must be analysed to predict the potential for *G. citricarpa* to infect fruit. This requires a much more complex study than the type of “compare locations” work on one species undertaken by Paul. Additional functionality to assist such a study is available in CLIMEX version 3 (Sutherst *et al.*, 2007).

The second factor can be taken into account by ensuring that predictions are restricted to areas of citrus cultivation and the information on the responses of *G. citricarpa* to cold conditions is taken into account.

For the third factor, the Panel undertook some exploratory analysis using leaf wetness models and the generic infection model (Magarey *et al.*, 2005) that is incorporated within the USA’s plant pest forecasting system NAPPFAST (Magarey *et al.*, 2007).

2.1.3.2. Limitations of the approach taken by Paul *et al.* (2005) and Paul (2006)

Limitations of the South African application of the CLIMEX Match Climates technique

The “match climates” results were presented only in Paul (2006), but unpublished and not referred to in documents submitted by the South African authorities. However the Panel undertook a brief review of this work because it helps underpin their conclusion that the climatic conditions suitable for *G. citricarpa* establishment are represented by the climate in north-eastern South Africa with considerable summer rainfall. The climate envelope study referred to in Annex 4, is not discussed further because, as the authors acknowledge, this was undertaken before the detailed survey of CBS in South Africa reported by Paul *et al.* (2005) had been made and there were errors in the input data.

The CLIMEX “match climates” technique does not take into account any biological information on the pest and therefore just provides a general index of climatic similarity. Limited by the functions available in CLIMEX version 1.1, Paul (2006) took all the year and all factors into account. This ignores the fact that the climates during key periods of crop phenology are critical to *G. citricarpa* and not the annual averages and patterns calculated by the CLIMEX algorithm. As such, the 60% threshold limit applied is unlikely to provide an accurate indicator of climatic suitability for *G. citricarpa*. Recent versions of CLIMEX allow comparisons to be made of climatic conditions during shorter periods but, although CLIMEX transposes climatic data so seasons in different hemispheres coincide, the new versions do not allow key periods in one part of the year at one location to be compared to different weeks at other locations. In addition, as noted above, the *G. citricarpa* infection process happens over a much shorter time scale than that used by CLIMEX so periods of climatic suitability may not be picked up.

A further difficulty lies with the choice of the 16 stations employed by Paul (2006) to represent the climate where CBS occurs in South Africa. Only stations in the north-east of the country were selected. These have similar climatic conditions with considerable summer rainfall. If stations representative of the three areas in the southern part of Eastern Cape Province where CBS is present had also been chosen, very different climatic match indices can be expected, since summer rainfall in these areas is much reduced. Although the “match climates” technique, like all climate envelope techniques, e.g. BIOCLIM, has limitations, it is still important to include all locations where the disease is present when trying to determine the range of climatic factors that are favourable for establishment before projecting them onto another part of the world, such as Europe. CLIMEX version 3 enables a regional climate match to be determined.

Instead of emulating the “match climates” technique by including locations in Eastern Cape Province and conducting the analysis with more recent data, due to the limitations of the technique noted above, it is more informative to plot monthly values of temperature, rainfall and relative humidity for different stations on the same graph. For illustrative purposes, data from five locations where citrus is grown were plotted:

- Nelspruit, Mapumalanga Province, South Africa (31°E, -25.5°S)
- Addo, Eastern Cape Province, South Africa (25.7°E, -33.5°S)
- Valencia, Spain (0.47°W, 39.5°N)
- Messina, Sicily, Italy (15.55°E, 38.19°N)
- Porto, Portugal (8.7°W, 41.1°N)

CBS is highly severe at Nelspruit (Paul *et al.*, 2005). Location information for the three areas where CBS is present in Eastern Cape Province is not given but can be inferred from the map in Paul *et al.* (2005). The westernmost area is assumed to be located around Addo since this is a known area of citrus production along the Sundays River valley (Villiers and Joubert, 2006) and fits with the drawing on the map. Citrus is grown at all three European locations. Valencia and Messina have a Mediterranean climate. Porto has a Lusitanian climate (Metzger *et al.*, 2005).

In order to compare climatic data for the same time period from these locations, the Panel chose the 1961-1990 monthly averages for the nearest 10 minute latitude-longitude grid cell in the Climatic Research Unit global climate dataset (New *et al.*, 2002) as imported into CLIMEX. For one location, Addo, the Panel was able to obtain average climatic data with an unknown time period from a website (http://www.saexplorer.co.za/south-africa/climate/addo_climate.asp) and daily data for January 2003-October 2008 (ARC, South Africa) for comparison. The similarities of these data with the data obtained from CLIMEX grid cell 11980, supports this approach (see figures 1-5). Grid cells 33236, 277048, 270642 and 288089 were chosen for Nelspruit, Valencia, Messina and Porto respectively. Graphs of monthly rainfall, maximum and minimum temperatures and relative humidity at 9AM are constructed. The data for the southern hemisphere locations were shifted 6 months to be comparable with northern hemisphere locations. The 3PM relative humidity graphs are not given since CLIMEX uses a simple multiple (0.85) of the relative humidity at 9AM.

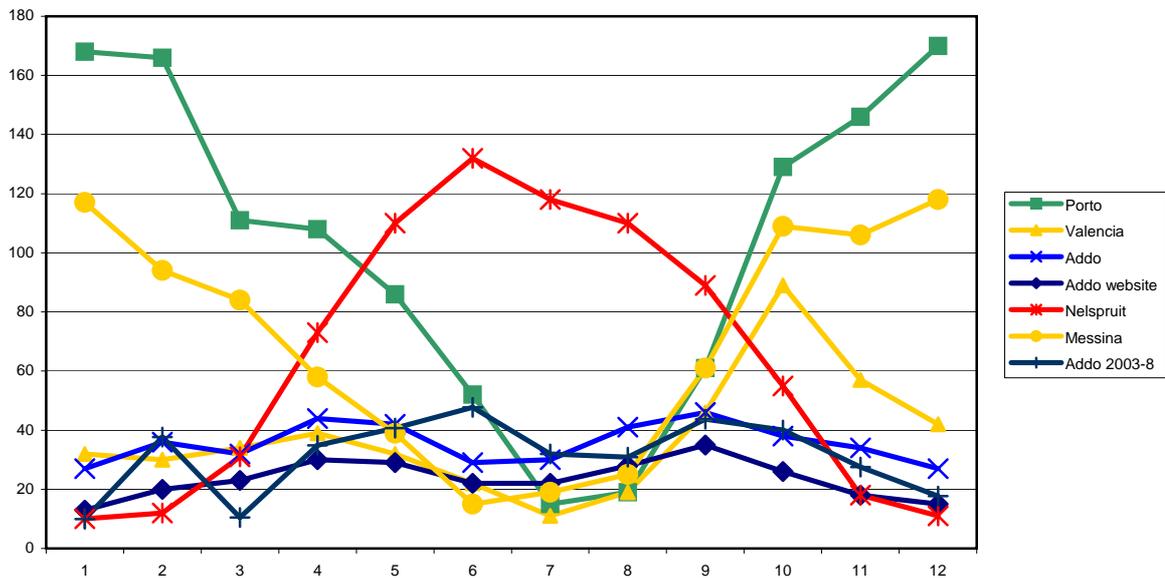


Figure 1. Monthly rainfall. Climate data extracted from grid cells nearest locations in Europe and South Africa (Source: CRU 1961-90 world climatology 10 minute lat-long resolution)

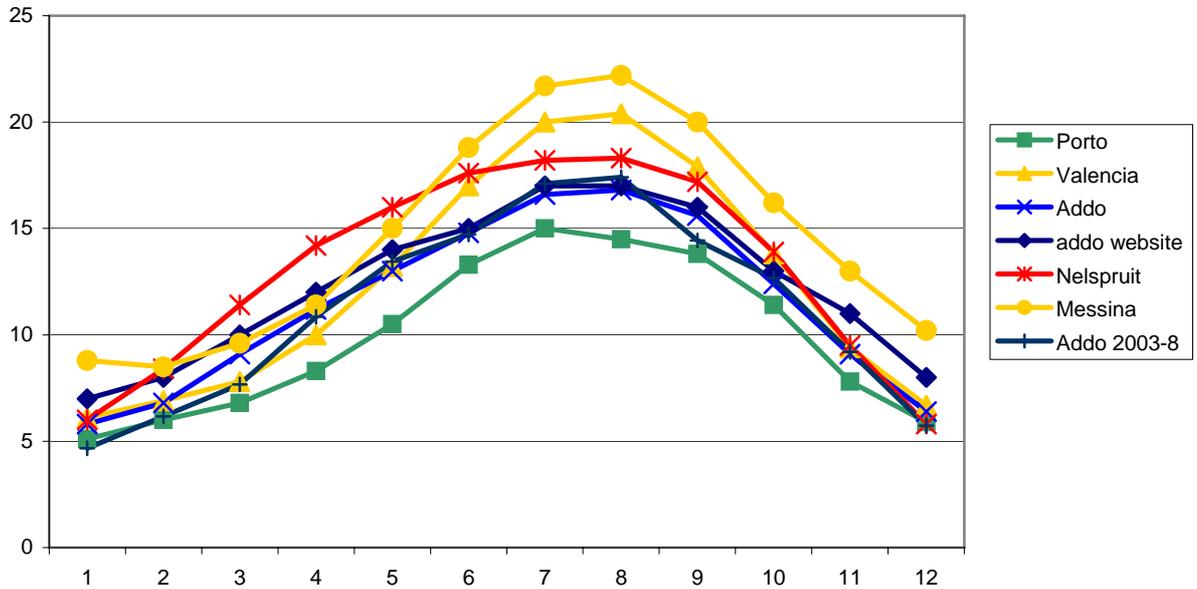


Figure 2. Monthly minimum air temperature. Climate data extracted from grid cells nearest locations in Europe and South Africa (Source: CRU 1961-90 world climatology 10 minute lat-long resolution).

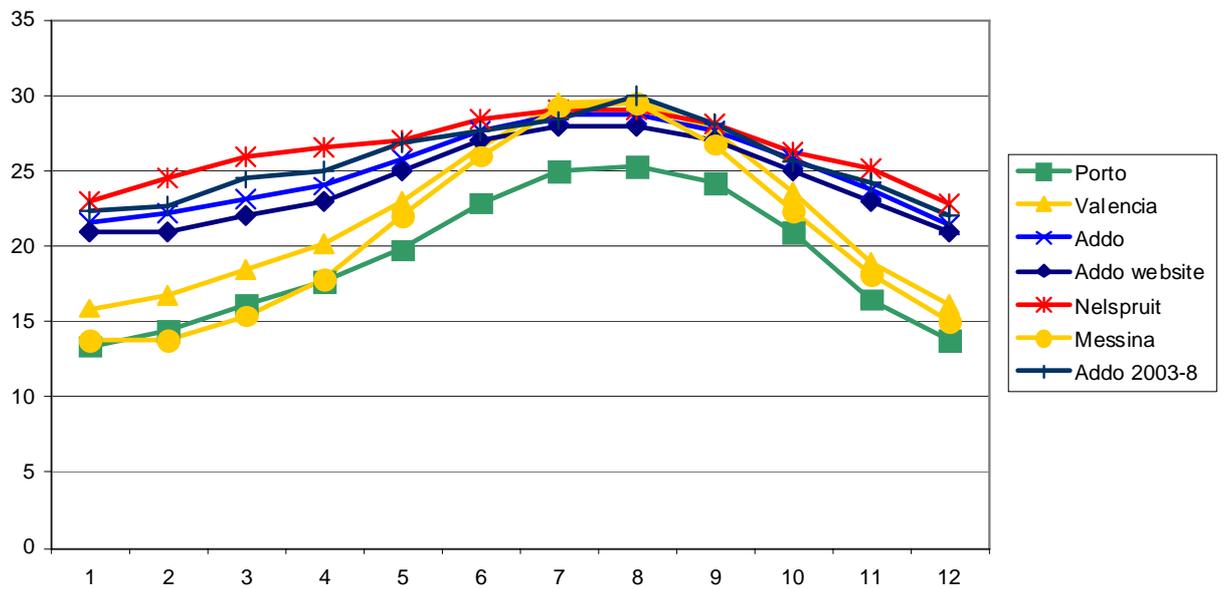


Figure 3. Monthly maximum air temperature. Climate data extracted from grid cells nearest locations in Europe and South Africa (Source: CRU 1961-90 world climatology 10 minute lat-long resolution).

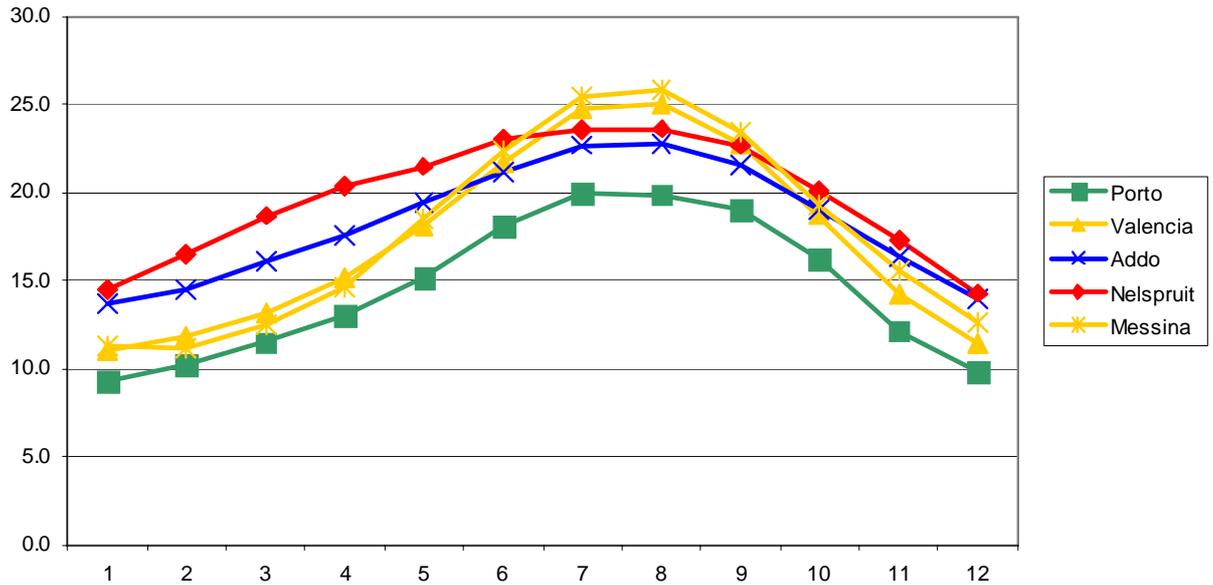


Figure 4. Mean monthly air temperature. Climate data extracted from grid cells nearest locations in Europe and South Africa (Source: CRU 1961-90 world climatology 10 minute lat-long resolution).

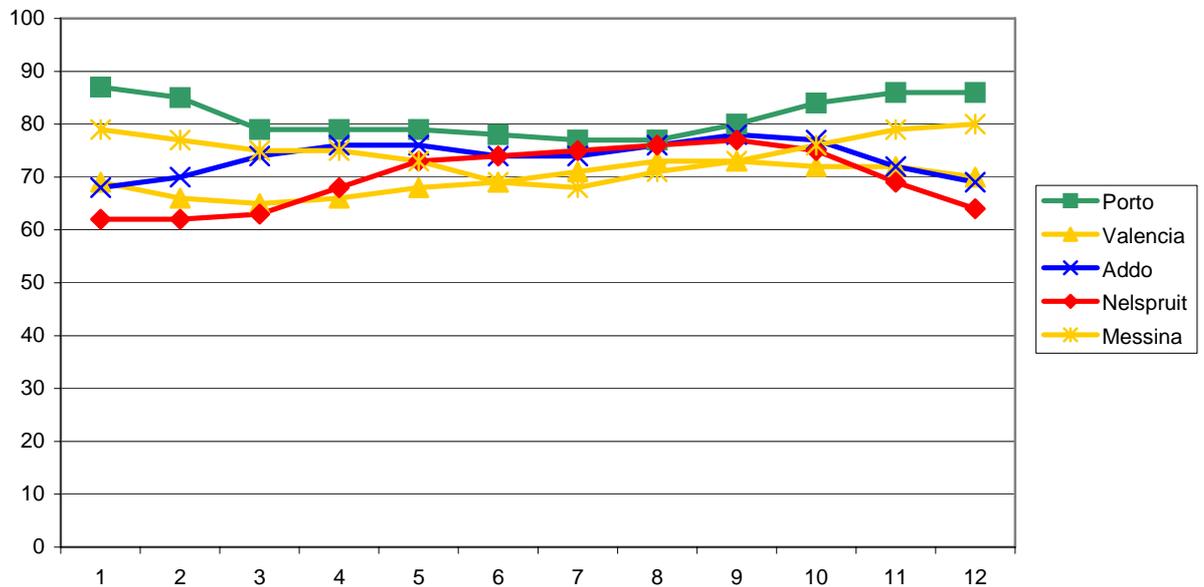


Figure 5. Monthly relative humidity at 9AM. Climate data extracted from grid cells nearest locations in Europe and South Africa (Source: CRU 1961-90 world climatology 10 minute lat-long resolution).

The climate data have also been plotted on a phenological time scale. This has been done to compare climatic conditions at the different locations when susceptible stages of the host are present. The same data as in the previous figures have been plotted to show the crop phenology for sweet oranges (*C. sinensis*) and mandarins (*C. reticulata*) (e.g. “f.s. -6” denotes six months before fruit set, and so on). Fruit set in the Mediterranean occurs around the beginning of May (Agustí, 2000) and in South Africa around the beginning of October (Villiers and Joubert, 2006).

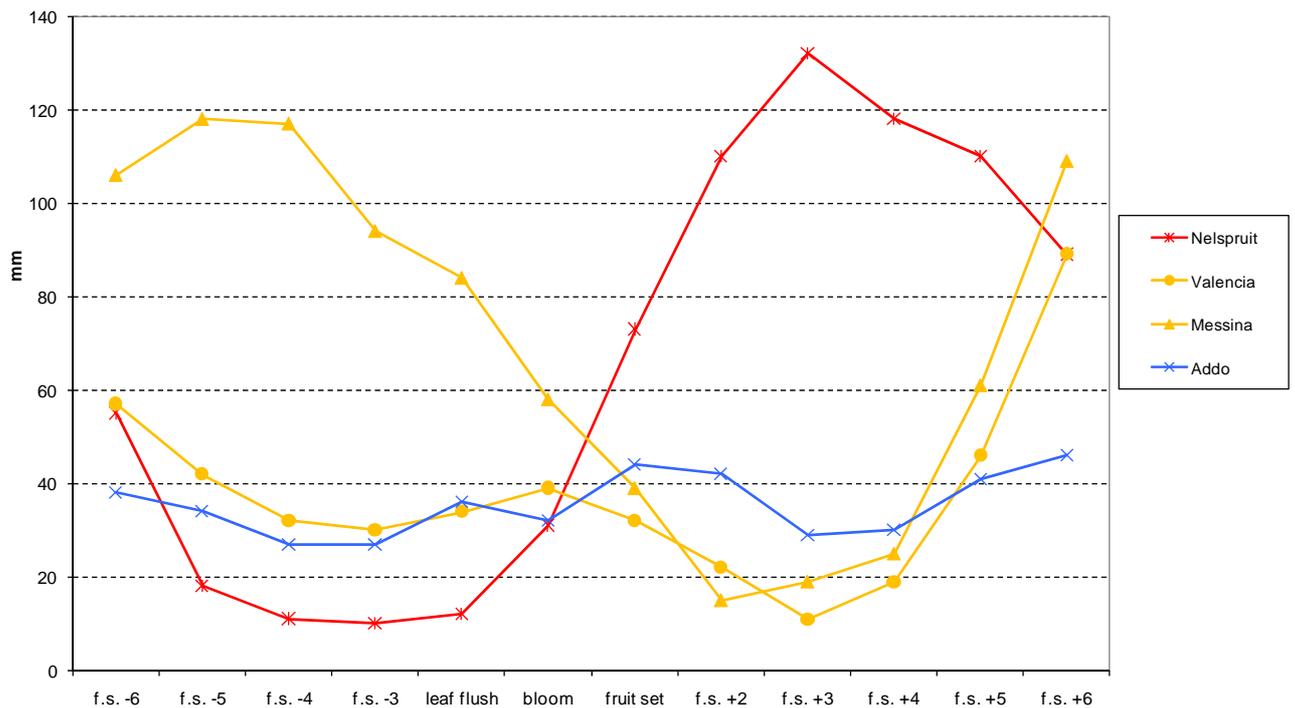


Figure 6. Monthly rainfall by citrus phenological stage. Climate data extracted from grid cell nearest locations in Europe and South Africa (Source: CRU 1961-90 world climatology 10 minute lat-long resolution).

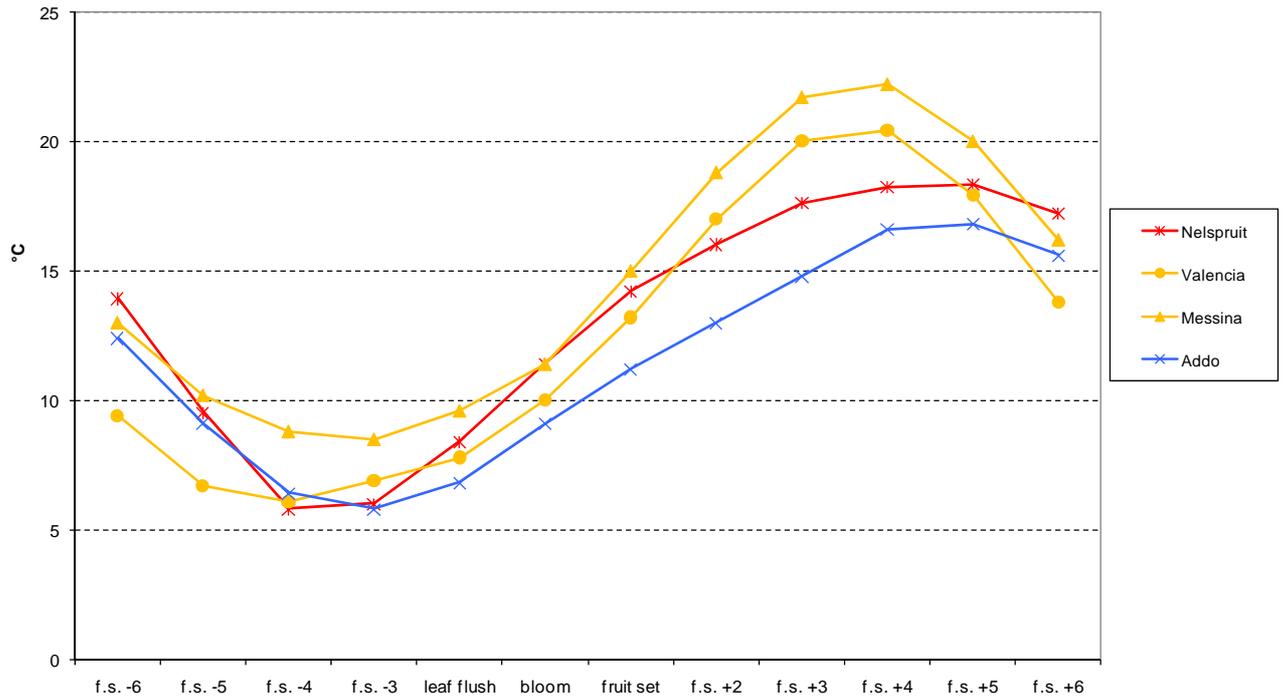


Figure 7. Monthly minimum air temperature by citrus phenological stage. Climate data extracted from grid cell nearest locations in Europe and South Africa (Source: CRU 1961-90 world climatology 10 minute lat-long resolution).

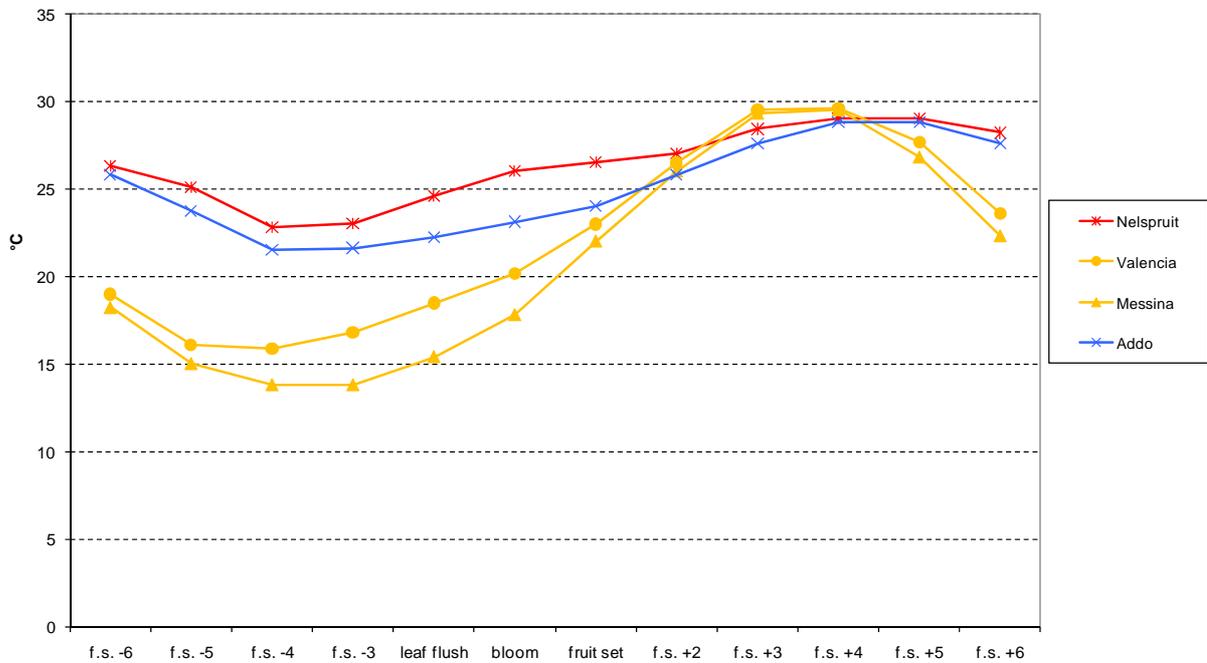


Figure 8. Monthly maximum air temperature by citrus phenological stage. Climate data extracted from grid cell nearest locations in Europe and South Africa (Source: CRU 1961-90 world climatology 10 minute lat-long resolution).

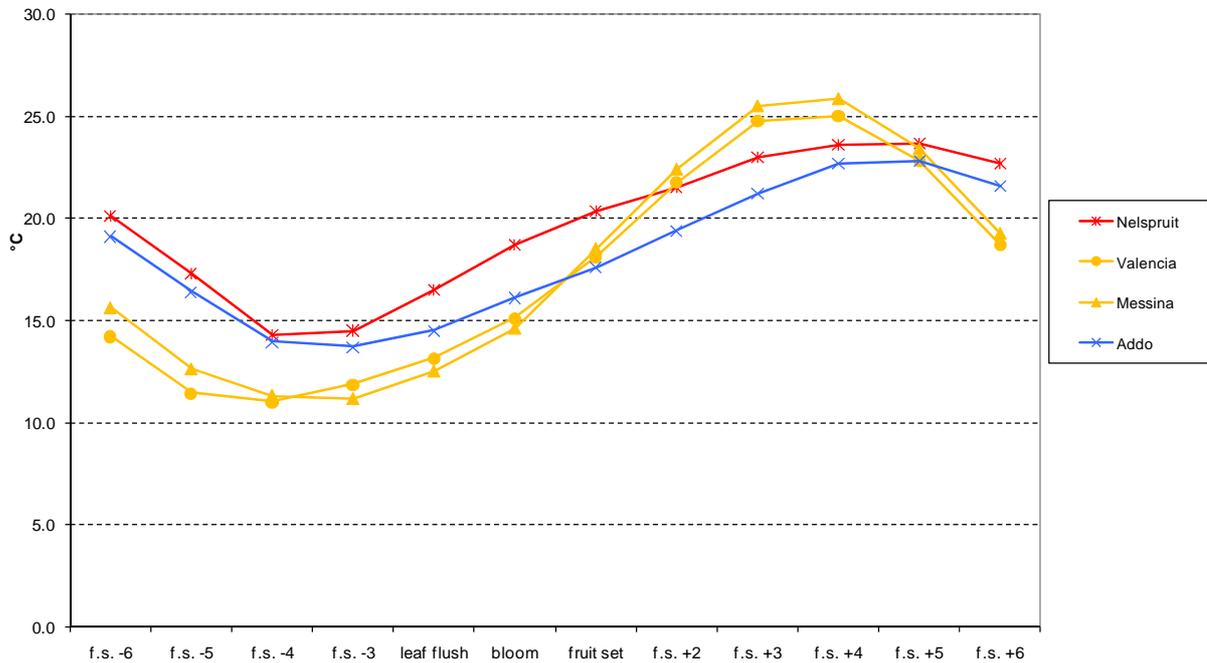


Figure 9. Monthly mean air temperature by citrus phenological stage. Climate data extracted from grid cell nearest locations in Europe and South Africa (Source: CRU 1961-90 world climatology 10 minute lat-long resolution).

The graphs confirm that the rainfall pattern is very different for Nelspruit, but similar for Addo, Valencia, Porto and Messina during the three hottest months of the year. However Porto has only two relatively dry months. Compared to Addo, Valencia has 20-50 mm less rain in the three hottest months but a wetter autumn. Messina has 10-40 mm less rain in the hottest months but a wetter autumn, winter and spring. Porto has a similar amount of rain in the hottest months but much more rain throughout the rest of the year. For minimum and maximum temperatures, Porto is coolest throughout the year. The South African sites have higher maximum temperatures in autumn, winter and spring, so, for example, Addo's mean monthly temperatures are approximately 3 °C higher than Valencia in winter. However monthly minima are similar throughout, although Messina and Valencia have higher minima in summer. Low temperatures and the absence of daily fluctuations have been demonstrated to delay the maturation of pseudothecia and ascospores on the leaf litter (Kiely, 1948; Kotzé, 1981; Lee and Huang, 1973). However, with the information available it is not possible to quantify the duration of this delay. Citrus leaves are shed all year around and, depending on temperature and wetting regimes, pseudothecia and ascospores can develop in as quickly as 40 days (Kotzé, 1981; Lee and Huang, 1973; McOnie, 1964c). Thus, the lower daily temperature maxima in the Mediterranean are likely to delay but not prevent ascospore production.

Limitations of the South African application of the CLIMEX Compare Locations technique

In addition to the general limitations of CLIMEX for predicting the potential distribution of *G. citricarpa* described above, the Panel has some concerns over the application of the CLIMEX “compare locations” technique used by Paul *et al.* (2005) and Paul (2006):

- The 1931-1960 climatic data employed are over 40 years out of date and are unlikely to reflect current conditions in CBS locations. In appendix A of Paul (2006), the 1961-1990 global climatology (New *et al.*, 1999) was employed but this dataset was not used to obtain the CLIMEX parameters and set the favourability thresholds. The 1961-90 dataset also does not summarise current climatic conditions.
- Thirty year averages mask the annual variability (Jarvis and Baker, 2001).
- Apart from a general description that parameters were adjusted iteratively to fit the current distribution of CBS with particular attention paid to “areas around Nelspruit in South Africa where the disease has been known to occur in serious epidemic proportions”, the procedure used to produce the parameters is not described in detail. It is therefore not clear to what extent parameters were varied to create an optimal synchrony between EIs at South African locations and the distribution and severity of CBS. However, their emphasis on areas around Nelspruit where the disease is particularly severe and the resulting map of ecoclimatic indices (EIs) for South Africa which gives very low or zero EIs at locations in Eastern Cape Province, such as Addo, where CBS is present but not severe, implies that the Eastern Cape locations were not taken into account in selecting the parameters. This is an important omission since the objective is to map the potential for CBS establishment not just severe impacts.
- The parameters selected produce very low or zero EIs at other South African locations where CBS has been recorded in addition to the three areas in Eastern Cape Province: principally along the Kwazulu-Natal coast. As Paul *et al.* (2005) note, presence in Taiwan is also not predicted. This may be due to the unrepresentative location of weather stations but very low EIs were also calculated at Darwin and Katherine in the Northern Territory of Australia where CBS has also been recorded.
- Cold stress parameters were used to constrain the distribution to north-eastern South Africa. However, to do this a very high 17 °C limiting low temperature and a cold stress threshold of 11°C had to be used and this bears little relation to what is known about the ability of *G. citricarpa* to survive and develop at low temperatures. *G. citricarpa* was readily isolated from fruit maintained at 8 °C for up to 40 days (Agostini *et al.*, 2006) and strains are usually stored in culture collections on dry filter papers at -20 °C (Peres *et al.*, 2007).
- Dry stress parameters are not included, a surprising omission considering the importance of moisture in the disease cycle.
- To provide support for the parameters selected, a sensitivity analysis would be helpful.
- Irrigation in many areas of the world is essential for viable commercial citrus production. Some methods of irrigation, e.g. by flooding the soil, may greatly enhance the suitability of locations for CBS that have low EIs. This is recognised by Paul *et al.* (2005).
- Thresholds of climatic suitability for the EI are arbitrary and species specific and therefore need to be set by the author (Stephens *et al.*, 2007). An EI threshold of 4 for climatic suitability for establishment is based on the absence of CBS in the south-western part of Western Cape Province and inland New South Wales. However, as noted above, it is present in other locations, e.g. Eastern Cape Province, with very low or zero EIs. As such,

the threshold of 4 is inappropriate and needs to be revised. Without reparameterising CLIMEX, it would be more appropriate to set the threshold for the climatic suitability for establishment at 1.

- The map legends do not give ranges so it must be assumed that an EI of “4” is equivalent to EIs between 1 and 4. With an EI of 4 or below, Paul *et al.* (2005) state that the climate is unfavourable for the persistence of the species. For EIs between 5 and 10, she considers the climate to be marginally suitable for disease development. However, logically, the next category should be described as “marginally suitable for the persistence of the species” since CLIMEX is modelling the potential distribution of species rather than the manifestation of disease. However, as noted above, by only taking into account the locations in north-eastern South Africa where the disease is serious when selecting the CLIMEX parameters, the thresholds chosen are likely to be too high for persistence.

2.1.4. Conclusions on the South African assessment of climate suitability

The Panel concluded that there are a number of difficulties with the application of CLIMEX by Paul *et al.* (2005) and that the CLIMEX programme itself has limitations for organisms like *G. citricarpa* because critical stages in a pest’s life cycle are dependent on climatic factors such as leaf wetness that are of short duration. To determine the importance of some of these factors, exploratory analyses were undertaken by running CLIMEX with recent data from EU weather stations and interpolated grids (section 2.2) and employing a generic infection model for foliar fungal pathogens which includes leaf wetness as an input variable (Magarey *et al.*, 2005) (see section 2.3).

2.2. Exploratory analysis of CLIMEX with new European data

To investigate the importance of some of the concerns noted above, exploratory analyses were undertaken to determine the importance of using:

- station data compared to interpolated climatic data in areas of citrus cultivation;
- recent climatic data (1990-2007 for station data; 1998-2007 for interpolated climatic data), including overall and yearly average values.

Although the Panel has serious concerns about the parameters selected by Paul *et al.* (2005), the Panel did not attempt to produce a new set or conduct a sensitivity analysis partly because of the time required and partly due to its general concerns over the degree to which the use of CLIMEX is appropriate to predict the potential distribution of this pathogen.

2.2.1. CLIMEX analysis with parameters from Paul *et al.* (2005) and EU station data (1990-2007)

The objective was to run CLIMEX using the same parameter values published by Paul *et al.* (2005) but with higher resolution interpolated data and recent climatic station data representative of the citrus growing areas in the EU.

2.2.1.1. Material and methods

To provide a general picture of the area of citrus crop production in the EU, the area in hectares planted with citrus in the regions of the EU was obtained from Eurostat and mapped (see Figure 10). To select the 50 km grid cells that represent the EU citrus growing area and identify reliable weather stations, a high resolution (1 km²) map of areas with more than 1% citrus tree coverage was provided by the Agr4cast group, Agriculture Unit, Institute for the protection and security of the citizens, Joint Research Center of the EU Commission (JRC)³, and overlaid with the 50 km grid boundaries and the locations of weather stations with greater and less than 80% of observations per year (see Figures 11-13).

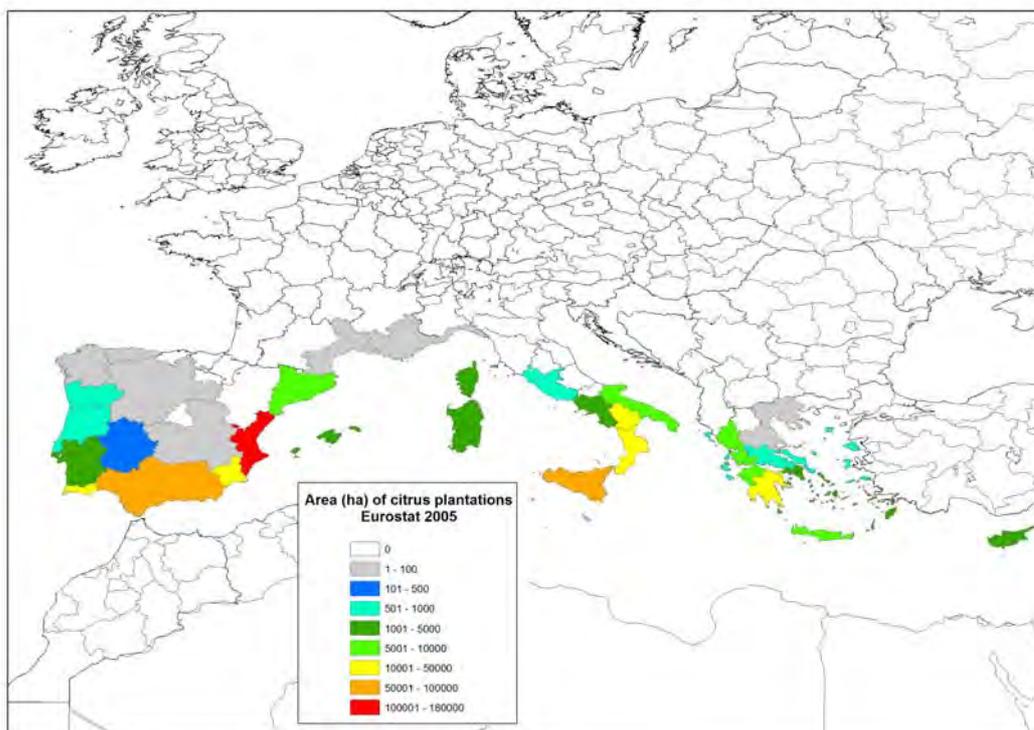


Figure 10. Area (hectares) of citrus plantations for EU Regions (Eurostat, year 2005).

³ Joint Research Center of the EU Commission, Institute for the protection and security of the citizens, Agriculture Unit, Agr4cast TP 483, Ispra (VA), Italy.

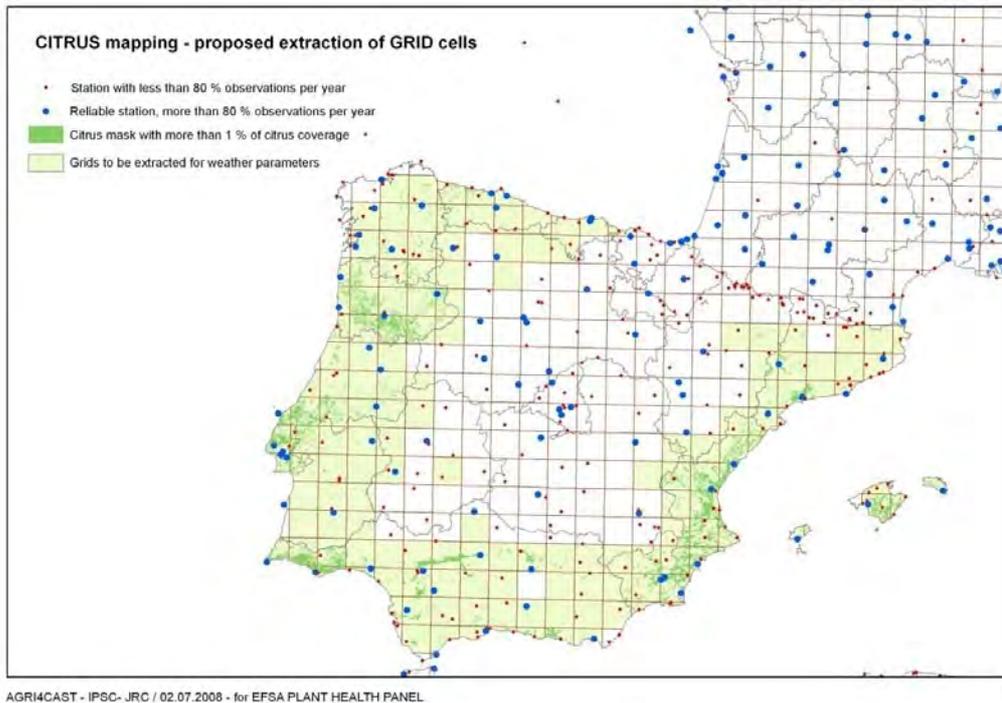


Figure 11. Area of citrus at 1 km resolution with greater than 1% citrus production, 50 km grids representing citrus production and locations of reliable weather stations in Spain and Portugal (JRC, 2008).

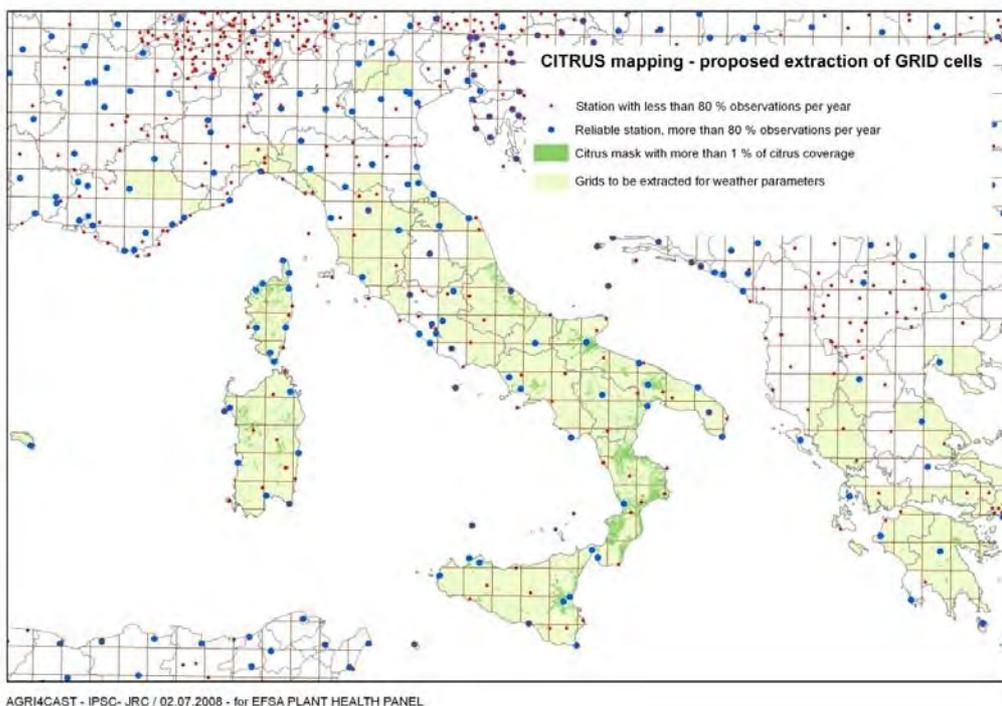


Figure 12. Area of citrus at 1 km resolution with greater than 1% citrus production, 50 km grids representing citrus production and locations of reliable weather stations in Italy and France (JRC, 2008).

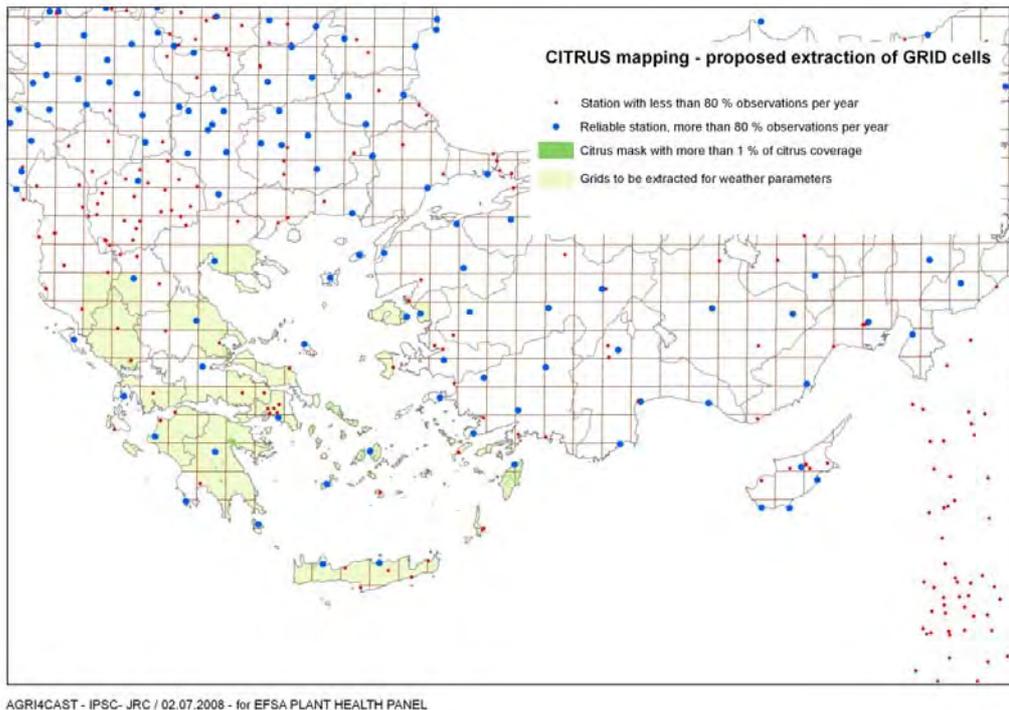


Figure 13. Area of citrus at 1 km resolution with greater than 1% citrus production, 50 km grids representing citrus production and locations of reliable weather stations in Greece (JRC, 2008).

The CLIMEX EI was then calculated with the CBS parameters published by Paul *et al.* (2005) and mapped for three datasets:

- (i) the 10 minute latitude \times longitude interpolated 1961-1990 Climatic Research Unit global monthly climatology (New *et al.*, 2002) available free with CLIMEX version 3. This is at a higher resolution than the 0.5° latitude \times longitude dataset used by Paul (2006). (Figure 14);
- (ii) daily data for 1990-2007 from 64 reliable weather stations in the citrus production areas of Europe with the meteorological parameters required by CLIMEX provided by the JRC and converted to CLIMEX. Twenty nine weather stations had a complete 18 year daily coverage (see Appendix Tables 1 and 2). The data were exported to ArcGIS (© ESRI) and mapped. (Figures 15-16);
- (iii) 418 grids at 50 km resolution selected to represent citrus cultivation in the EU with ten years (1998-2007) of interpolated daily data converted to CLIMEX. JRC produced an additional interpolation for relative humidity (see Appendix section 2.) to ensure the data could be loaded into CLIMEX. Maps of the CLIMEX ecoclimatic index (EI) were produced for the average climate during the period 1998-2007, for each year from 2007-1998 and to show the number of years the EI was above zero, 5 and 11 during the 10 year period (Figures 17-20).

2.2.1.2. Results: CLIMEX maps produced following analysis with recent European data

The maps representing the CLIMEX ecoclimatic index in Europe are summarised in the following table. Maps for each of the ten years (1998-2007) of ecoclimatic index are given in Appendix Fig. 1-10.

Table 2. Summary of CLIMEX maps for EU.

Figure	Climatic dataset	Resolution	Time scale	Locations	Map
Fig. 14	Climatic Research Unit	Monthly 10° lat x lon	1961-90	EU-wide	Ecoclimatic index >0
Fig. 15	JRC	Daily station data	1990-2007	29	Ecoclimatic index >0
Fig. 16	JRC	Daily station data	1990-2007	64	Ecoclimatic index >0
Fig. 17	JRC	Daily 50 km	1998-2007 average	418	Ecoclimatic index >0
Fig. 18	JRC	Daily 50 km	1998-2007 average	418	Years with ecoclimatic index >0
Fig. 19	JRC	Daily 50 km	1998-2007 average	418	Years with Ecoclimatic Index ≥ 5
Fig. 20	JRC	Daily 50 km	1998-2007 average	418	Years with ecoclimatic index ≥ 11
Appendix Fig. 1-10.	JRC	Daily 50 km	1998-2007 (10 annual maps)	418	Ecoclimatic index >0

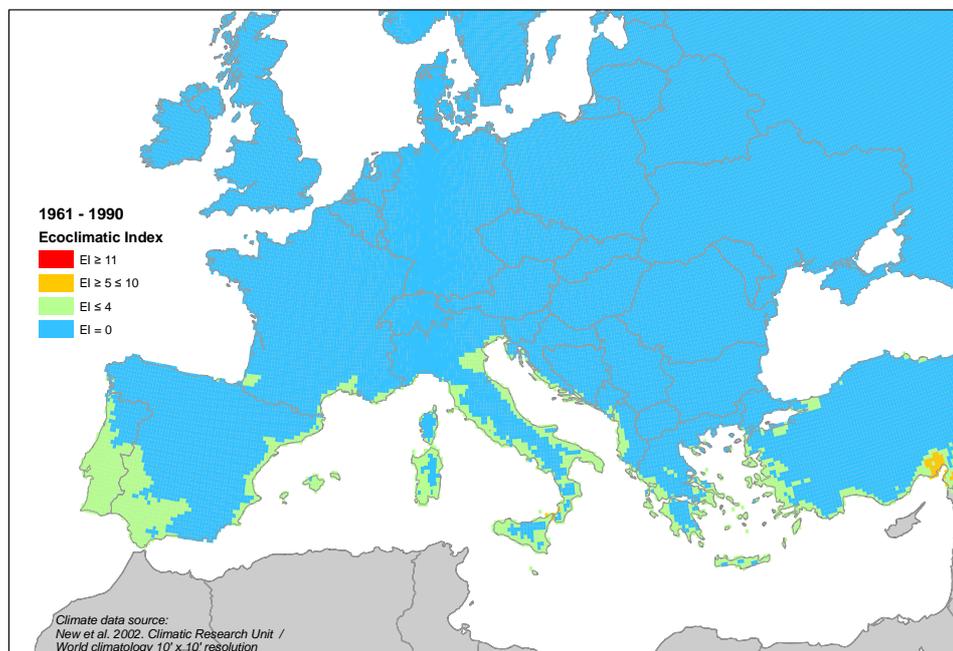


Figure. 14 CLIMEX ecoclimatic index for *G. citricarpa* based on the CRU 1961 – 1990 ten minute latitude × longitude spatial resolution with parameters from Paul *et al.* (2005).

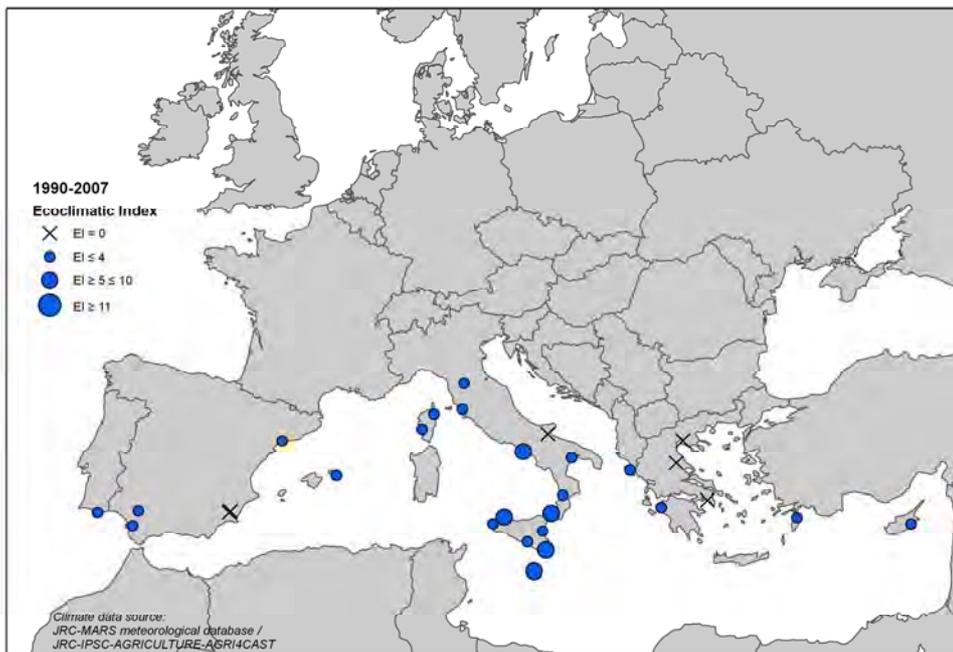


Figure 15. CLIMEX ecoclimatic index for *G. citricarpa* based on the 29 weather stations having complete data for the 18 year period 1990-2007 from the JRC-MARS meteorological database.

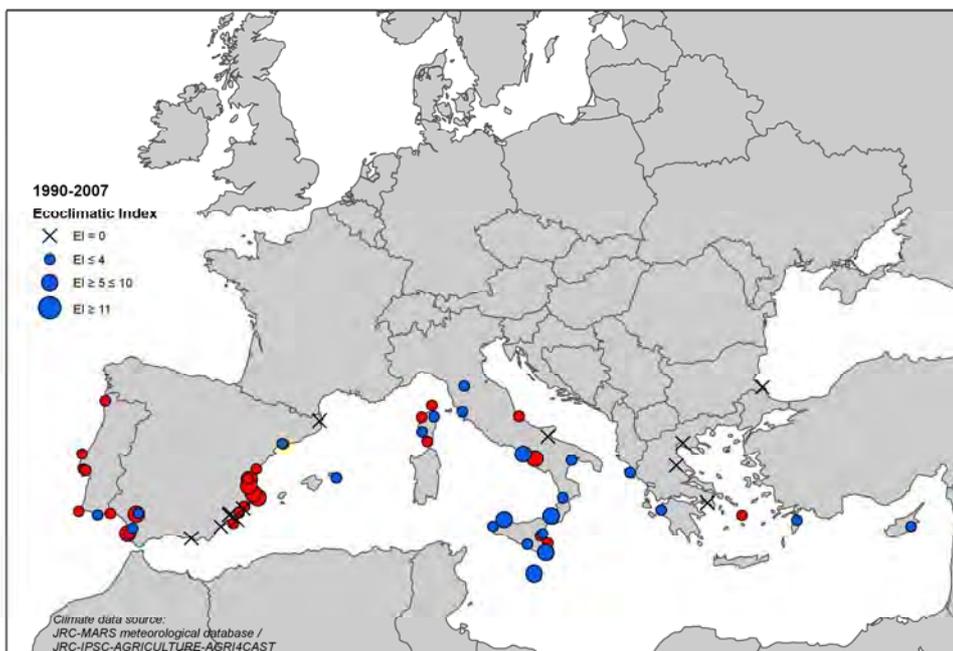


Figure 16. CLIMEX ecoclimatic index for *G. citricarpa* based on the 29 weather stations (blue colour) with complete data, and for 35 stations with incomplete data (red colour) for the 18 year period 1990-2007 from the JRC-MARS meteorological database.

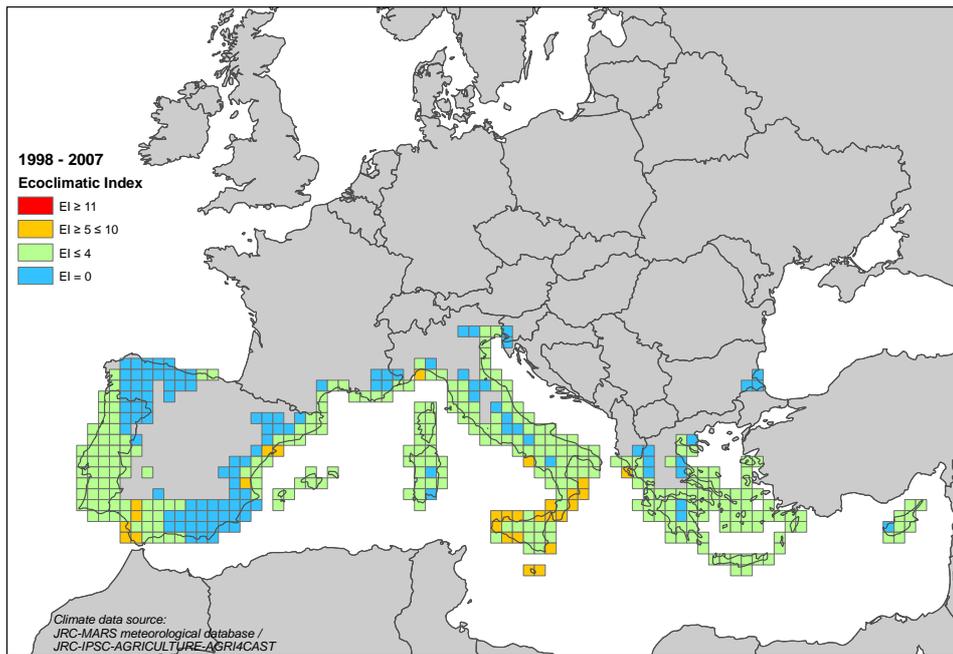


Figure 17. CLIMEX ecoclimatic index for *G. citricarpa* based on mean 1988-2007 climatic data from the JRC-MARS meteorological database interpolated to 418 grids at 50 km resolution representing the area of citrus production in the EU year period.

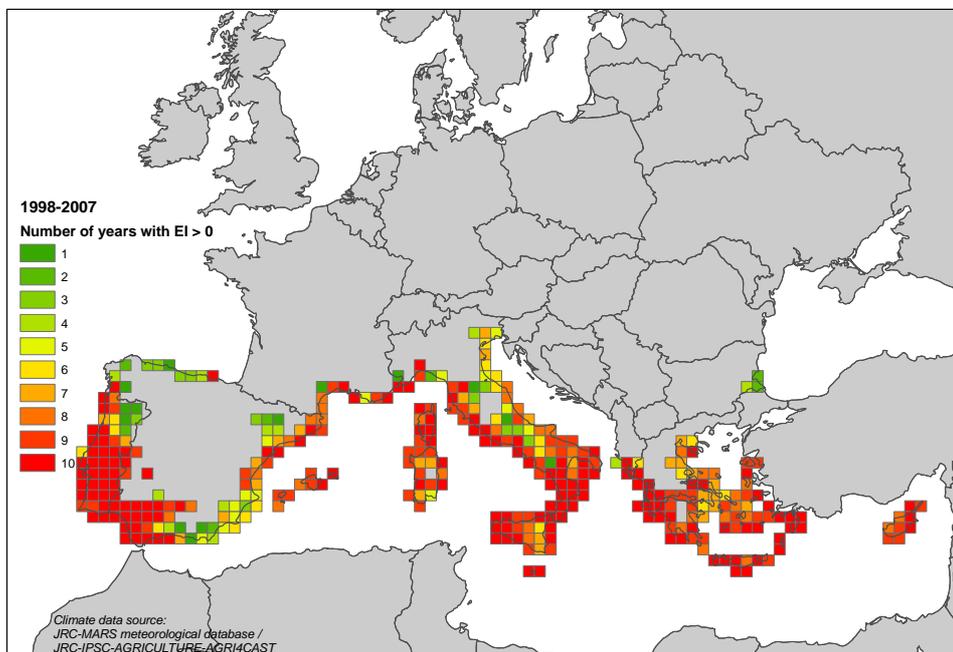


Figure 18. Number of years from 1998-2007 for which the CLIMEX ecoclimatic index for *G. citricarpa* is greater than zero based on climatic data from JRC-MARS meteorological database interpolated to 418 grids at 50 km resolution representing the area of citrus production in the EU year period.

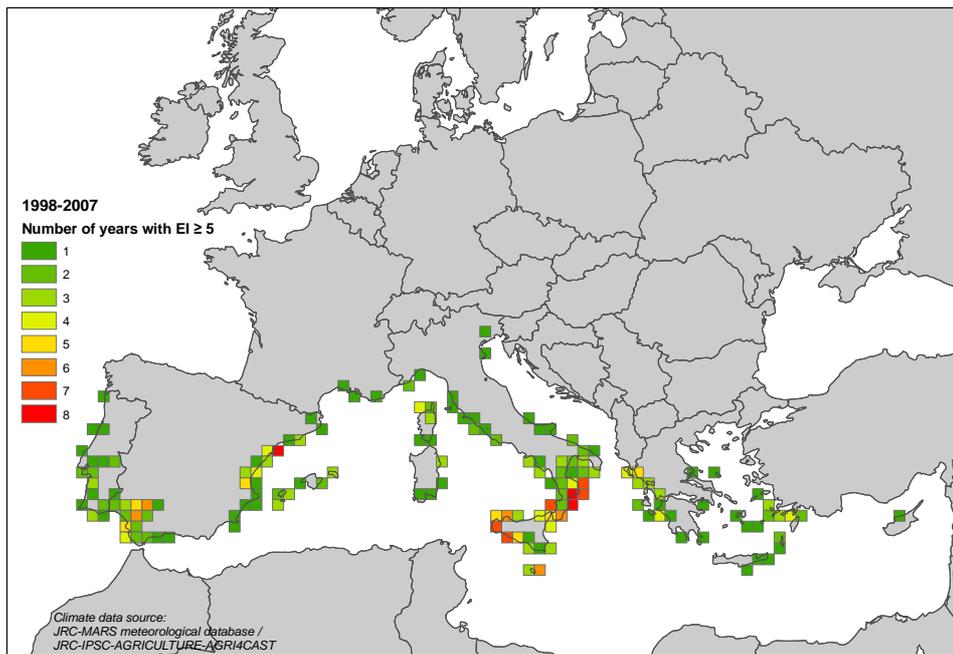


Figure 19. Number of years from 1998-2007 for which the CLIMEX ecoclimatic index for *G. citricarpa* is greater than 5 based on climatic data from the JRC-MARS meteorological database interpolated to 418 grids at 50 km resolution representing the area of citrus production in the EU year period.

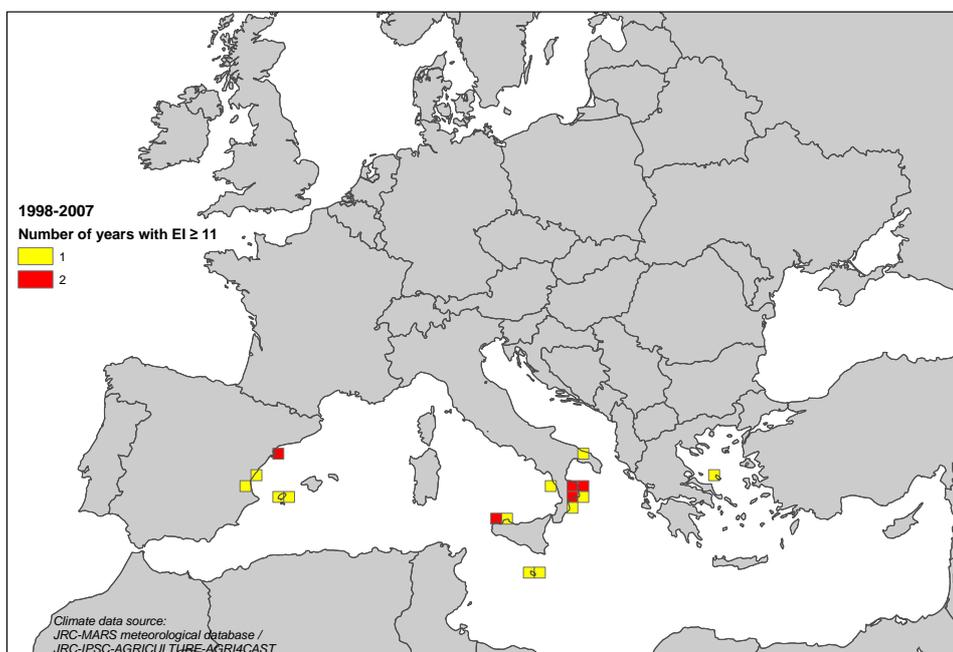


Figure 20. Number of years from 1998-2007 for which the CLIMEX ecoclimatic index for *G. citricarpa* is greater than 5 based on climatic data from the JRC-MARS meteorological database interpolated to 418 grids at 50 km resolution representing the area of citrus production in the EU year period.

2.2.1.3. Summary of the results of the CLIMEX analysis with recent European data

Using the 0.5° latitude × longitude interpolated 1961-1990 CRU global climate dataset, Paul (2006) found only one location in Turkey with an ecoclimatic index greater than 5. Using the 10 minute latitude × longitude interpolated 1961-1990 CRU global climate dataset, the Panel found 28 grid cells with an ecoclimatic index greater than 5. Four of these were in the straits of Messina (Sicily), the remainder in south-east Turkey (near Adana). Additional locations were found on the Eastern Mediterranean coast.

For the 1990-2007 JRC-MARSTAT weather station data imported to CLIMEX, 12 out of 64 locations had ecoclimatic indices $EI \geq 5 \leq 10$, determined to be marginally suitable for CBS development by Paul *et al.* (2005). The remaining 42 stations had either $EI = 0$ (12 stations) or $EI > 0 \leq 4$ (40 stations). No stations were predicted to have conditions favourable for disease development ($EI \geq 11$) (Appendix Table 1).

For the locations with complete data, i.e. data from all 18 years 1990- 2007, 5 out of 29 stations were predicted to be marginally suitable for CBS development, i.e. with ecoclimatic indices $EI \geq 5 \leq 10$. The remaining 24 stations had either $EI = 0$ (6 stations) or $EI > 0 \leq 4$ (18 stations). (Appendix Table 2).

For the JRC interpolated data (average 1998-2007), 28 out of 418 grid cells had ecoclimatic indices $EI \geq 5 \leq 10$, determined to be marginally suitable for citrus black spot disease development by Paul *et al.* (2005). The remaining 390 cells had either $EI = 0$ (108 grid cells) or $EI > 0 \leq 4$ (282 grid cells). No grid cells were predicted to have conditions favourable for disease development ($EI \geq 11$). One hundred and fifty two grid cells had an $EI > 0$ in all 10 years. ($EI > 1 = 27$, $EI > 2 = 6$, $EI > 3 = 0$).

2.2.1.4. Conclusions of the CLIMEX analysis with recent European data

In conclusion, it is clear that, with the same CLIMEX parameter values selected by Paul *et al.* (2005) and the same thresholds for favourability but with recent weather station and gridded data averaged over the last 10-18 years, some parts of the EU citrus production area are “marginally suitable for disease development”. No areas of EU citrus production are “favourable or highly favourable for disease development”. In some years and in some locations these thresholds are exceeded, indicating that in these years and locations, climate is “favourable for disease development”. However a number of problems have been identified. The parameter values selected to run CLIMEX and the CLIMEX ecoclimatic index thresholds chosen for persistence and disease development by Paul *et al.* (2005) appear to be based only on locations in north-eastern South Africa where the disease is serious and do not take into account the situation in the Eastern Cape Province, where the pathogen is present but where it is assumed the disease is not so important. This has major implications for the extrapolation to Europe because at least one of the Eastern Cape Province locations has temperature and rainfall conditions which are relatively similar to some citrus growing areas of the EU.

In addition, the lower limiting temperature and the cold stress temperature threshold parameters for CLIMEX are set very high by Paul *et al.* (2005) and are not related to published information on cold temperature survival for *G. citricarpa* (Agostini *et al.*, 2006; Peres *et al.*, 2007). If these temperature thresholds are reduced to more biologically appropriate levels, this will increase the ecoclimatic index at European locations.

Citrus orchards in Europe generally have lower maximum winter temperatures (but similar minimum temperatures) compared to those in South Africa. This is unlikely to affect survival but may delay the maturation of pseudothecia and ascospores (Kotzé, 1981; Lee and Huang, 1973). The time required for ascospore maturation under a broad range of temperature and wetting regimes has not been studied and so, outside endemic areas, it is difficult to estimate

the potential periods of ascospore release. However the maturation of ascospores on infected leaves shed during late autumn, winter and early spring occurs almost simultaneously in early summer (Kotzé, 1963; Lee and Huang, 1973; McOnie, 1964c). In some cases, leaf litter may decompose before ascospores mature (Kotzé, 1981) but severe epidemics have also been reported in areas where ascospores were produced only on leaves shed in spring and early summer, because leaves abscised in winter were completely decomposed at the time of infection (Lee and Huang, 1973). The effect of any delay in development caused by lower winter temperatures in Europe is unlikely to prevent the production of spores during all the vulnerable phenological stages of the host, assuming suitable environmental conditions. This aspect is investigated further in the section on leaf wetness.

The CLIMEX parameter values need to be modified to take these factors into account but, even so, it is clear that the thresholds for pathogen persistence are set too high and that, therefore, the establishment potential for *G. citricarpa* in the EU is greater than that estimated by Paul *et al.* (2005). Many of the locations where significant ecoclimatic indices have been calculated are in areas of extensive citrus cultivation. The Panel cannot therefore agree with the conclusion by Paul *et al.* (2005) that the climate of the EU is unsuitable for *G. citricarpa* establishment.

Further work needs to be undertaken to determine the extent to which serious impacts are likely to occur if the pathogen became established in the EU. Evidence from locations, such as those in Eastern Cape Province, where *G. citricarpa* is presumed to cause less serious epidemics compared to north-eastern South Africa will be invaluable.

2.3. Modelling climate suitability for *G. citricarpa* by the simple generic infection model for foliar fungal plant pathogens.

CLIMEX cannot readily be used to analyse specific periods of the year when the host is at a susceptible stage and inoculum is potentially available. Moreover, it cannot directly take into account the effect of leaf wetness, a critical environmental variable for the successful infection of most fruit and foliage fungal pathogens including *G. citricarpa* (Kotzé, 1963 and 1981).

Therefore, additional simulations were performed using a different model; the “simple generic infection model for foliar fungal plant pathogens” developed by Magarey *et al.* (2005). This model requires estimates of the three cardinal temperatures and of two wetness duration thresholds, and computes the leaf surface wetness duration requirement. It is especially helpful for modeling pathogens for which extensive epidemiological data are unavailable (Magarey *et al.*, 2005). The generic infection model has been recently implemented in the NAPPFAST system and is being used by USDA-APHIS to create pest risk maps (Magarey *et al.*, 2007). The model predicts climate suitability based on the environmental requirements for infection by the target pathogen. The outputs must therefore also take into account periods of host susceptibility and the potential availability of inoculum.

In the present study, pycnidiospore and ascospore infection were modelled separately, since they do not have the same climatic requirements and play different roles in the introduction and spread of the pathogen into a new area. In the absence of trade in propagation material, prohibited from entering the EU (Council Directive 2000/29/EC; see section 4.4.1.2.), *G. citricarpa* can enter only through imports of infected citrus fruit. The fungus sporulates on the fruit rind in the form of pycnidiospores, which can be disseminated short distances by water splash. Thus, the first infection cycle at the point of entry will necessarily be caused by pycnidiospores disseminating the pathogen from imported infected fruit to a susceptible host. Once the disease is established in one specific location, the further spread of the disease during subsequent growing seasons will depend on recurrent infection cycles by airborne ascospores.

2.3.1. The simple generic infection model for foliar fungal plant pathogens

The generic infection model (Magarey *et al.*, 2005) is based upon a temperature response function (Wang and Engel, 1998; Yin *et al.*, 1995) which is scaled to the surface wetness duration requirement. The model output represents the wetness duration required to achieve a critical disease intensity at a given temperature. The wetness duration requirement ($W_{(T)}$) at temperature T is computed as follows:

$$W_{(T)} = \min[W_{\min}/f(T), W_{\max}]$$

$$\text{with } f(T) = \left(\frac{T_{\max} - T}{T_{\max} - T_{\text{opt}}} \right) \left(\frac{T - T_{\min}}{T_{\text{opt}} - T_{\min}} \right)^{(T_{\text{opt}} - T_{\min}) / (T_{\max} - T_{\text{opt}})}, \text{ where } T_{\min} \text{ is the minimum temperature}$$

for infection, T_{\max} is the maximum temperature for infection, T_{opt} is the optimum temperature for infection, W_{\min} is the minimum value of wetness duration requirement for the critical disease threshold at any temperature, W_{\max} is the upper boundary on the value of $W_{(T)}$ because wetness is not always a rate-limiting factor, D_{50} is the duration of a dry period at relative humidities <95% that will result in a 50% reduction in disease compared with a continuous wetness period. T_{\min} , T_{\max} , T_{opt} , W_{\min} , W_{\max} , D_{50} are the six model parameters. The algorithms and parameters (see also section 2.3.2) used by the Panel for the simulation of ascospores and pycnidiospores infections are detailed in Appendix (Tables 12 and 14).

2.3.2. Parameter values for *G. citricarpa*

Magarey *et al.* (2005) provide parameter values for many foliar fungal plant pathogens but not for *G. citricarpa*. Parameter values used in Magarey and Borchert (2003) to estimate the infection periods for *G. citricarpa* were derived from infection data for pycnidiospores of the grape black rot pathogen, *Guignardia bidwellii* (Ellis) Viala and Ravaz (Spotts, 1977). Regarding the use of *G. bidwellii* parameter values, the Panel notes that high infection efficiencies with *G. bidwellii* at relatively low temperatures (10-15 °C) have been reported, both with ascospores and pycnidiospores (Ferrin and Ramsdell, 1977; Spotts, 1977). However the germination of *G. citricarpa* spores under this temperature regime is very poor (Kotzé, 1963; Noronha, 2002), thus much lower infection efficiencies than those reported for *G. bidwellii* would be expected. In addition, Magarey and Borchert (2003) stated that there is a good correlation between the temperature limits for the infection by pycnidiospores of *G. bidwellii* (10-32 °C) and those reported by Lee and Huang (1973) for the development of *G. citricarpa* ascomata (7-35 °C). In the case of *G. citricarpa*, the environmental requirements for ascospores production and infection are completely different. Ascomata and ascospores are produced in the leaf litter after periods of alternate wetting and drying driven by rainfall, irrigation or dew (Kiely, 1948; Lee and Huang, 1973; McOnie, 1964c). As long as the leaf litter does not decompose, the fungus is able to withstand a broad range of temperatures (Lee and Huang, 1973). However the temperature range for infection seems to be narrower (Timmer, 1999). For these reasons, therefore, the parameter values for *G. bidwellii* do not appear to be appropriate for *G. citricarpa*.

The Panel has reviewed the information available in the literature about the biology of *G. citricarpa* and has defined possible parameter values for this pathogen, both for infection by pycnidiospores and ascospores (see table below). As extensive studies on the relationship between temperature/wetness regimes and the infection of *G. citricarpa* are not available in the literature, parameters values were mainly obtained from published data on the germination of *G. citricarpa* spores (Kotzé, 1963; Noronha, 2002). Spore germination is required for infection,

thus environmental conditions that are not conducive for spore germination are also limiting for infection. Consequently, parameter values of T_{min} , T_{max} , W_{min} for spore germination should be comparable to those for infection. However the relationship between spore germination and disease intensity is non-linear (Gäumann, 1950). In most cases, high levels of disease intensity are observed even when the rate of spore germination is not at its maximum. Therefore, the value of T_{opt} for spore germination and infection do not necessarily coincide.

Data from Kotzé (1963) on the germination of *G. citricarpa* ascospores on potato dextrose agar media (PDA) under different combinations of temperature and wetness durations were used for setting the parameters values for infection by ascospores. Timossi *et al.* (2003) also provided data on ascospore germination, however the response to temperature and wetness duration in this study differs substantially from that reported by Kotzé (1963). Isolates used by Timossi *et al.* (2003) produced ascospores on artificial media which, according to Baayen *et al.* (2002), only occur with the homothallic non-pathogenic species *G. mangiferae*. No conclusive data on the identification of these isolates, such as PCR testing or the presence of yellow pigments in oatmeal agar, were provided. Therefore, isolates used by Timossi *et al.* (2003) were possibly misidentified. Kiely (1948) and McOnie (1967) also evaluated the germination of ascospores under different wetness durations, but only at 25 °C. McOnie (1967) obtained peak germination approaching 100% within 24 hours of incubation on PDA. The rate of appressoria formation was extremely variable, but most of them were observed from 24 to 48 hours of incubation. Contrary to the results obtained by Kotzé (1963) and McOnie (1967), Kiely (1948) did not obtain any germination after 24 hours of incubation on PDA and the highest rates of ascospores germination were observed only after 48 hours. However, since only one temperature was tested, the Panel considers that the data from Kiely (1948) and McOnie (1967) are of limited value for setting the parameters in the generic infection model. By inoculating fruit in the field, McOnie (1967) demonstrated that ascospores can infect with at least 15 hours of continuous wetness. However no records of the temperatures during the experiment were reported.

For pycnidiospores, data from Noronha (2002) on pycnidiospore germination and appressoria formation on polystyrene slides under different temperatures and incubation periods were used. Also Mendes *et al.* (2005) provided data on pycnidiospores germination, but the isolates tested were the same as those used by Timossi *et al.* (2003), which were possibly misidentified (see above). Preliminary experimental data from pycnidiospores infection studies on lemon seedlings were also considered (IAM-UPV, 2008).

The parameter values identified from these studies for the application in the generic infection model for foliar fungal plant pathogens are presented in Table 3. However, due to the small number of experimental data available, the Panel considers that there is high uncertainty regarding the parameter values, especially T_{min} , T_{opt} , W_{max} and D_{50} . Therefore, a sensitivity analysis was performed for these parameters (section 2.3.5).

Table 3. **Parameter values for pycnidiospores and ascospores of *G. citricarpa*.**

	T_{min}	T_{max}	T_{opt}	W_{min}	W_{max}	D_{50}
Pycnidiospores	10 °C (15 °C)	35 °C	25 °C (30 °C)	12 h (14 h)	35 h (48 h)	0, 3, 14 h
Ascospores	15 °C (12 °C)	35 °C	27 °C (29.5 °C)	15 h	38 h (46 h)	0, 3, 14 h

Note: the selected values are given in the upper row. The values between brackets in the lower row are the parameter values used in the sensitivity analysis.

With regard to T_{min} , Kotzé (1963) reported germination of ascospores at 15 °C. However this was the lowest temperature tested in the experiment so it is not possible to exclude infection at

lower temperatures. Thus, for sensitivity analysis a T_{min} of 12 °C was also considered [i.e. 20% less than T_{min} as selected from Kotzé (1963)].

For pycnidiospores, Noronha (2002) obtained 20 % of appressoria formation at 10 °C after 36 hours of incubation. Since this temperature may still be too low for infection, a T_{min} value of 15 °C (same as ascospores) was included in the sensitivity analysis.

As indicated by Magarey *et al.* (2005), T_{max} may be set at 35 °C when, as in the case of *G. citricarpa*, there is no information on the upper temperature limit for infection.

For both ascospores and pycnidiospores (Kotzé, 1963; Noronha, 2002), maximum rates of germination were obtained at temperatures higher than the T_{opt} values compiled by Magarey *et al.* (2005) for an extensive list of foliar fungal pathogens from temperate as well as from tropical areas. Thus, taking into account the non-linear relationship between spore germination and infection stated above, values for T_{opt} were selected accordingly.

With regard to the T_{opt} for ascospores, Kotzé (1963) obtained the highest germination rate at 29.5 °C, which was also the highest temperature tested. However the highest value for T_{opt} reported by Magarey *et al.* (2005) is 28 °C. The optimal temperature for the growth of *G. citricarpa* on liquid basal synthetic medium is 27 °C (Kotzé, 1981) and the optimal temperature for hyphal growth is 25-28 °C (Chiu, 1955). Therefore, the Panel used a value of 27 °C for T_{opt} for *G. citricarpa* ascospores and decided to include the T_{opt} value of 29.5 °C in the sensitivity analysis.

For pycnidiospores, Noronha (2002) obtained the highest rates of appressoria formation between 25 and 30 °C, with peaks of appressoria formation at 25 °C for most incubation periods. A peak of appressoria formation at 30°C was obtained only in the incubation period of 24 hours. Therefore, T_{opt} for pycnidiospores was set at 25 °C but a value of 30 °C was included in the sensitivity analysis.

With regard to W_{min} , McOnie (1967) demonstrated that ascospores can infect with at least 15 hours of continuous wetness. This value is supported by the finding of Kotzé (1963), who obtained 15.7% germination of ascospores after 15 hours of incubation at 29.5 °C, showing consistency between germination and infection data.

With regard to the selected W_{min} value for pycnidiospores, an appressoria formation rate of approximately 30% was observed after 12 hours of incubation (Noronha, 2002). Preliminary results from inoculations on lemon seedlings showed high levels of infection by *G. citricarpa* pycnidiospores with 14 hours of leaf wetness duration at 22.5 °C (IAM-UPV, 2008). Thus, W_{min} was set at 12 hours and a value of 14 hours was considered in the sensitivity analysis.

A W_{max} value of 38h was selected for ascospores according to the results of Kotzé (1963). As longer wetness durations were however not considered in this study, a W_{max} value of 46 hours [i.e. 20% more than the W_{max} value selected] was included in the sensitivity analysis.

No significant increase in the rate of appressoria formation from pycnidiospores was observed after 35 hours of incubation for most temperatures with the exception of 15 °C, where a peak was observed after 48 hours (Noronha, 2002). Therefore, a value of 35 hours was selected for W_{max} and a value of 48 hours was considered in the sensitivity analysis.

No information was found in the literature on the sensitivity of *G. citricarpa* to dry interruptions during infection. However, even in endemic areas such as Brazil where the

disease is particularly severe, wetness durations during the infection periods are shown to be in the order of 10 hours per day (Reis *et al.*, 2006). This suggests a lower requirement for leaf wetness duration than that reported in the literature or a potential for withstanding periods of dryness during the infection process. Therefore, the following values were considered for D_{50} : 0 hours for no interruption; 3 hours as a value which is often found in the literature as being a generally acceptable period of leaf wetness interruption (Rossi *et al.* 2007; Xu and Butt, 1993); 14 hours as a long interruption according to the meteorological data shown by Reis *et al.* (2006).

In the case of pycnidiospores, the Panel considered that the requirement for splash dispersal needs to be integrated in the model by considering only those potential infections starting with a rain event. The other possible starting events for splash dispersal of pycnidiospores (see section 3.1.5) are difficult to quantify and were therefore not considered in the implementation of this model.

2.3.3. Modelling climate suitability for *G. citricarpa* infection: preliminary tests with climatic data from the current pathogen distribution and from disease-free areas

2.3.3.1. Objective

Preliminary tests of the generic infection model, using the parameters values identified for *G. citricarpa*, were performed with climatic data from areas where *G. citricarpa* occurs outside Europe and from European citrus growing areas, where the citrus black spot disease is not known to occur. The aim was to assess the capability of the model to predict a high number of potential infections by *G. citricarpa* in sites where the disease is present. The results obtained for these sites were compared with simulations performed for several disease free sites in the EU citrus growing areas, using climatic data from the EU interpolated grid and from EU agrometeorological stations.

2.3.3.2. Material and methods

From areas of *G. citricarpa* current distribution outside Europe, climatic data from the following sites could be collected in the time frame available: Gairdah (Miles *et al.*, 2004; Paul *et al.*, 2005) and Maryborough (Paul *et al.*, 2005) in Queensland, Australia; Tucuman, El Comenar and San Miguel de Tucuman from Tucuman area in Argentina (Foguet *et al.*, 1985); Nelspruit and Addo in South Africa (Paul *et al.*, 2005; see section 2.1.3.2.). For the EU citrus growing areas, climatic data from four agrometeorological stations and seven EU grid cells were also analysed (Table 4). Data from the extra-European sites and from the EU agrometeorological stations included daily values of maximum and minimum air relative humidity. The weather data series from the EU grid did not include daily values of maximum and minimum air relative humidity, hence air relative humidity was estimated as described in Appendix section 2.2.2. For all sites, hourly data (including air relative humidity) were generated following the procedure described in Appendix section 2.2. and used as inputs to the generic infection model.

A complete model run included the following steps:

1. estimate / generate hourly values of weather variables;
2. estimate leaf wetness using the SWEB model;
3. run the generic infection model for ascospores and pycnidiospores of *G. citricarpa* with $D_{50}=0$, $D_{50}=3$ and $D_{50}=14$.

Table 4 Selected sites from areas where *G. citricarpa* occurs and from EU disease-free areas: geographical location and sample years analysed.

Site	Latitude	Longitude	Sample years analysed
Gaindah, Queensland (AU)	-25.6	151.6	2000-2003
Maryborough, Queensland (AU)	-25.5	152.7	2000-2002
Tucuman, Argentina	-26.8	-65.2	2004-2005
San Miguel de Tucuman, Argentina	-26.48	-65.13	2000
El Comenar, Argentina	-26.46	-65.10	2006-2007
Nelspruit, South Africa	-25.5	31	2003-2007
Addo, South Africa	-33.6	25.7	2003-2007
Sicily, Italy - GRID	37.70	14.93	2006-2007
Portugal (North) - GRID	42.08	-8.81	2006-2007
Portugal (South) - GRID	37.97	-8.82	2006-2007
Greece (South) – GRID	37.07	21.80	2006-2007
Greece (Center) – GRID	39.63	22.71	2006-2007
Valencia (Spain) - GRID	39.22	-0.58	2006-2007
Spain (North) - GRID	42.75	-5.47	2006-2007
Riposto, Italy	37.43	15.11	2006-2007
Siracusa, Italy	36.58	15.17	2006-2007
Caronia Buzza, Italy	38.02	14.28	2006-2007
Misilmeri, Italy	38.02	13.27	2006-2007

2.3.3.3. Results

Results of simulations are shown in Fig.21-26 and must be seen as sample comparisons of various steps of the analysis of climate suitability for *G. citricarpa* infection using the procedure implemented in this opinion. Although not exhaustive, due to the limited sample data set used, the graphs show that the simulation procedure yielded high values of potential infection at sites where the disease is endemic. The graphs also show a lower level for potential infection at EU sites.

The results obtained for potential infection events by ascospores are shown for the selected sites from areas where CBS occurs and from disease-free areas in fig. 21, 22 and 23 when $D_{50}=0, 3$ and 14h respectively. The number of potential infection events per year is shown on the y-axis for each site.

The results obtained for potential infection events by pycnidiospores are shown for the selected sites from areas where CBS occurs and from disease-free areas in fig. 24, 25 and 27 when $D_{50}=0, 3$ and 14 hours respectively. The number of potential infection events per year is shown on the y-axis for each site.

In relative terms, high values were obtained for sites from areas where CBS occurs outside the EU, confirming the climatic suitability of these areas and showing that the model behaves realistically. Generally, lower values were obtained for disease-free EU sites. However some of these were comparable to those from sites of current *G. citricarpa* occurrence (e.g. Addo in South Africa).

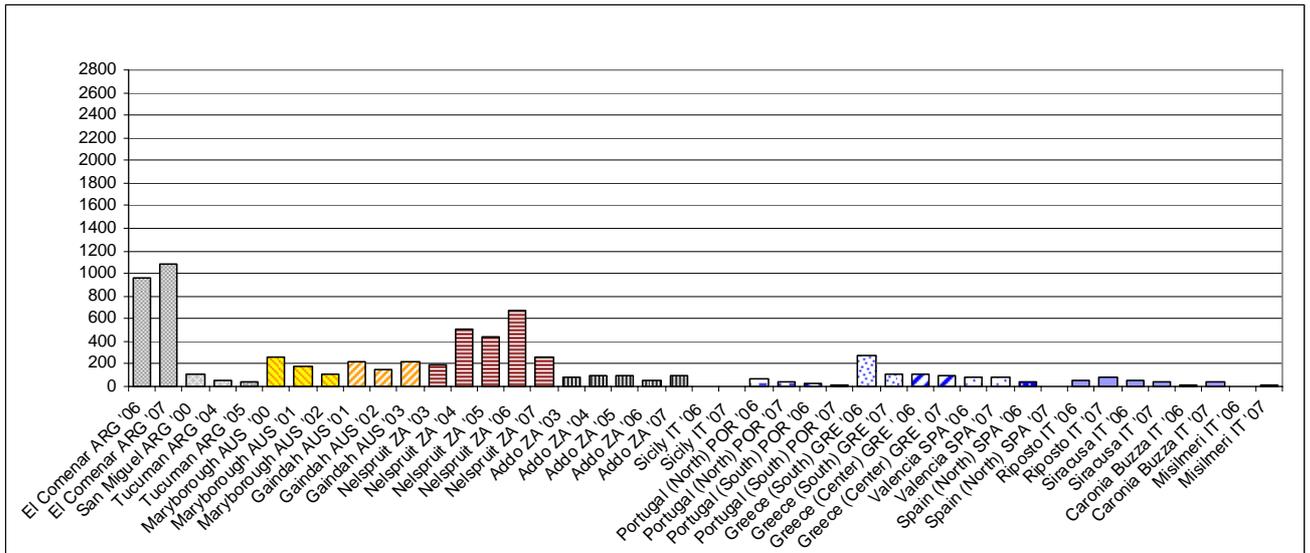


Figure 21. Number of potential infection events for *G. citricarpa* ascospores simulated for sites from areas where CBS occurs outside Europe and from disease-free sites in the EU with D50=0.

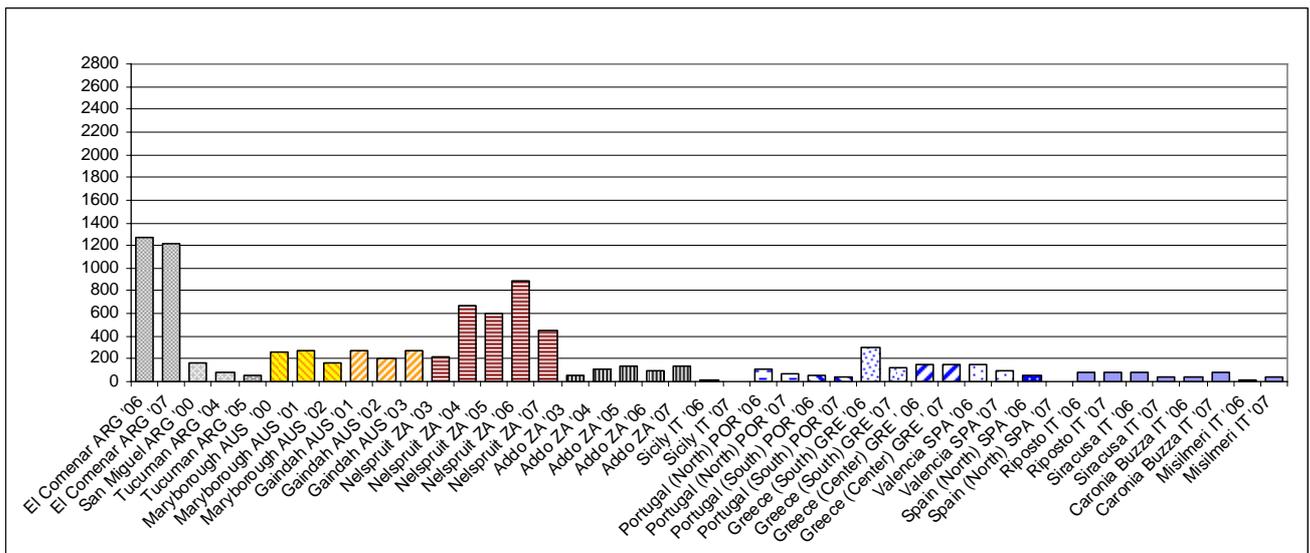


Figure 22. Number of potential infection events for *G. citricarpa* ascospores simulated for sites from areas where CBS occurs outside Europe and from disease-free sites in the EU with D50=3.

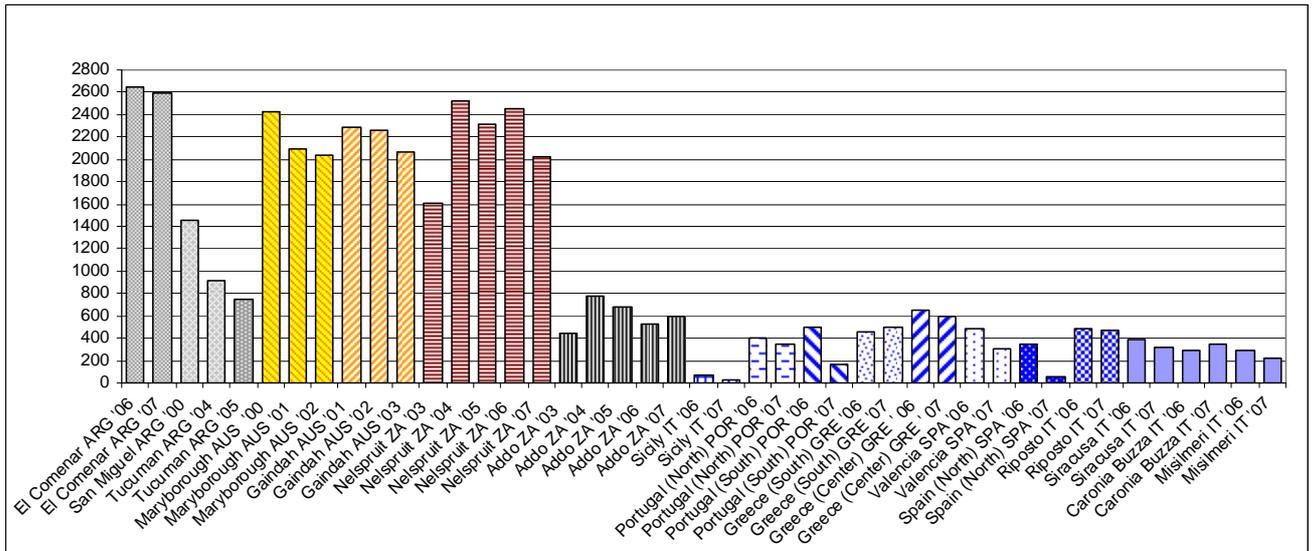


Figure 23. Number of potential infection events for *G. citricarpa* ascospores simulated for sites from areas where CBS occurs outside Europe and from disease-free sites in the EU with $D_{50}=14$.

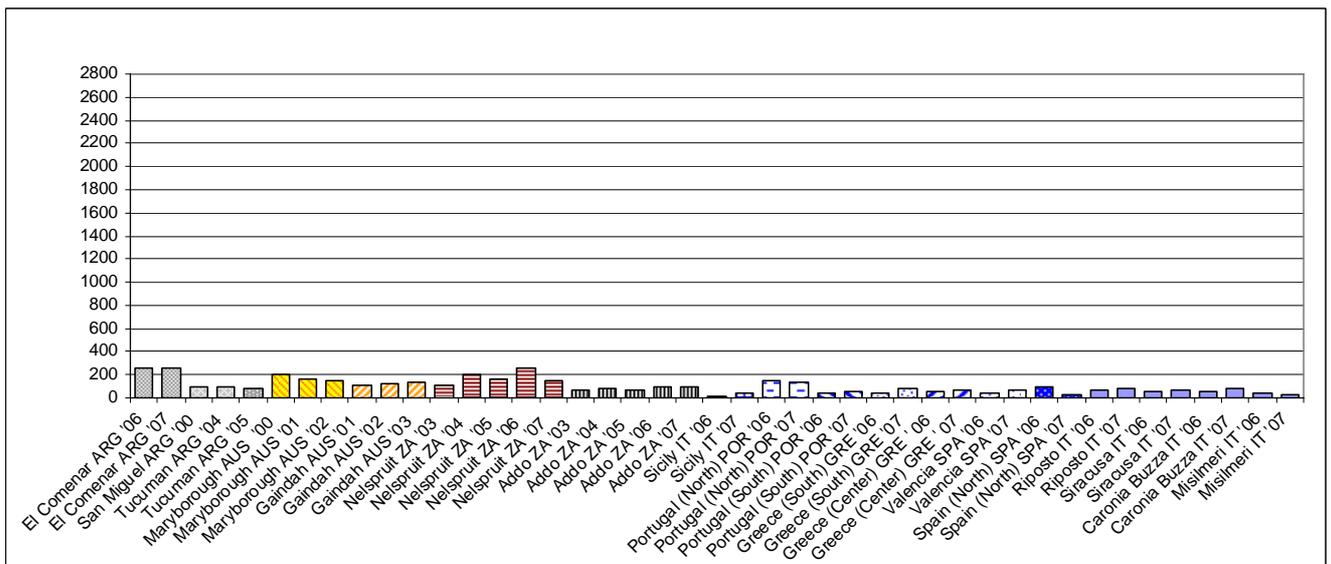


Figure 24. Number of potential infection events for *G. citricarpa* pycnidiospores simulated for sites from areas where CBS occurs outside Europe and from disease-free sites in the EU with $D_{50}=0$.

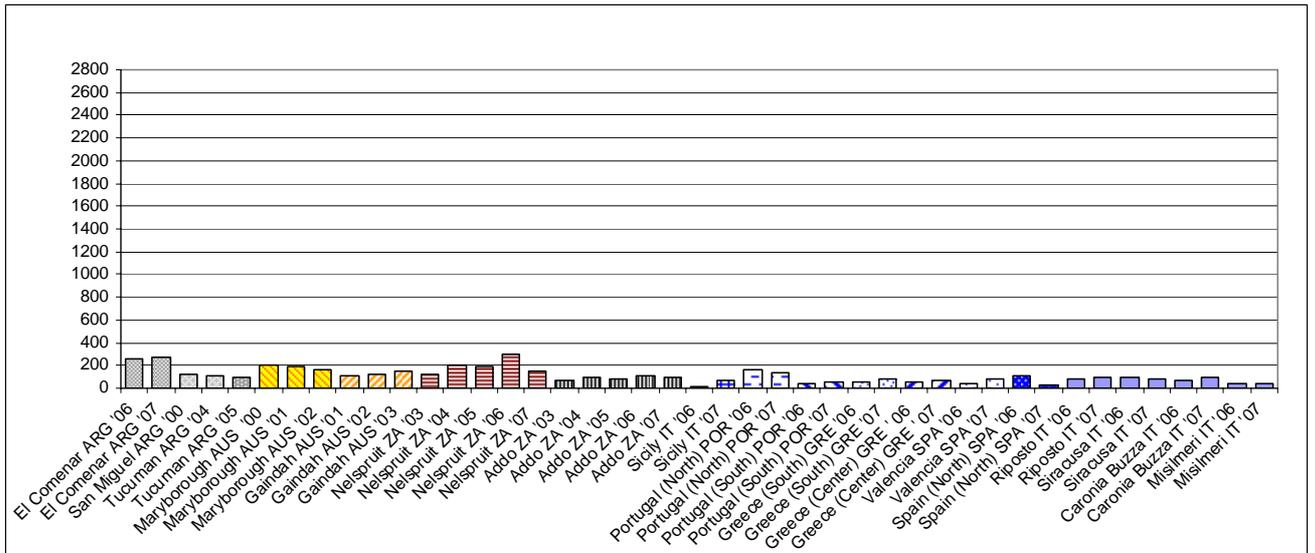


Figure 25. Number of potential infection events for *G. citricarpa* pycnidiospores simulated for sites from areas where CBS occurs outside Europe and from disease-free sites in the EU with $D_{50}=3$.

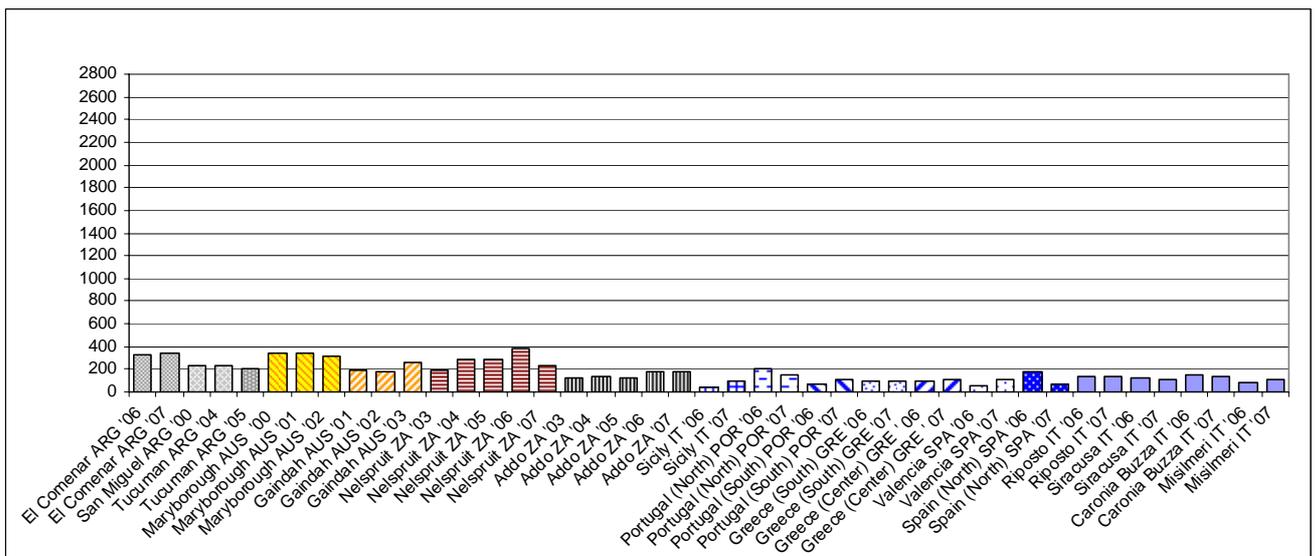


Figure 26. Number of potential infection events for *G. citricarpa* pycnidiospores simulated for sites from areas where CBS occurs outside Europe and from disease-free sites in the EU with $D_{50}=14$.

2.3.4. Modelling climate suitability for *G. citricarpa* infection with interpolated climatic data for the EU citrus growing areas

2.3.4.1. Objective

After the preliminary test, the generic infection model was applied to all the 50 km grid cells representing citrus production in the EU to obtain predictions of climate suitability for infection by both pycnidiospores and ascospores of *G. citricarpa*.

2.3.4.2. Material and methods

The model was run for the 418 cells of the citrus growing areas of the EU (see section 2.2.1.1. and Appendix 2.1.) with the set of most likely parameter values identified in section 2.3.2 with $D_{50}=0$, $D_{50}=3$ and $D_{50}=14$ (Table 3). The generation of hourly meteorological data, including the estimation of leaf wetness through the SWEB model, is described in Appendix sections 2.2 and 2.3. The percentages of hours with potentially successful infection by ascospores and by pycnidiospores were computed separately for each month from March to October over the time period 1998-2007. The period from November to February was not analysed due to time constraints and also considering that the temperature would be generally too low to sustain infections. In the case of pycnidiospores, to satisfy the requirement for splash dispersal, only those potential infections starting with a rain event were considered.

2.3.4.3. Results

The model output provides the number of potential infection events. A potential infection event corresponds to an hour with possible infection. Results are presented as percentages of hours with potential successful infection per month for April, May, September and October, and also for the period March-October over the time period 1998-2007. In the months of June, July and August, generally a limited number or no potential infection events were found (data not shown).

Pycnidiospores

The percentages of hours with potential successful infection by *G. citricarpa* pycnidiospores from April to October over the period 1998-2007 are shown in Fig. 27, with $D_{50}=0$, $D_{50}=3$ and $D_{50}=14$. With the parameter value $D_{50}=0$, i.e. when no interruption of leaf wetness is allowed, many Portuguese, Italian and French and part of the Spanish and Greek citrus growing regions have more than 0.5% of total time favourable to complete potential infections by pycnidiospores. The highest percentages are observed in the southern Italian regions of Sicily and Calabria and along the Atlantic coast of Portugal and Spain. Generally, compared to $D_{50}=0$, no visible differences in the simulation outputs are observed with $D_{50}=3$, i.e. when an interruption of leaf wetness of up to 3 hours is tolerated to complete an infection cycle, (Fig. 27, 28 and 29). With the exception of April, with $D_{50}=14$, i.e. when up to 14 hours of continuous dry period is allowed to complete an infection cycle, a considerable increase in the percentages of hours with potential successful infection by *G. citricarpa* pycnidiospores is observed. (Fig. 27, 28 and 29).

According to the model simulations, climate is generally unsuitable for infection from June to August for all D_{50} values (data not shown). In April and May respectively, up to 5% and 10% of the total number of hours provided potential successful infection by *G. citricarpa* pycnidiospores in part of the citrus growing areas (Fig. 28). September is, on average, the most favourable month for pycnidiospores infection, while in October lower percentages are observed (Fig. 29).

Ascospores

The results of the simulations for *G. citricarpa* ascospore infection from April to October over the period 1998-2007 are shown in Fig. 27, with $D_{50}=0$, $D_{50}=3$ and $D_{50}=14$. The results with the three D_{50} values for the months of April and May are shown in Fig. 30 and for the months of September and October in Fig. 31.

According to the model simulations, climate is generally unsuitable for infection from June to August for all D_{50} values (data not shown). The month of April shows almost no potential infection because temperatures are limiting, whereas May infections are limited by leaf wetness. September is, on average, the most favourable month followed by October. October offers a greater opportunity than September in terms of leaf wetness, but infection can be limited by temperature during this month.

The simulations show that infection from ascospores can occur, though not at all locations and with magnitudes smaller than in the areas of the pathogen current distribution outside Europe tested so far with the same model (section 3.3.3.). In particular, both with a D_{50} value of 0 and 14, the number of infection events per month can be ranked in descending order as September, October, May and April. June and August show only sporadic potential infection events and almost none are predicted to occur in July.

Even using the most conservative value of D_{50} (0 hours) to estimate infections, potential infection events are still predicted to occur in the EU. When results from both the EU and other areas are taken into account it is clear that: (a) the procedure used is sensitive to year by year variation; (b) the risk of infection is always noticeably positive for sites where CBS is endemic and (c) EU sites show a smaller number of potential infections and some sites show a number of potential infection events either equal or close to zero.

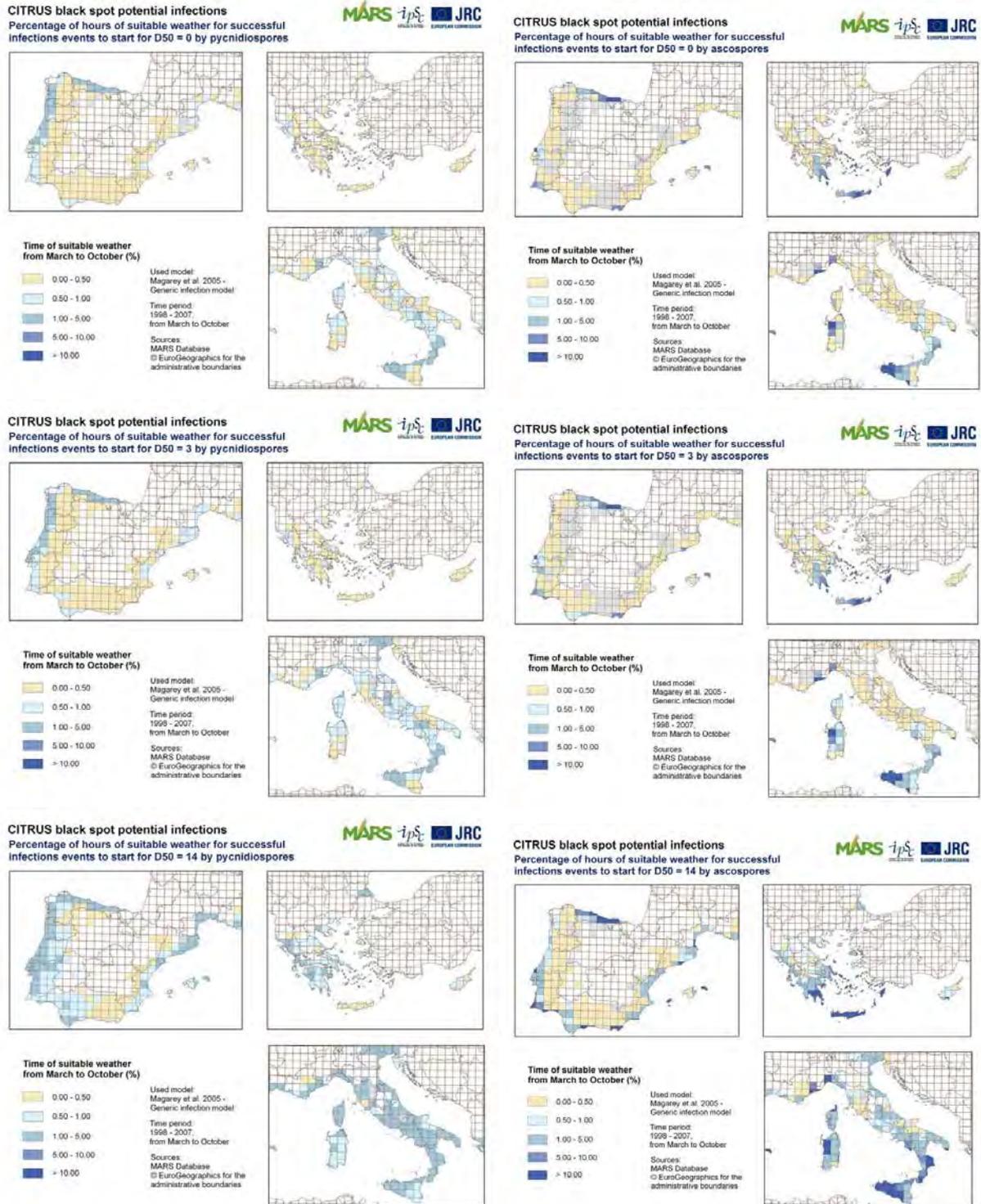


Fig. 27. Percentages of hours with potential successful infection by *G. citricarpa* pycnidiospores (left) and ascospores (right) from April to October over the period 1998-2007, with (from top to down) $D_{50}=0$, $D_{50}=3$ and $D_{50}=14$ (Note: in grids with grey colour the percentage is equal to 0).

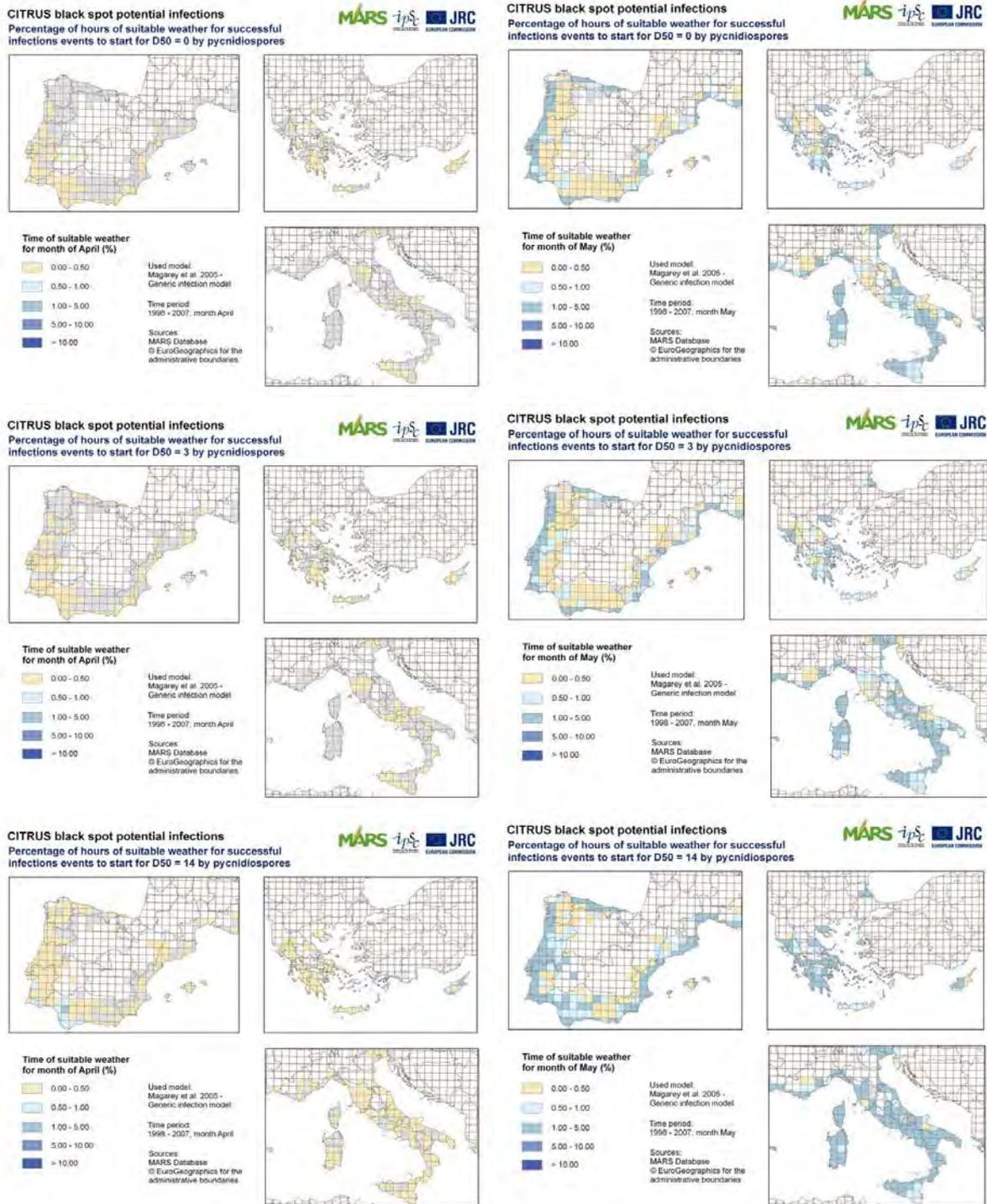


Fig. 28. Percentages of hours with potential successful infection by *G. citricarpa* pycnidiospores per month for April and May over the period 1998-2007, with (from top to down) $D_{50}=0$, $D_{50}=3$ and $D_{50}=14$ (Note: in grids with grey colour the percentage is equal to 0).

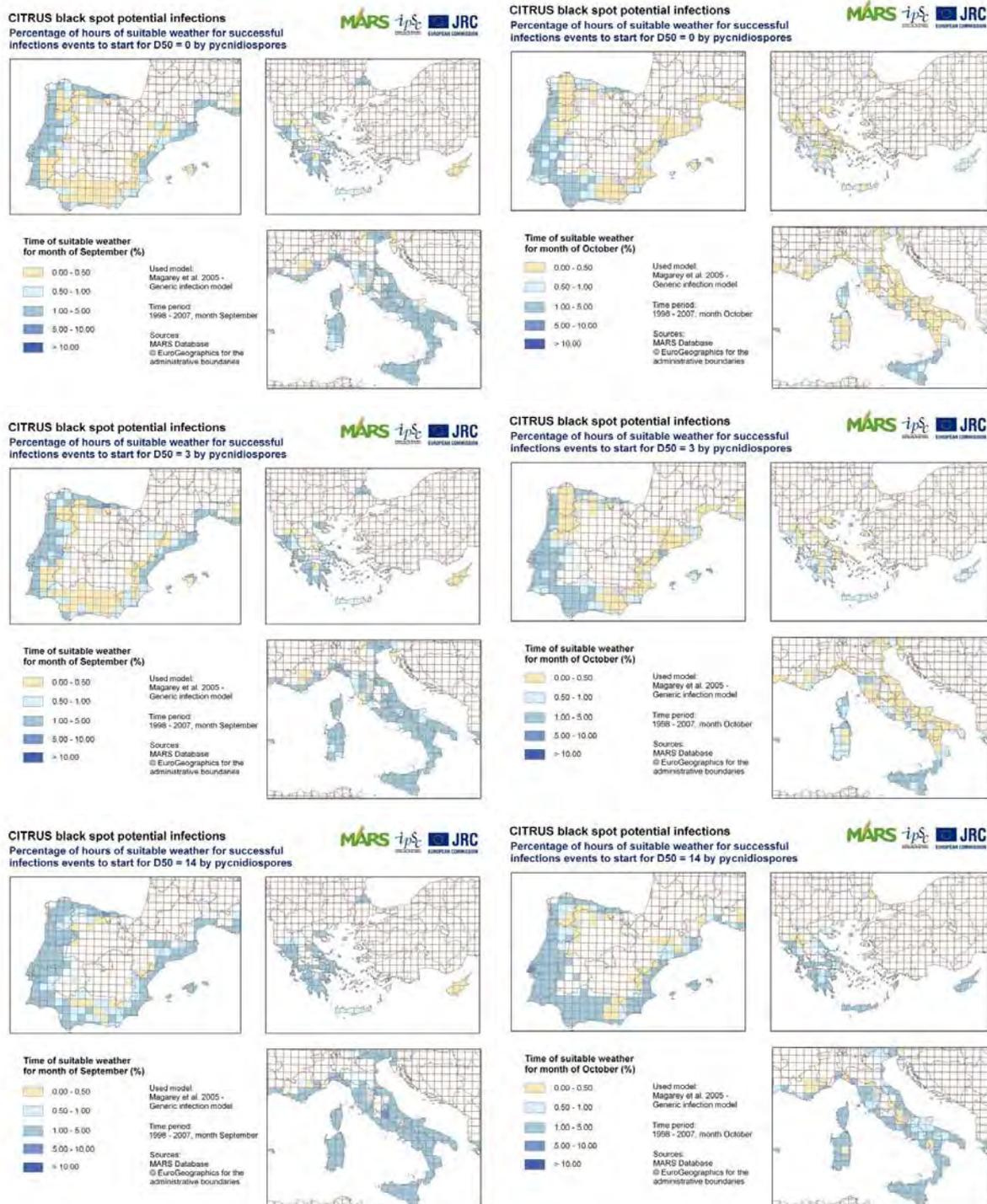


Fig. 29. Percentages of hours with potential successful infection by *G. citricarpa* pycnidiospores per month for September and October over the period 1998-2007, with (from top to down) $D_{50}=0$, $D_{50}=3$ and $D_{50}=14$ (Note: in grids with grey colour the percentage is equal to 0).

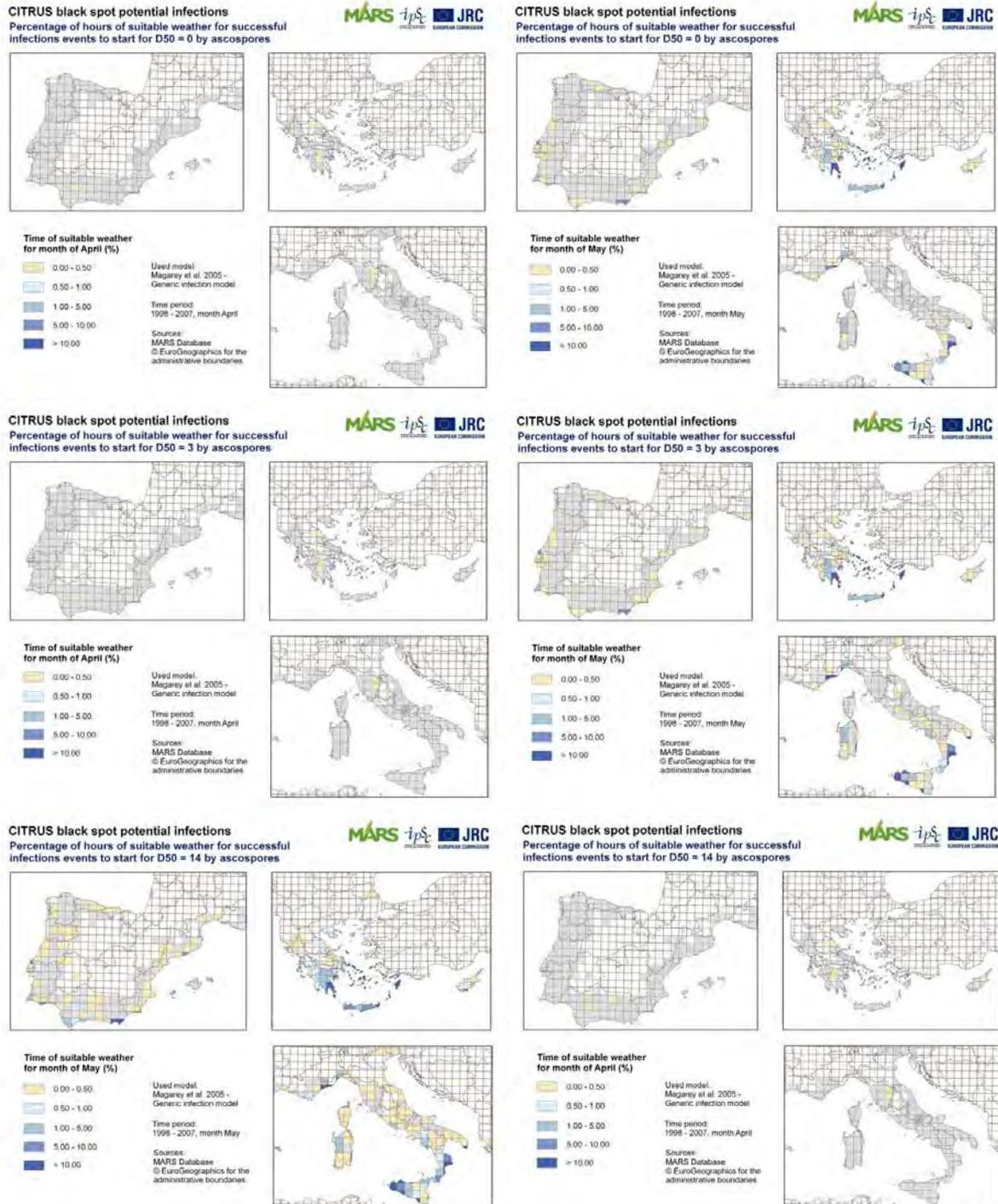


Fig. 30. Percentages of hours with potential successful infection by *G. citricarpa* ascospores per month for April and May over the period 1998-2007, with (from top to down) $D_{50}=0$, $D_{50}=3$ and $D_{50}=14$ (Note: in grids with grey colour the percentage is equal to 0).

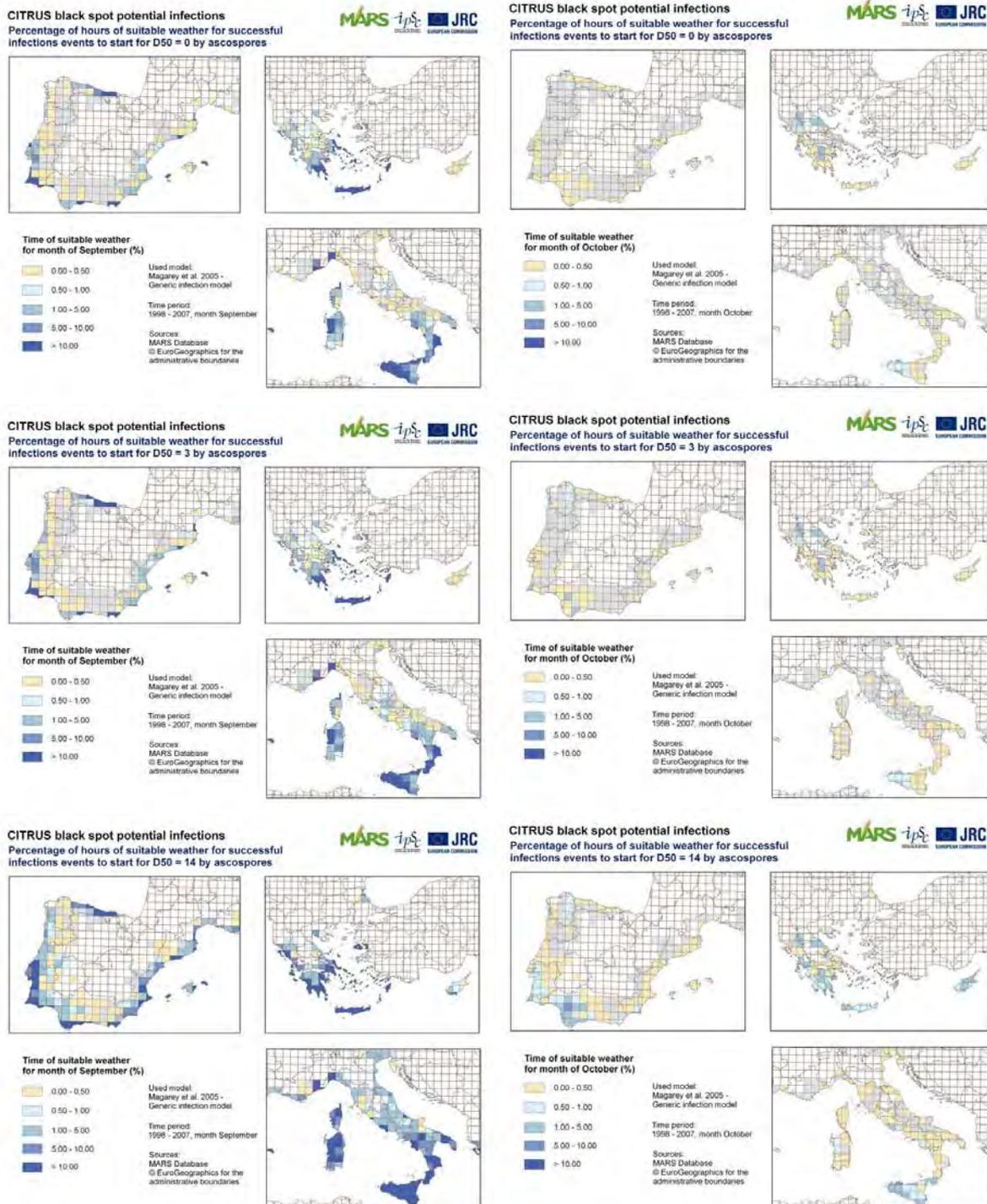


Fig. 31. Percentages of hours with potential successful infection by *G. citricarpa* ascospores per month for September and October over the period 1998-2007, with (from top to down) $D_{50}=0$, $D_{50}=3$ and $D_{50}=14$ (Note: in grids with grey colour the percentage is equal to 0).

2.3.5. Modelling climate suitability for *G. citricarpa* infection with data from agrometeorological stations, including sensitivity analysis

2.3.5.1. Objective

In section 3.3.4, the climatic suitability for *G. citricarpa* infection in EU citrus-growing areas was analysed using the European 50 km-grid of interpolated climatic data. This approach allowed us to analyse climate suitability at the continental scale but not to take into account some specific microclimatic features of citrus orchards.

The objective of this section is to analyse the climate suitability of several Spanish and Italian sites using on-site measured temperature and leaf wetness data obtained from agrometeorological stations. Wetness durations obtained from the station data were compared to wetness duration requirements computed with the generic infection model (Magarey *et al.*, 2005) implemented at an hourly time step for both ascospores and pycnidiospores.

As the number of sites and years considered in this second series of simulations was low and the computation time was rapid, it was possible to analyse in detail the sensitivity of the model outputs to different parameter values. The ranges of parameter values considered for the sensitivity analysis were as described in section 2.3.2.

2.3.5.2. Material and methods

Data

Meteorological data were obtained from 14 Spanish sites: Tivenys (TA), Amposta (TA), Burriana (CS), Xilxes (CS), Chiva (VA), Monserrat (VA), Alzira (VA), Javea (AL), Molina (MU), Palma (MU), Rinconada (SE), Aznalcazar (SE), Almonte (HU), Isla Christina (HU). The dataset included approximately one year of hourly temperature, rainfall, and leaf wetness for each site, except Chiva where approximately three years of data were available (Table 5).

At the Spanish stations, temperature, relative humidity, rainfall and surface wetness were monitored with automated weather stations (Watch Dog Data logger 450 (Temp. ± 0.6 °C, RH $\pm 3\%$)), equipped with a tipping bucket rain gauge ($\pm 3\%$) and a resistance-type leaf wetness sensor (Spectrum Technologies Inc., Plainfield, IL, USA). Environmental monitors were located between two trees on the central row of each orchard at 1.5-2 m above the soil surface. Electronic wetness sensors faced north at an angle of 30° to the horizontal. The wetness threshold was adjusted at the beginning of the study using a paper string wetness recorder (Bazier, Jules Richard; Argenteuil, France) and field observations.

Meteorological data were collected from 10 Italian sites (table 5) in Sicily and Calabria: Gioia Tauro (RC) and San Marco Argentaro (CS) from Calabria; Lentini (SR), Siracusa (SR), Misilmeri (PA), Mineo (CT), Caronia (ME), Paternò (CT), Ribera (AG) and Riposto from Sicily. The agrometeorological stations are located in citrus growing areas but outside the citrus groves. The dataset included several years of hourly temperature, rainfall, and leaf wetness for each site. As hourly rainfall measurements were not available for two Italian stations, the model for pycnidiospore infections was run for only eight of the stations.

In Calabria, temperature, relative humidity, rainfall and surface wetness were monitored with automated weather stations (Silimet, Modena, Italy) equipped with a data logger (Ad-2/A-22 or Ad-2/B with 16-32 channels), sensors for temperature and relative humidity (precision: Temp. ± 0.2 °C; RH $\pm 2\%$), a tipping bucket rain gauge (precision $\pm 2\%$) and a resistance-type leaf wetness sensor (facing north at an angle of 30° to horizontal). The stations in Sicily had

automated weather stations produced by MTX (Modena, Italy) and equipped with a data logger (model WST1800), sensors for wind speed (MTX – VVE, precision +/- 0.5 m/s since 10 m/s; +/- 1.0 m/s since 50 m/s), wind direction (MTX – VDI), solar radiation (PHILIPP SCHENK – 8102), air temperature (MTX – TAM, precision +/- 0.15 °C), relative humidity (MTX – UAM, precision +/- 1,5 %), rainfall (MTX – PPR, precision +/- 4%) and leaf wetness (MTX – BFO).

Table 5 Characteristics of the agro-meteorological stations from Spain and Italy. The region/province are indicated between brackets (For Spain: TA=Tarragona; CS=Castellón; VA=Valencia; AL=Alicante; MU=Murcia; SE=Sevilla; HU=Huelva. For Italy: SR=Siracusa; PA=Palermo; CT=Catania; ME=Messina; AG=Agrigento; CS=Cosenza; RC=Reggio Calabria).

Location	Y UTM	X UTM	Elevation (m)	Period analysed
Spain: Almonte (HU)	4114272	184666	90	25/09/07-25/09/08
Spain: Alzira (VA)	4334760	722126	<10	28/09/07-21/11/08
Spain: Amposta (TA)	4510397	304833	<5	04/10/07-19/10/08
Spain: Aznalcazar (SE)	4128347	212005	60	24/09/07-12/08/08
Spain: Burriana (CS)	4421714	244070	<5	20/09/07-05/11/08
Spain: Chiva (VA)	4371650	706371	270	01/01/03-31/12/05
Spain: Isla Christina (HU)	4129782	116275	<5	20/09/07-15/09/08
Spain: Javea (AL)	4295957	254855	<5	20/09/07-18/07/08
Spain: Molina (MU)	4220042	656964	90	17/09/07-08/10/08
Spain: Monserrat (VA)	4360256	711620	170	01/12/07-13/08/08
Spain: Palma (MU)	4170088	677739	<5	24/09/07-08/10/08
Spain: Rinconada (SE)	4152969	235594	<10	24/09/07-07/08/08
Spain: Tivenys (TA)	4550356	289017	40	01/10/07 - 19/10/08
Spain: Xilxes (CS)	4405558	742602	<5	13/11/07 - 05/11/08
Italy: San Marco Argentaro (CS)	4378412	596228	400	01/01/06 - 31/12/07
Italy: Gioia Tauro (RC)	4252800	578565	20	01/01/06 - 31/12/07
Italy: Misilmeri (PA)	4210500	363375	160	01/01/02 - 01/09/08
Italy: Caronia (ME)	4209150	455207	50	22/02/02 - 01/09/08
Italy: Riposto (CT)	4170912	517486	50	01/01/02 - 01/09/08
Italy: Paternò (CT)	4152071	487181	100	01/01/02 - 01/09/08
Italy: Ribera (AG)	4144951	346639	30	01/01/02 - 01/09/08
Italy: Lentini (SR)	4132840	493420	50	25/01/02 - 01/09/08
Italy: Mineo (CT)	4130521	475760	200	01/01/02 - 01/09/08
Italy: Siracusa (SR)	4101770	514148	90	07/01/02 - 01/09/08

Simulations

The epidemiological model was first run with the parameter values already used for simulating climate suitability for the European 50 km-grid (see section 2.3.2.), i.e with the so-called “most-likely” parameter values (Table 3). The percentages of hours with potentially successful infection by ascospores and by pycnidiospores were computed separately for each month. In the case of pycnidiospores, to satisfy the requirement for splash dispersal, only those potential infections starting with a rain event were considered (section 2.3.2.).

The model was then re-run with other combinations of parameter values (see annex) in order to analyse the sensitivity of the model output to changes in the parameter values. The total number of parameter combinations was equal to 48 for pycnidiospores and 24 for ascospores.

The number of parameter combinations was lower for ascospores because there is less uncertainty about parameter W_{min} for this type of spore (Table 3).

The uncertainty associated with the model output was analysed by calculating the minimum, median and maximum percentages of time with potential successful infection for each month. The simulations were also used to compute first-order and total sensitivity indices for each uncertain parameter. The *first-order sensitivity index* of a given parameter represents the main effect of this parameter on the output variable and measures the variance reduction that would be achieved by fixing (or reducing the range of) that parameter. The values of this index can be used to identify the parameters that deserve an accurate estimation. This is called ‘Factor Prioritisation setting’ by Saltelli *et al.* (2004). The sum of these indices over parameters is lower than or equal to 1 (or 100 if the indices are expressed in %). The *total sensitivity index* of a given parameter is the sum of all effects (first- and higher-order) involving this parameter. It takes into account the interactions between this parameter and the other uncertain model parameters. The total sensitivity index can be described as the expected fraction of variance that would be left if only the parameter itself was to stay undetermined. It can be used for model reduction purposes; when a factor does not have any effect either on its own or in cooperation with others. It can be considered as non-influential and can be fixed to any value within its range of uncertainty. This is called ‘Factor Fixing setting’ by Saltelli *et al.* (2004). The sum of these indices over parameters is equal to or higher than 1 (or 100 if the indices are expressed in %). As the simulations were based on a full factorial design, the sensitivity indices were computed from an ANOVA (Monod *et al.* 2006).

2.3.5.3. Results

i) Results obtained with the «most likely» parameter values

Pycnidiospores

Figure 32 shows the percentages of hours with potential successful infection by pycnidiospores for each month and each station in Spain and Italy. The parameters T_{min} , T_{max} , T_{opt} , W_{min} and W_{max} were set equal to the most likely values defined in Table 3 and three values were successively used for D_{50} . Each curve shows the evolution of the percentage of time with potential infection across each month for stations in Spain or in Italy. Curves are smoother for Italy than for Spain because the results obtained in all Spanish stations, except Chiva, are only based on one year of data. The results obtained for Italy should be considered as more accurate because they were derived from several years of data.

Figure 32 shows that, when $D_{50}=0$, the percentage of time with possible infection is higher than 5% at only one Spanish station (Alzira) in February and October. The percentage is increased when higher values of D_{50} are used. Thus, when $D_{50}=14h$, the percentage of time with possible infection is higher than 5% at several stations in Spain in February, April, May, September, and October. The variability across stations is very high in Spain for all the values of D_{50} . For example, when $D_{50}=14h$, the percentage ranges from 0 to 14.3% in February, from 0 to 12.6% in May, and from 0.5 to 10.6% in October.

At the Italian stations, the highest values are reached in September, October, November and December. Smaller peaks are also simulated in March and April. The percentage of time with potential successful infection is never higher than 5% when $D_{50}=0$ or $D_{50}=3h$, but can be higher than 5% in October, November and December when $D_{50}=14h$. The variability across stations is not very high. For example, when $D_{50}=14h$, the percentage ranges from 3 to 5.2% in October.

Ascospores

Figure 33 shows the percentages of hours with potential successful infection by ascospores for each month and each station in Spain and Italy. The parameters T_{min} , T_{max} , T_{opt} , W_{min} and W_{max} were set equal to the most likely values defined in Table 3 and three values were used successively for D_{50} . As before, each curve shows the evolution of the percentage of time with potential infection across the months for a station in Spain and Italy.

Figure 33 shows that in Spain and when $D_{50}=0$, the percentage of time with potential successful infection is never higher than 8%. It is higher than 1% only in April, July, September and October and is lower than 1% in all stations during November, December, January, February and March. The percentages increase in Spain when D_{50} is set to a higher value. For example, when $D_{50}=14$ h, the percentage of time with possible infection can be higher than 10% between April in October in some stations. The variability across stations is high in Spain, especially when $D_{50}=14$; with this parameter value, the percentage ranges from 0 to 33% in July.

The percentage of time with potential successful infection tends to be higher in the Italian stations than in Spain; it is higher than 10% in some Italian stations between August and October even when $D_{50}=0$. The highest values are reached between August and October. Smaller peaks are also simulated in May and June. The lowest values are reached during winter; the percentage of time with potential successful infection is always lower than 7% between December and March, even with $D_{50}=14$ h. The variability across stations is important. For example, when $D_{50}=14$ h, the percentage ranges from 0.8 to 46% in October.

ii) Sensitivity to T_{min} , T_{opt} , W_{min} , W_{max} and D_{50} (Figures 3-6 and Table 1)

Pycnidiospores

Figures 34 and 35 show the percentages of time with potential successful infection by pycnidiospores for each month and each station in Spain and Italy. These percentages were derived from 48 combinations of values of T_{min} , T_{opt} , W_{max} and D_{50} (see detailed results in Appendix, Table 15). Minimum (lowest dashed line), median (continuous line) and maximum (highest dashed line) percentages obtained for the parameter combinations are displayed for each station in a separate graph. These values show the ranges of variation of the simulations induced by parameter uncertainty.

Here again, the curves are smoother for Italian stations due to the higher number of data available at these stations. Peaks are simulated in the Spanish stations at different periods of the year, especially in February, April, May, and October. In Italy, the highest percentages are reached between October and December. In 16 out of the 22 stations considered for pycnidiospore infection, the maximum simulated percentage value was higher than 5% for at least one month.

In all stations, the minimum, median, and maximum are equal or very close to zero in July and August. The risk of infection by pycnidiospores is thus clearly low during this period of the year. This is due to the low frequency of rainfall during summer. For the other months, the differences between the maximum and minimum values simulated by the model are much larger and, thus, results are more uncertain. For example, in Siracusa (Italy), the percentage of

potential successful infection ranges from zero to 5% in November depending on the parameter values used.

Ascospores

Figures 36 and 37 show the percentages of time with potential successful infection by ascospores for each month and each station in Spain and Italy. These percentages were derived from 24 combinations of values of T_{min} , T_{opt} , W_{max} and D_{50} (see detailed results in Appendix, Table 13). Minimum (lowest dashed line), median (continuous line) and maximum (highest dashed line) percentages obtained over the parameter combinations are displayed for each station in a separate graph.

In all stations but two (Chiva in Spain and San Marco Argentaro in Italy), the maximum simulated percentage was higher than 20% for at least one month. Depending on the station the highest values are obtained between May and October in Spain and between July and October in Italy. The only exception is Isla Christina in Spain. The maximum percentages are lower than 10% in most stations during winter due to the effect of low temperatures.

The minimum percentages of potential successful infection are equal or close to zero for all months in all stations. As the maximum percentages are often high between April and October, the differences between the maximum and minimum values simulated by the model are important in almost all stations during this period. For example, for Alzira (Spain), the percentage of potential successful infection ranges from zero to 47% in October depending on the set of parameter values. This result shows that the simulation of ascospore infection is highly uncertain.

Sensitivity indices

Table 6 shows the sensitivity indices obtained for the parameters and for the two types of infection. The highest sensitivity indices were for D_{50} but the sensitivity indices for T_{min} were not negligible, especially for pycnidiospores. D_{50} and T_{min} are thus the two main sources of uncertainty in our results. Both parameters should be key targets of future research.

Table 6. **First-order and total sensitivity indices (%) computed for pycnidiospores and ascospores.**

Parameter	Pycnidiospores		Ascospores	
	First-order	Total	First-order	Total
D_{50}	55.60	66.76	85.41	90.89
T_{min}	31.14	42.47	8.50	13.92
T_{opt}	0.98	1.09	0.27	0.36
W_{max}	0.65	1.07	0.22	0.32
W_{min}	0.03	0.07	-	-

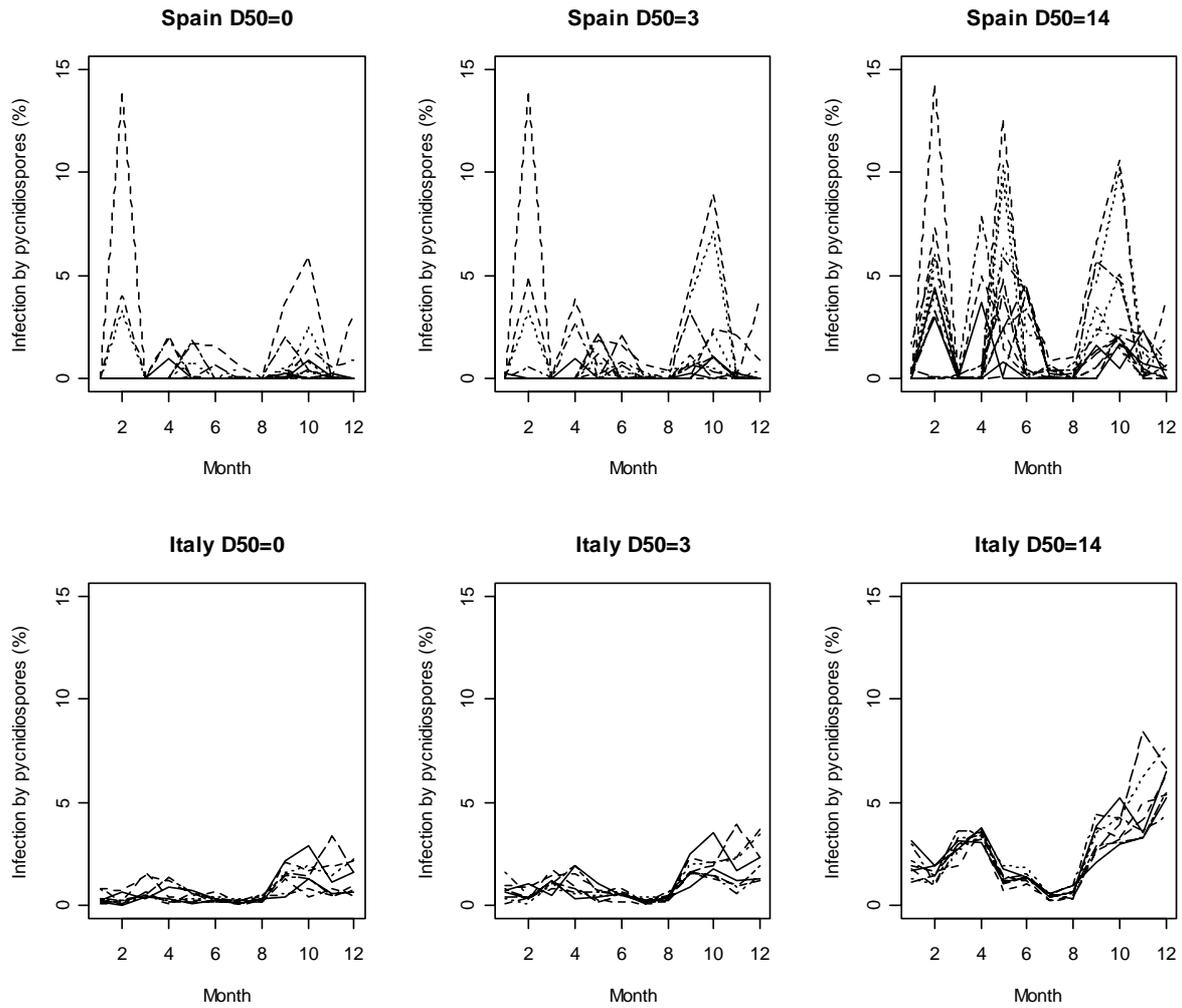


Figure 32. Percentage of hours with potential successful infection by pycnidiospores of *G. citricarpa* for each month and each station in Spain and Italy. The parameters T_{\min} , T_{\max} , T_{opt} , W_{\min} and W_{\max} were set equal to the most likely values and three values were successively used for D_{50} . Each curve corresponds to a given station.

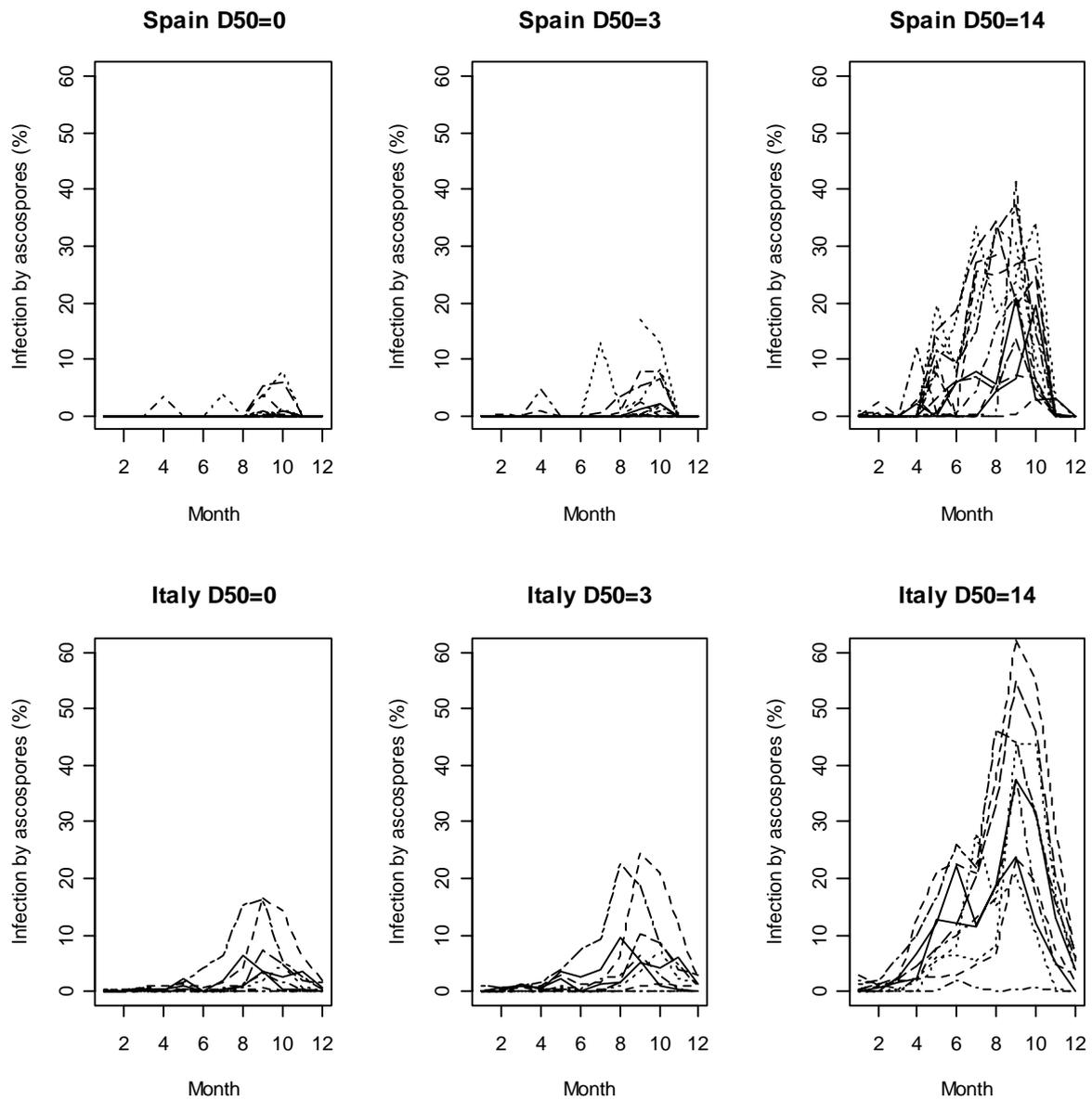


Figure 33. Percentage of hours with potential successful infection by ascospores of *G. citricarpa* for each month and each station in Spain and Italy. The parameters T_{\min} , T_{\max} , T_{opt} , W_{\min} and W_{\max} were set equal to the most likely values and three values were successively used for D_{50} . Each curve corresponds to a given station.

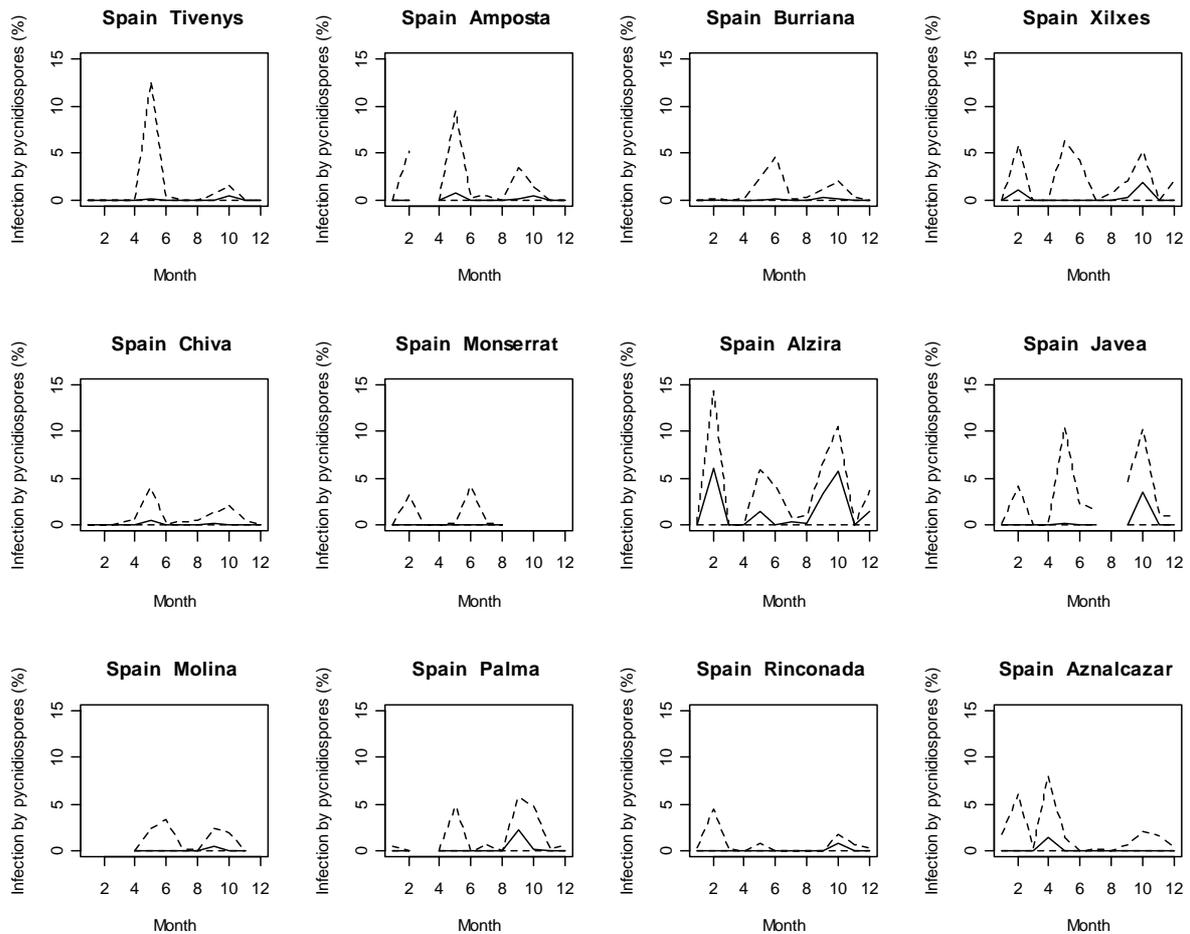


Figure 34. Percentage of hours with potential successful infection by pycnidiospores of *G. citricarpa* for each month and 12 stations in Spain. The model was run with 48 combinations of parameter values. Minimum (lowest dashed line), median (continuous line) and maximum (highest dashed line) values obtained for each month over the parameter combinations are displayed for each station.

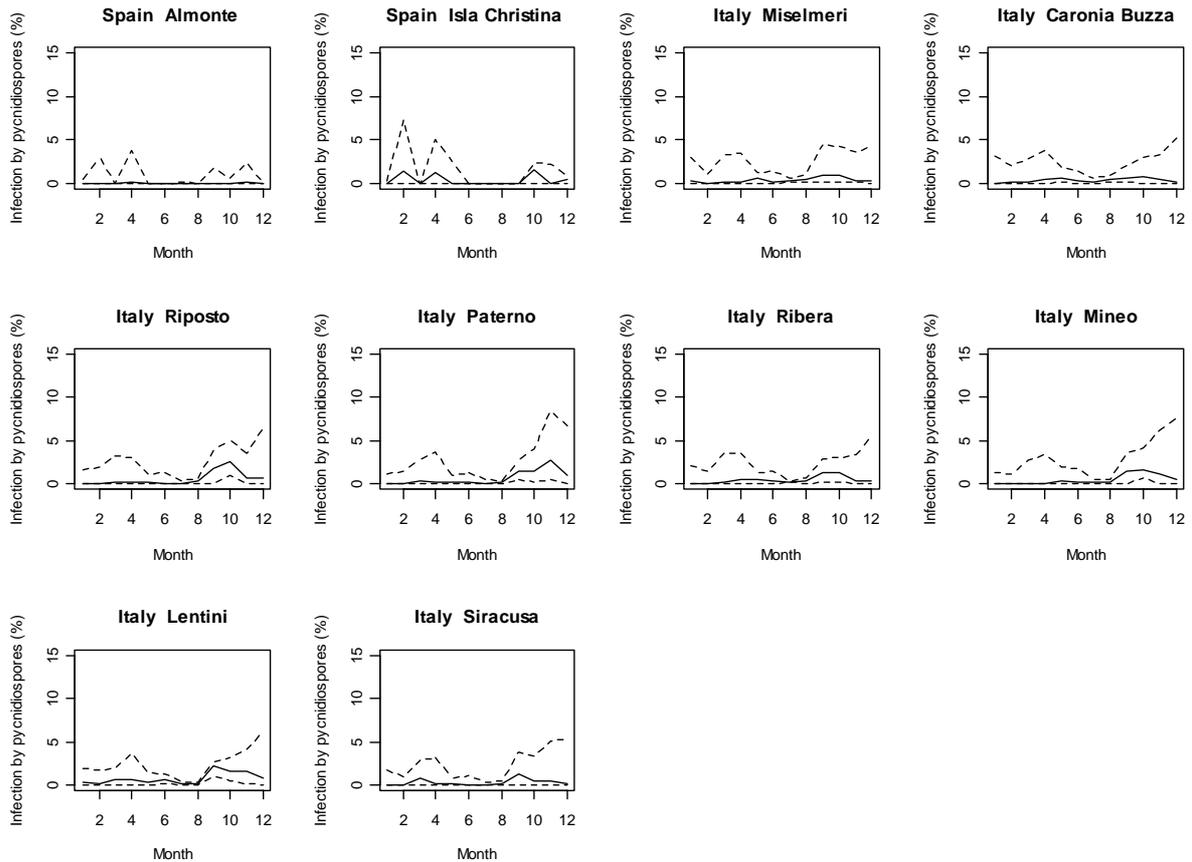


Figure 35. Percentage of hours with potential successful infection by pycnidiospores of *G. citricarpa* for each month and for two stations in Spain (Almonte and Isla Christina) and 10 stations in Italy. The model was run with 48 combinations of parameter values. Minimum (lowest dashed line), median (continuous line) and maximum (highest dashed line) values obtained for each month over the parameter combinations are displayed for each station.

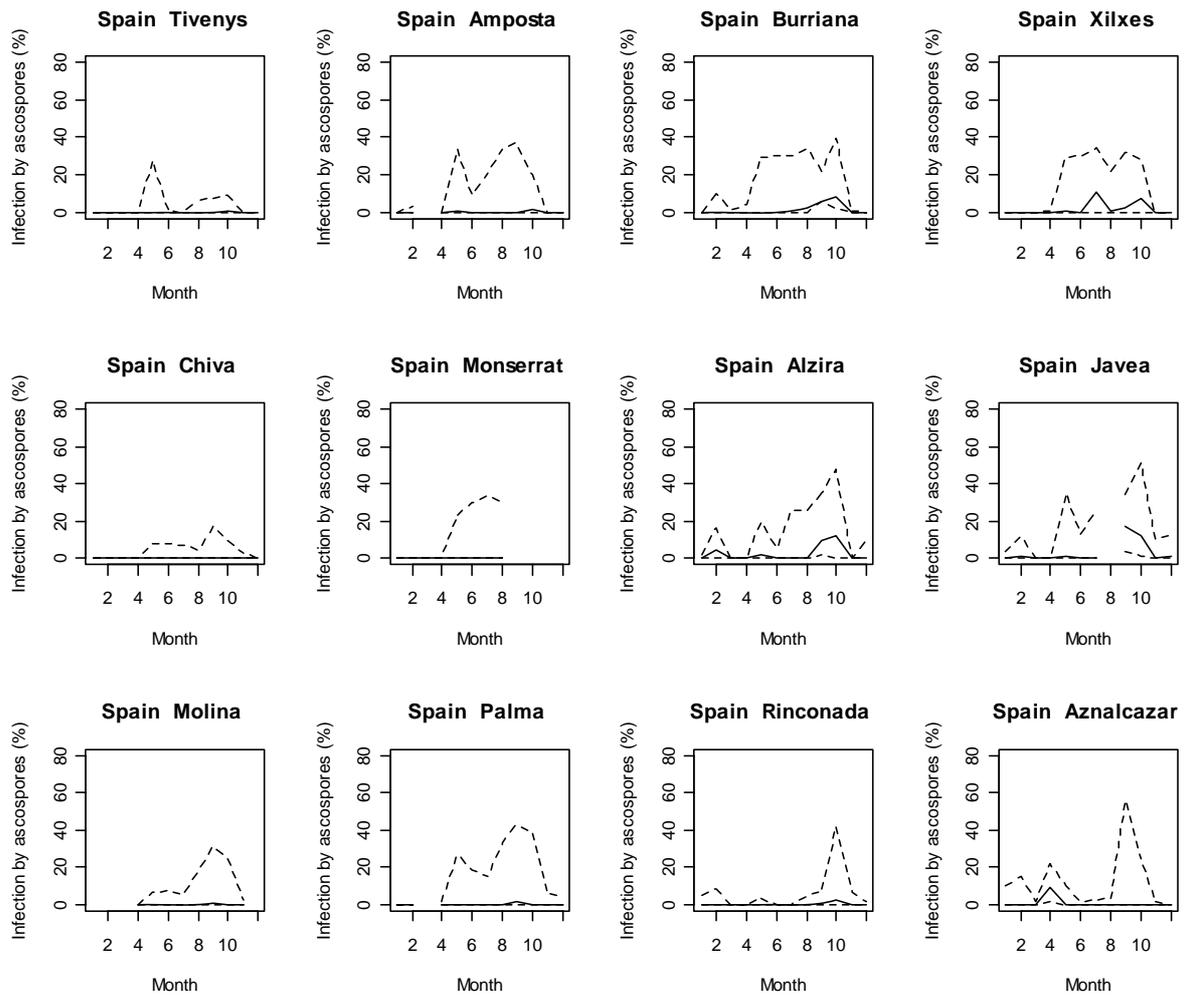


Figure 36. Percentage of hours with potential successful infection by ascospores of *G. citricarpa* for each month and 12 stations in Spain. The model was run with 24 combinations of parameter values. Minimum (lowest dashed line), median (continuous line) and maximum (highest dashed line) values obtained for each month over the parameter combinations are displayed for each station.

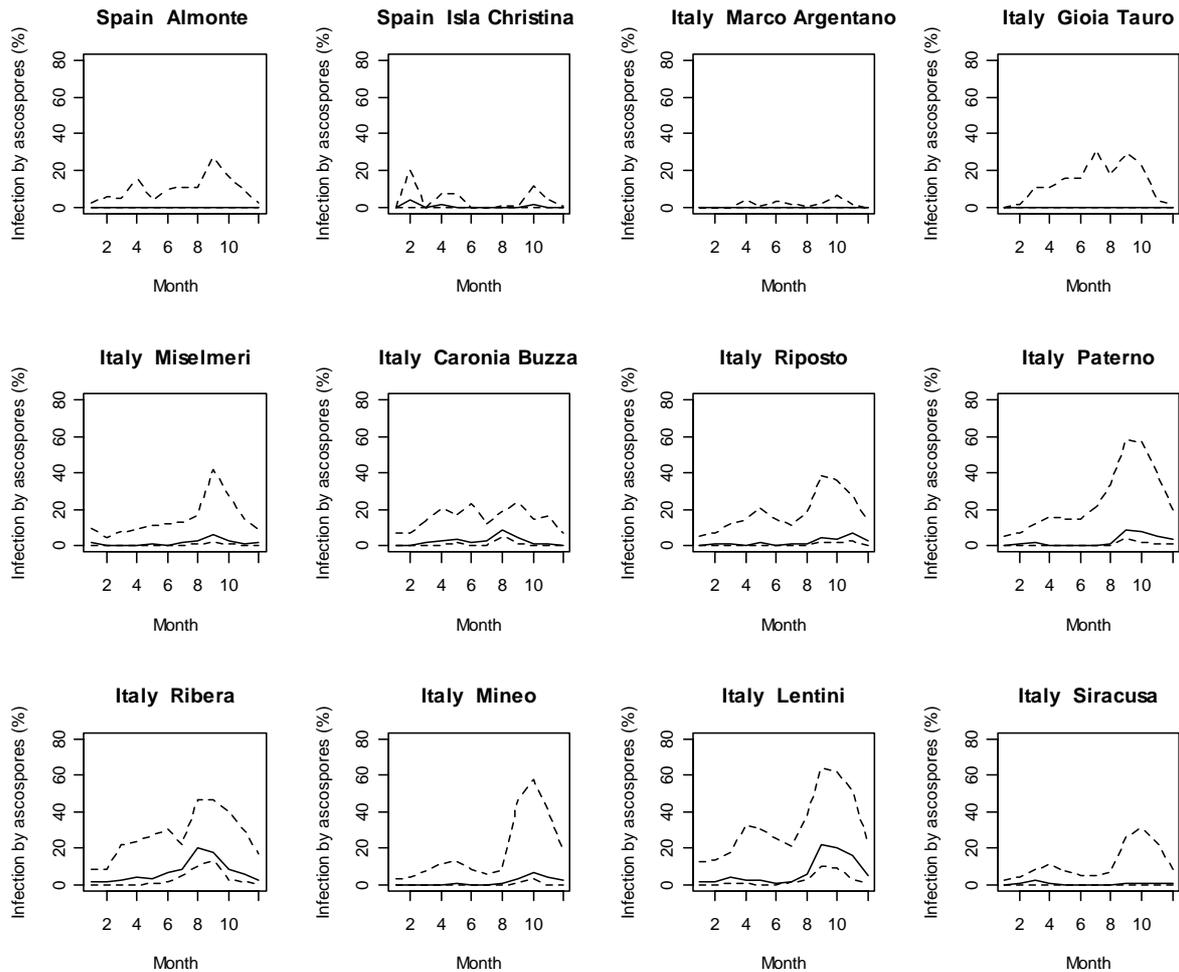


Figure 37. Percentage of hours with potential successful infection by ascospores of *G. citricarpa* for each month for two stations in Spain (Almonte and Isla Christina) and eight stations in Italy. The model was run with 24 combinations of parameter values. Minimum (lowest dashed line), median (continuous line) and maximum (highest dashed line) values obtained for each month over the parameter combinations are displayed for each station.

2.3.6. Conclusions on the implementation of the generic infection model (Magarey *et al.*, 2005)

Exploratory studies of the potential infection of citrus by both *G. citricarpa* pycnidiospores and ascospores in areas of citrus production in the EU have been undertaken by the Panel using a generic infection model (Magarey *et al.*, 2005). To take into account the considerable level of uncertainty concerning the parameter values required to run the model, best estimates were obtained from the literature and subject to a sensitivity analysis.

Pycnidiospores are critical to *G. citricarpa* establishment only for the first infection event following entry. The climatic conditions at any location therefore only need to remain suitable for the time required to complete one infection. The results obtained from the model with data from both the 50 km grids representing EU citrus cultivation and agro-meteorological stations in citrus growing areas of Spain and Italy showed a similar picture. Although the uncertainty is

high, climatic conditions favourable for pycnidiospore infection occur in many of the locations where citrus is grown in the EU particularly in September and October.

After the first infection event by pycnidiospores, the further spread of the disease depends on subsequent infection cycles by ascospores. Thus, for successful establishment in a given area, the prevalent climate should be suitable for ascospore infection. In the 50 km grid, climatic conditions were found to be potentially favourable for infection by ascospores in April-May and late September-October, but were generally not favourable in June-August. These results were confirmed by the simulations performed with data from the agro-meteorological stations in Spain and Italy.

Although, these exploratory studies indicate that during some months of the year, especially September and October, climatic conditions are suitable for pycnidiospore and ascospore infection in the citrus production areas of the EU, it is also important to determine whether there is synchrony between the times of year when the climate is suitable for infection and the host is susceptible. Fruit are susceptible to infection by *G. citricarpa* for up to 4-6 months after petal fall (Baldassari *et al.*, 2006; Kotzé, 1981, McOnie, 1964b; Reis *et al.*, 2003) whereas lemon leaves remain susceptible for up to 10 months (Truter *et al.*, 2004; 2007). Therefore, in spring, young citrus fruit and leaves are particularly vulnerable to infection, but susceptible fruit and leaves would still be present during the potential periods of infection by pycnidiospores and ascospores in September and October. Lemon has secondary flowerings until September (Agustí, 2000), so, during this period, these fruit would be particularly susceptible to infection by pycnidiospores and ascospores.

The importance of cold winter temperatures for the potential establishment of *G. citricarpa* in the EU also needs to be considered. Paul *et al.* (2005) concluded that the main factor limiting the distribution of the disease was cold stress in the form of continuous winter days with temperatures too low to allow the survival of the pathogen. However *G. citricarpa* has been isolated from fruit maintained at 8 °C for up to 40 days (Agostini *et al.*, 2006) and strains are usually stored in culture collections on dry filter papers at -20°C (Peres *et al.*, 2007). In section 2.1.3., it was shown that minimum winter temperatures in EU citrus growing locations, such as Valencia, Messina and Porto, were similar to those at Addo in Eastern Cape Province in an area where CBS has been reported. It is therefore unlikely that winter temperatures in areas of EU citrus production will prevent *G. citricarpa* from surviving. However the maturation of pseudothecia and ascospores in the leaf litter (Kiely, 1948; Kotzé, 1981; Lee and Huang, 1973) may be delayed by cool winter temperatures potentially causing asynchrony between the pathogen and the host. If lower maximum temperatures in winter and spring in the EU delay the onset of ascospore release until after mid-summer, no infection would take place in spring despite that the availability of both suitable climate and susceptible host tissues. However, during September and October, environmental conditions would again be potentially favourable for infection and susceptible fruit, especially lemons, and leaves would be present. It is therefore unlikely that, by itself, any delay in the production of ascospores would be sufficient to prevent infection.

One other factor needs to be taken into account. Although *G. citricarpa* readily overwinters in green leaves and in the leaf litter, fallen leaves may decompose before the ascospores mature. However citrus leaves are shed all year around and mature pseudothecia can develop on the leaf litter in only 40 days. Severe epidemics can still arise from ascospores produced on leaves shed in spring and early summer (Kotzé, 1963; Lee and Huang, 1973). As such the cooler maximum temperatures in the EU citrus growing areas are unlikely to prevent establishment of *G. citricarpa*.

2.4. General conclusions on climate suitability

The Panel reviewed the approach taken by South Africa to determine whether citrus production areas in the EU are climatically suitable for the establishment of *G. citricarpa* using the computer pest distribution modelling software, CLIMEX. Problems were identified both with the application of CLIMEX by South Africa and their choice of model.

The selection of parameters to run the “compare locations” CLIMEX model was found to be based only on locations in north-eastern South Africa where severe epidemics of CBS have been recorded. However it did not take into account the areas of Eastern Cape Province where CBS is less severe but still occurs despite very different climatic conditions. Climatic data for one location where CBS occurs, Addo in Eastern Cape Province, were found to be relatively similar to several places where citrus is grown in the EU. In addition, the lower limiting temperatures and cold stress parameters selected were found to be unrelated to findings in the literature. The problems identified with parameter selection have two main implications for the estimates of potential establishment of *G. citricarpa* in the EU. Firstly these generate lower values of the ecoclimatic index (EI), the indicator of establishment potential, than should be the case and secondly they indicate that the EI thresholds for the suitability of establishment chosen by Paul *et al.* (2005) are too high. Nevertheless, even with the same parameters and EI thresholds of Paul *et al.* (2005) but with recent data from weather stations and interpolated 50 km grids, in some years and some EU citrus growing locations the climate was found to be “favourable for disease development”.

Although the parameter fitting approach taken by CLIMEX does tend to integrate the complex processes that determine whether a species can survive in a particular location, it has a limited ability to predict the potential distribution of organisms that are critically dependent on wetness because the dew point is not included in the parameter set and suitable infection periods can be of a shorter duration (hours) than the minimum CLIMEX time step (weeks). To explore, these issues, a generic infection model (Magarey *et al.*, 2005) was employed once a suitable method for estimating leaf wetness had been selected. Sensitivity analyses were conducted to take into account the uncertainty on many parameter values for *G. citricarpa*.

The model predicted numerous pycnidiospore and ascospore infection events over a ten year period (1998-2007) at agro-meteorological stations and in 50 km grids, even when the most conservative values for key parameters such as the tolerance to dry interruptions (D_{50}) were utilised. Almost no infection events were predicted in summer (June-August) but significant numbers occurred at many locations in the spring (April-May) and autumn (September-October). Leaf infection can occur throughout the year if the climate is suitable but fruit susceptibility is limited to the 4-6 months after fruit set. Lemons have secondary flowerings until September, so those developing fruits would be particularly susceptible in autumn. Even if the onset of ascospores was delayed by the effect of cooler Mediterranean winter temperatures and the spring infection period was missed, susceptible leaves and fruit would be present in the autumn infection period, especially in the case of lemons.

Based on (a) the evaluation of the application of CLIMEX Paul *et al.* (2005), (b) the limitations of CLIMEX in predicting the potential distribution of pathogens such as *G. citricarpa*, (c) the relative climatic similarities between locations where CBS occurs in Eastern Cape Province and where citrus is grown in the EU and (d) the results of the application of a generic infection model for foliar fungal pathogens, the Panel cannot agree with the conclusion by Paul *et al.* (2005) that the climate of the EU is unsuitable for the establishment of *G. citricarpa*.

3. Likelihood of an introduction, leading to an establishment, of *G. citricarpa* to the EU citrus growing areas on citrus black spot infected citrus fruits

3.1. Probability of entry

3.1.1. Identification of the pathways

According to the Terms of Reference (see p. 7), only the fruit pathway needs to be considered.

The Panel notes that passenger traffic should also have been considered in the South African pest risk assessment. Directive 2000/29/EC Annex IV Paragraph 16.4, which describes the special requirements with regards to the introduction and movement of *G. citricarpa* on citrus fruit, applies both to commercial trade and to the movement of citrus fruit by passenger traffic.

3.1.2. Probability of pest being associated with fruit at origin

Prevalence of pest in source area

According to the Pest Risk Assessment conducted by South Africa (Annex 1) (hereinafter referred as “the document”), CBS occurs in sub-tropical regions of South Africa that experience summer rainfall, and has never become established in regions with a Mediterranean climate, such as the Western Cape Province.

In subsequent exchange of technical information between the SA-WG and the EU-WG:

- The EU-WG (Annex 2) noted that, based on the literature, (i) CBS has already established in areas with various environmental conditions, such as cool, misty, dry, hot, semi-arid, sub-tropical regions, etc. (Wager, 1952), and (ii) too much hope has been placed in the past on climate as a limiting factor (Kotzé, 1981).
- Paul *et al.* (2003) (Annex 4) stated that the disease-free status of the Western Cape was recognised by both the EU (Commission Decision 98/83/EC) and the USDA. The Western Cape Province was declared free of CBS based on the results of 17,200 microbial isolations made from citrus fruit and leaves in its south western citrus production area (Venter *et al.*, 1995).
- The EU-WG (Annex 6) referred to South African legislation from 2005, restricting the movement of citrus from some districts to others within the Western Cape Province, due to CBS. The SA-WG (Annex 7) clarified that these restrictions concern the movement of citrus propagation material and not that of fruit. The SA-WG said that these districts were not included because citrus was not grown commercially, for export.

The Panel notes that:

- the reference (Venter *et al.*, 1995) cited in Annex 4 concerning data from a South African report, where certain districts in the Western Cape Province of South Africa were extensively tested and certified CBS negative is unpublished and cannot therefore be evaluated.

and considers that the pest is widespread within the citrus-producing areas of South Africa with the possible exception of the Western Cape Province for which the Panel can not express its

opinion as the document did not include scientific evidence on the CBS-free status of this province.

Occurrence of the pest in a life stage, which would be associated with commodities, containers or conveyances

According to the document, only pycnidia and pycnidiospores of *P. citricarpa* (the anamorph of *G. citricarpa*) may be associated with symptomatic citrus fruit.

The EU-WG (Annex 2) reported that, in addition to pycnidia and pycnidiospores, latent mycelium of the pathogen may also be associated with the fruit pathway. It further noted that living stages of the organism have been intercepted on fruit imported into the EU from CBS-infested areas (Annex 2) including South Africa (Annex 6), supporting the likelihood of fruit as a pathway.

The SA-WG (Annex 3) agreed that pycnidiospores or latent mycelium may be present in the fruit. However the SA-WG stated that (i) pycnidiospores are short-lived and, therefore, they did not play a role in the long distance spread of the disease, and (ii) latent mycelium was not infective. Concerning interceptions, the SA-WG (Annex 3) stated that the complete elimination of a regulated organism (i.e. zero tolerance) was not feasible and, therefore, CBS infected fruit from South Africa may be intercepted in the EU. The SA-WG (Annex 7) noted that the EU-WG provided no scientific justification for the development of pycnidia from latent mycelium.

The Panel found additional information according to which *G. citricarpa* has a long incubation period on citrus fruit, which, depending on the citrus variety, temperature and tree condition, varies from 2 to 10 months (McOnie, 1967) or from 3 to 12 months (Kellerman and Kotzé, 1977). Therefore, the pathogen may also be present in asymptomatic citrus fruit as latent mycelium, which as the citrus fruit rind approaches maturity, is progressively more capable of developing satisfactorily in the rind tissues and so producing more active and serious lesions (Kiely, 1948). The production of pycnidia and pycnidiospores of *G. citricarpa* on these lesions could be induced by certain temperature and light conditions (Brodrick and Rabie, 1970).

The Panel in agreement with the SA-WG and the EU-WG considers that (i) pycnidia with pycnidiospores of *G. citricarpa* produced on CBS fruit lesions, and (ii) latent mycelium present in infected, but asymptomatic, fruit are associated with the citrus fruit pathway. It further considers that the latent mycelium is not by itself infective, however, it is capable of producing lesions in which pycnidia with pycnidiospores may develop following exposure of the fruit to certain environmental conditions.

Volume and frequency of movement along the pathway

According to the document, considerable quantities of citrus fruit may enter the PRA area and more specifically approx. 65% of the 900,000 tonnes of citrus fruit exported from South Africa annually are destined for the EU market. However no indication was given in the document or in any of the subsequent SA-WG reports (Annexes 3, 4, 5 & 7) as to the frequency of the movement of fruit along the pathway.

The EU-WG in Annex 6 provided data (Eurostat, 2006) on the quantities of citrus fruit imported into the EU from South Africa during the period January 2003-August 2006. However the EU-WG provided no data on the frequency of those imports.

The Panel found additional information according to which:

- for the period 1999-2007, the quantity of citrus fruit imported into the PRA area from South Africa varied from 407,000 to 639,000 tonnes per annum (Eurostat, 2008);
- citrus fruit from South Africa are imported into the PRA area during the period April-November (Eurostat 2008), (Fig. 38);
- the frequency of imports varies between years. For example, variability among years in the number of consignments is observed in the Netherlands for citrus fruit import at Rotterdam harbor (PD, 2008).

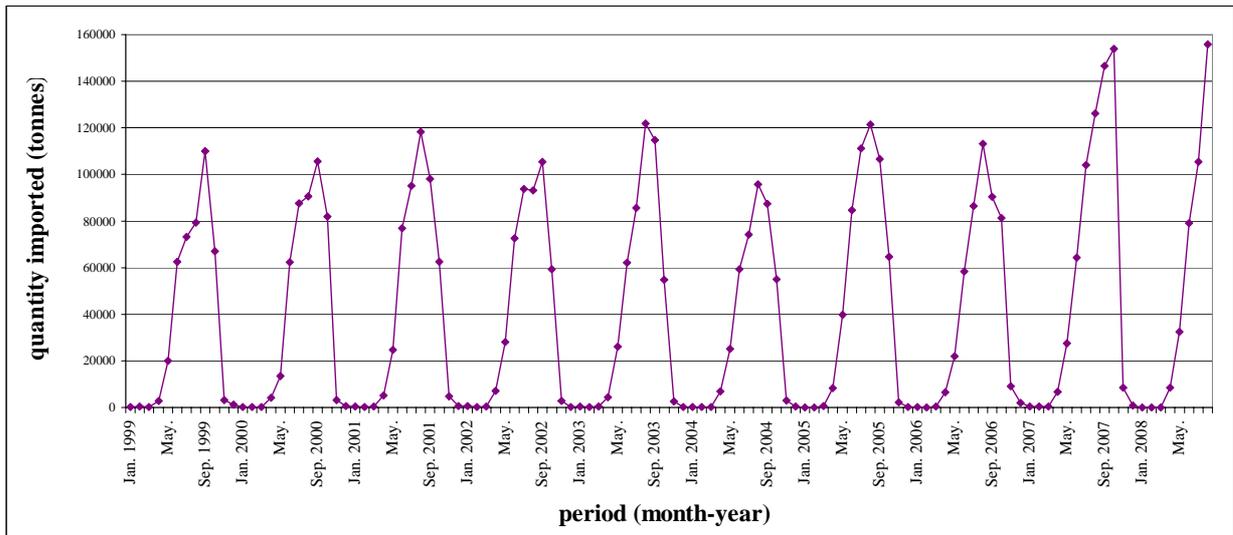


Figure 38. Quantity (tonnes) of citrus fruit imported monthly into the PRA area from South Africa during the period January 1999-August 2008 (Source: Eurostat, 2008).

The Panel, in agreement with the document, considers that significant quantities of citrus fruit from South Africa will enter the PRA area annually. The frequency of those imports is variable depending on the year and the EU-MS.

Seasonal timing

The document stated that citrus fruit may enter the PRA area during the period April to September. However no information was given in the document concerning the period of the year when fruit became infected by the pathogen and when disease symptoms and pycnidia of *G. citricarpa* developed on the fruit lesions under South African conditions.

The Panel notes that:

- citrus fruit are susceptible to infection for a period of 4–6 months from fruit set, after which fruit become resistant (Baldassari *et al.*, 2006; Kotzé, 1981 and 2000; McOnie, 1964b; Reis *et al.*, 2003; Spósito *et al.*, 2008);
- under the South African conditions, citrus flowering occurs in September. The first CBS symptoms on fruit appear in April and pycnidia formation in fruit lesions starts in mid-May (Kotzé, 1981);
- citrus fruit from South Africa are imported into the PRA area from April to November (Figure 38).

Based on the above, the Panel considers that citrus fruit from infested orchards in South Africa have already been exposed to the pathogen by the time these fruit are imported into the PRA area.

Pest management, cultural and commercial procedures applied at the place of origin

According to the document, the pest management measures applied in South African orchards and packinghouses mitigate the phytosanitary risk of CBS posed by citrus fruit exported from South Africa. These measures included:

- monitoring of ascospore release in the different citrus-producing regions using spore traps;
- chemical pre-harvest treatments with a preventive action, such as dithiocarbamates, in order to reduce inoculum;
- depending on spore counts, systemic chemicals, such as benzimidazoles, with a preventive and curative action, used pre-harvest during periods of peak spore release;
- harvesting of fruit after colour-break, which implies that most infections will have expressed symptoms (rind lesions) enabling the culling of infected fruit in the packinghouse;
- dipping of fruit in orthophenylphenol which destroys any spores on the surface of the fruit;
- waxing of fruit using products that also contain post-harvest chemicals that suppress subsequent pycnidiospore development.

In subsequent exchange of technical information between the EU-WG and the SA-WG, it was noted by the EU-WG (Annex 2) that the chemical treatments described had a suppressive and not an eradicated effect on the pathogen. Pycnidia are capable of producing several crops of pycnidiospores and, according to the EU-WG, it was uncertain whether crops produced after the application of treatments would be affected. The EU-WG (Annexes 2 and 6) also questioned the efficacy of the South African packinghouse procedures, existing regulations and the spray programme in maintaining fungicide sensitivity.

The SA-WG (Annex 3) acknowledged the non-eradication and non-residual effect of the specific pest management procedures but said that they are part of a systems approach. Regarding fungicide resistance, the SA-WG (Annex 3) noted that new chemicals (e.g. strobilurins) have since been registered and used to counter the development of resistance. It also acknowledged that (i) post-harvest chemical treatments kill exposed pycnidiospores present on fruit at time of treatment, and (ii) under favourable conditions in the EU, pycnidia can form on fruit treated against CBS.

The Panel notes that:

- It is unclear how ascospores of *G. citricarpa* and *G. mangiferae* can be differentiated using spore trapping as, according to Baayen *et al.* (2002) and Kotzé's comments (Annex 3), the two species can not be distinguished based on ascospore morphology.
- No data was provided on the efficacy of pre-harvest sprays or the monitoring of benzimidazole resistant *G. citricarpa* strains in South Africa. According to Agostini *et al.*, (2006), pre-harvest sprays were ineffective in eliminating quiescent infections on fruit.
- Harvesting fruit after colour-break in order to ensure that most infections will have expressed symptoms by that time, does not take into account information available in the literature, according to which,

- (i) Pre- and post-harvest symptom development on fruit depends on a range of factors (see Introduction)
 - (ii) Of the six different types of fruit symptoms, freckle spot appears after colour break (Bonants *et al.*, 2003; Kotzé, 1981), whereas virulent spot appears on mature fruit towards the end of the season or even in storage (Kotzé, 2000). In addition, symptomless fruit at harvest may develop symptoms during transport or storage (Agostini *et al.*, 2006; Kotzé, 1963) (*see* Introduction).
- Fruit harvested prior to or at colour-break and subjected to artificial colour change procedures (de-greening) (Terblanche, 1999), may not have developed CBS symptoms at harvest time.
 - Culling of infected fruit in the packinghouse is unlikely to be effective as, with the exception of hard spot with pycnidia, CBS lesions are small (<1 mm or 1-3 mm, in diameter), morphologically diverse (see Introduction) and can resemble those caused by other citrus pathogens, mechanical or insect damage (Kotzé, 2000; Snowdon, 1990). In addition, culling in the packinghouse will not detect latent infections.
 - The pathogen remains viable on infected fruit receiving a wide range of pre- and/or post-harvest treatments and once CBS symptoms are present on fruit, it is highly likely that the pathogen is viable (Agostini *et al.*, 2006).

The Panel considers that the management procedures described in the document are designed to reduce the risk of infected fruit being exported from South Africa to the PRA area, but they do not eliminate the pathogen. This is further supported by the fact that citrus fruit from South Africa continue to be intercepted by the EU-MS.

Conclusion on the probability of the pest being associated with fruit at origin

The Panel after taking into consideration that:

- the pathogen is present and widespread in the source area, with the possible exception of the Western Cape Province, as the document did not include scientific evidence on its CBS-free status;
- both pycnidia with pycnidiospores and latent mycelium of the organism are associated with the fruit pathway;
- large volumes of citrus fruit from South Africa enter the PRA area annually;
- citrus fruit from South Africa are imported into the PRA area during the period April-November, by which time they have already been exposed to the pathogen in the South African orchards;
- the pre- and post-harvest management procedures applied in South Africa do not eliminate the pathogen on infected fruit, and
- infected citrus fruit from South Africa continues to be intercepted in the PRA area throughout the importation period;

concludes that the probability of the organism being associated with the citrus fruit in South Africa is high.

3.1.3. Probability of survival during transport or storage

Speed and conditions of transport and duration of the life cycle of the pest in relation to the time in transport and storage

The document stated that treated fruit is shipped under temperatures which are detrimental to the development of the fungus. In a subsequent report (Annex 3), the SA-WG noted that the cold chain, which is the second component of the post-harvest treatments, has a suppressive effect on the pathogen.

The Panel notes that no indication is given in the document or in any of the subsequent South African reports (Annexes 3, 4, 5 and 7) of the duration of the fruit transport from the citrus orchards in South Africa to the EU markets or on the conditions, especially the temperatures, prevailing during transport.

The Panel found additional information according to which:

- fruit packed at the Letaba Estates takes 2-3 days to reach the port from the packinghouse (<http://www.up.ac.za/academic/fabi/citrus/blackspot.html>).
- the time between packing of citrus fruit in South Africa and arrival at the export markets is at least 3 weeks (Terblanche, 1999);
- transport from South Africa citrus orchards to ports is not usually part of the cold chain (Mather, 1999) and fruit sometimes develops symptoms during this period (Paul, 2006);
- fruit exported from South Africa is shipped in chilled containers, at a normal refrigeration temperature of 4 °C unless a lower temperature is required by the importing country (Mabiletsa, 2006);
- low temperatures and light intensities occurring during transport and storage of citrus fruit suppress symptom development. However, if those fruit are moved to a higher temperature, symptoms develop rapidly (Agostini *et al.*, 2006; Brodrick and Rabie 1970; Kotzé, 1988; Wager, 1952);
- Symptomatic fruit can be a source of viable pycnidiospore inoculum for several months, as the sporogenous layers of pycnidia are regenerative and numerous crops of pycnidiospores can be produced following regular wetting of the fruit (Kiely, 1948; Truter *et al.*, 2007; Wager, 1952).

The Panel, based on the above, considers that in terms of duration and conditions of transport and storage, the pathogen in the form of (i) pycnidia with pycnidiospores in lesions of symptomatic fruit or (ii) latent mycelium present in asymptomatic fruit, will remain viable during the time taken for transport and storage of citrus fruit.

Vulnerability of the life-stages during transport and storage

The document stated that pycnidiospores of *G. citricarpa* are short-lived. The EU-WG noted (Annex 2) that pycnidiospores taken from a slowly mummified and dried up CBS-infected orange fruit survived for up to four or, in some cases, five months (Wager, 1949) and that the results of Korf *et al.* (2001) referred to mature pycnidiospores, whereas many crops of pycnidiospores may be produced by a pycnidium. Therefore, the EU-WG considered that more information was needed on the survival of the other crops of pycnidiospores.

The Panel notes that:

- The statement that pycnidiospores of *G. citricarpa* are short-lived is not supported by scientific evidence and no indication is given either in the document or in any of the subsequent South African reports (Annexes 3, 4, 5 and 7) as to the vulnerability of the organism during transport and storage.
- The pathogen has been isolated from infected fruit maintained for 3 weeks at 4.5, 10 or 25 °C (Korf *et al.*, 2001). Similarly, Agostini *et al.*, (2006) showed that the pathogen could be readily isolated from infected citrus fruit stored at 8 °C and ambient temperatures (15-25 °C) for more than 40 days.
- Low transport temperatures may prolong the survival of pycnidiospores, as according to Korf *et al.*, (2001), mature pycnidiospores harvested from infected fruit were still viable after 3 weeks of storage of the fruit at 4.5 or 10 °C, but they lost their viability when the fruit were stored at 25 °C. Kiely (1948) showed that the viability of freshly exuded pycnidiospores incubated at 25 °C was decreased by 60 and 100% after four days and three months, respectively.
- Low temperatures (<20 °C) during transport and storage do not seem to affect the survival of latent mycelium of the pathogen, as symptoms of the disease quickly develop as soon as these fruit are transferred to higher temperatures (Kotzé, 1988; Wager, 1952).
- Splash-dispersed spores are surrounded by mucilage that protects them from desiccation and loss of viability during dry conditions (Fournet, 1969; Marconi, 1964 and 1967). In the case of *G. citricarpa*, Kiely (1948) reported that fresh crops of pycnidiospores are produced by the pycnidia displacing the pycnidiospores that had been formed earlier under less favourable conditions.
- Agostini *et al.*, (2006) showed that the pathogen was viable on fruit stored at different moisture conditions for a period of 40 days.

The Panel, after taking into account the above information and the fact that living stages of the pathogen have been intercepted on citrus fruit imported from South Africa into the EU, considers that pycnidiospores within pycnidia and latent mycelium present in infected citrus fruit are unlikely to be adversely affected by storage and transport conditions although their activity may be suppressed in the short term by the low temperatures and the low light intensities occurring during transport and storage.

Prevalence of pest likely to be associated with a consignment

The document stated that the incidence of the organism on fruit was low due to the combined effects of pre-harvest controls and culling. Elsewhere in the same document it was mentioned that the quantities of the live pathogen was low due to post-harvest controls, senescence of

lesions prior to packing, packinghouse selection, packinghouse treatments and shipping temperatures.

The EU-WG (Annexes 2 and 6), presented official data (Eurostat; Europhyt) detailing the number of citrus fruit consignments from South Africa which were found to be infected by *G. citricarpa* during the periods 1996-2001 and 2003-2006.

The Panel notes that:

- The percentage of citrus fruit intercepted because of *G. citricarpa* (Europhyt, 2008) compared with total citrus imports for EU MS (Eurostat, 2008), for the period January 2005-November 2008, ranged between 0 and 1.12%, with the exception of Slovenia in 2005 (Fig. 39).
- The monthly percentage of intercepted consignments of citrus fruit because of *G. citricarpa* over the total number of consignments imported from South Africa into the Netherlands (Rotterdam harbor) varied considerably during the period January 2005-November 2008 (Fig. 40).
- Under Council Directive 2000/29/EC, there is a 'zero tolerance' for *G. citricarpa* on citrus fruit consignments. Therefore, no data exists on the total number of citrus fruit that are infected by the organism in individual consignments imported from South Africa into the PRA area.

Based on the above, the Panel considers that the prevalence of the pathogen in citrus fruit consignments imported from South Africa into the PRA area is not known and therefore, the statement made in the document on the low incidence of the organism on fruit cannot be evaluated.

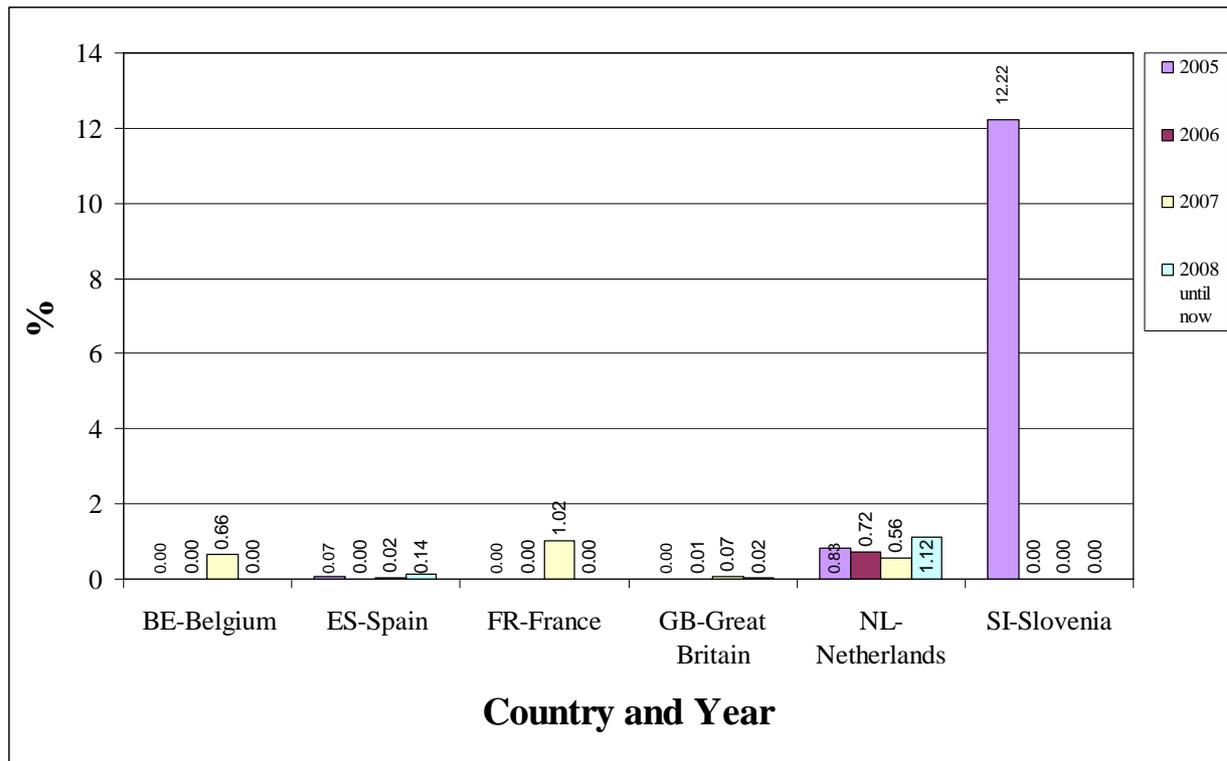


Figure 39. Percentage of South African citrus fruit intercepted because of *G. citricarpa* (Europhyt, 2008) over the total imported quantities into the PRA area from South Africa (Eurostat, 2008) during the period January 2005-November 2008.

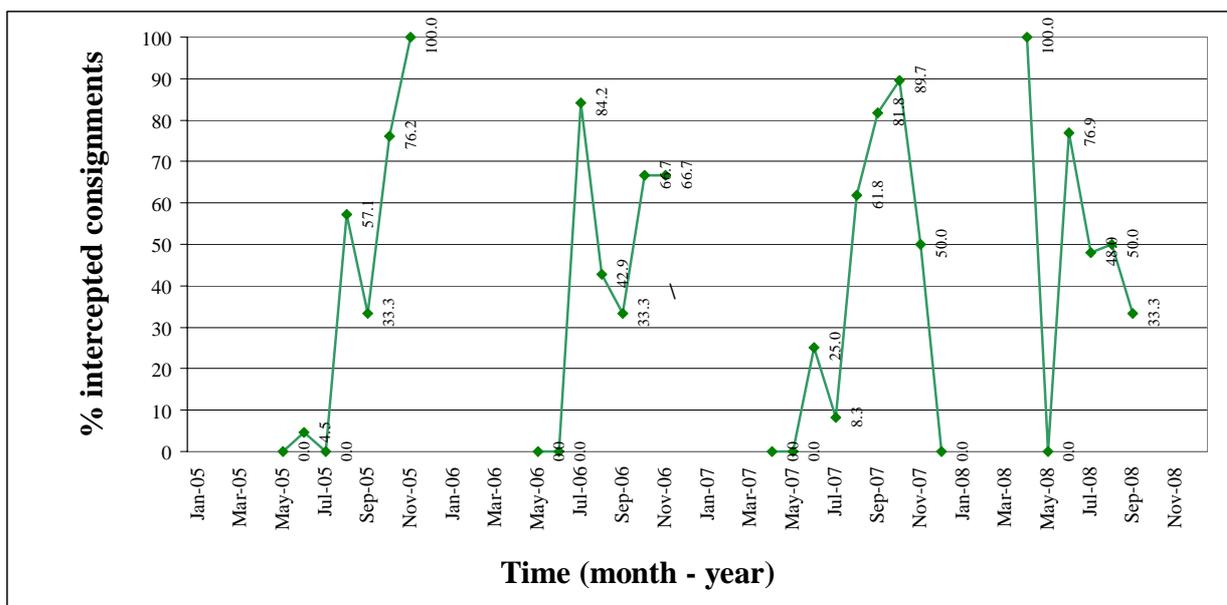


Figure 40. Monthly percentage of intercepted consignments of citrus fruit because of *G. citricarpa* over the total number of citrus fruit consignments imported from South Africa into the Netherlands (Rotterdam harbor), during the period January 2005-November 2008 (Source: PD, 2008).

Commercial procedures applied to the consignments in country of origin, destination, transport or storage

The Panel notes that:

- Information on the commercial procedures applied to the citrus fruit consignments in South Africa is provided in section 'Pest management, cultural and commercial procedures applied at the place of origin'.
- Information on the commercial procedures applied during fruit transport and storage in South Africa is included in section 'Speed and conditions of transport and duration of the life cycle of the pest in relation to the time in transport and storage'.
- Citrus fruit in the PRA area are transported and stored in refrigerated and controlled atmosphere facilities (e.g. containers, storage rooms, etc.) (http://www.tis-gdv.de/tis_e/ware/obst/).
- Low temperatures and light intensities suppress the development of symptoms on infected citrus fruit. However, if those fruit are moved to a higher temperature, symptoms develop rapidly (Agostini *et al.*, 2006; Brodrick and Rabie 1970; Kotzé, 1988; Wager, 1952).

The Panel considers that the commercial procedures applied to citrus fruit consignments prior to their export from South Africa or after their import into the PRA area may suppress the development of symptoms but they are unlikely to affect the survival of the pathogen.

Conclusion on the probability of survival during transport or storage

The document provided no estimate of the probability of the pest surviving transport and storage conditions.

The Panel, after taking into account that:

- pycnidiospores within pycnidia and latent mycelium present in infected citrus fruit are unlikely to be adversely affected by transport and storage conditions although their activity may be suppressed in the short term by the low temperatures and the low light intensities occurring during transport and storage, and
- living stages of the pathogen continue to be intercepted on citrus fruit imported from South Africa into the PRA area,

concludes that the probability of the organism surviving transport and storage is high.

3.1.4. Probability of surviving existing pest management procedures

The SA-WG provided no estimate of the probability of the pest surviving existing management procedures. The document stated that the organism caused obvious rind lesions which should be readily detectable. However the document also stated that isolation and incubation of the organism is necessary.

The EU-WG (Annexes 2 and 6) stated that living stages of the pathogen have been intercepted on several occasions on fruit of South African origin imported into the EU and provided data on the number of interceptions during the periods 1996-2001 and 2003-2006.

The Panel notes that:

- Pre-harvest treatments may reduce the disease level in the orchard, but they do not eliminate the pathogen (see section 4.4 'Evaluation of alternative measures').

- Culling of fruit in the field or in the packinghouse is unlikely to be effective in eliminating infected fruit (see section 'Pest management, cultural and commercial procedures applied at the place of origin').
- Citrus fruit harvested green and subjected to artificial colour change procedure (de-greening) (Terblanche, 1999), may not have developed CBS symptoms at harvest time (see section 'Pest management, cultural and commercial procedures applied at the place of origin').
- Latently infected fruit are asymptomatic, and probably also go undetected at border inspection. CBS has an incubation period of 2-12 months on citrus fruit (Kellerman and Kotzé, 1977; McOnie, 1967). Post-harvest development of symptoms is greatly influenced by the temperature and light intensity in the packinghouse, during transit to the market or in storage (Brodrick and Rabie, 1970; Kotzé, 1981; Wager, 1952).

The Panel considers that the existing pest management procedures provide some safe-guard against the entry of the pathogen into the PRA area. However, given the range of CBS symptoms on fruit, the unevenness of their development, the presence of latent infections and the suppressive effects of pre- and post-harvest treatments on symptom development, the Panel concludes that the probability of *G. citricarpa* surviving existing pest management procedures is high for fruit with latent infections and inconspicuous symptoms and low to moderate for fruit with hard spot lesions with pycnidia.

3.1.5. Probability of transfer to a suitable host

Dispersal mechanisms, including vectors to allow movement from the pathway to a suitable host

According to the South African document, ascospores are the main source of inoculum. CBS infected fruit only produced pycnidia and water-dispersed pycnidiospores, which, according to the document, are not considered to be an important source of inoculum in the disease cycle either through downward movement of rain water or splashing upwards. The document further noted that pycnidiospores are short-lived, are produced over a short period, at a specific developmental stage of infection and under specific environmental conditions, are sticky (mucilage) and require running water for dissemination and exposure to specific stimuli for germination. In addition, the document described a number of risk mitigating factors that limit the infective potential of pycnidiospores. These included infected fruit being placed in a tree canopy, exposed to specific environmental conditions and possessing lesions at a specific stage of maturity for infection to take place.

In subsequent exchange of technical information between the EU-WG and the SA-WG, the EU-WG (Annexes 2 and 6) noted that ascospores are the most efficient means of dissemination of the organism in areas where the disease has established but that pycnidiospores may be as important as ascospores when the disease is initially introduced into an area (Whiteside, 1967) as:

- citrus orchards in the PRA area are irrigated (Annex 2) and irrigation, particularly sprinkler irrigation, is equivalent to rain in the spread of various fungal and bacterial diseases;
- the role of insects in pycnidiospores spread was not discussed in the document (Annex 2);

- the possibility of pycnidiospores being splashed upward from infected fruit and/or peel on orchard floor onto the lower reaches of the tree canopy is very likely (Annex 6);
- citrus are omni-present in the EU Mediterranean area;
- the low-hanging growth habit of citrus trees in some of the Mediterranean orchards (Annex 2 and 6) favour splash dispersal from the ground;
- susceptible young leaves and fruit are distributed within the whole tree canopy;
- specific stimuli are not required for pycnidiospore germination under field conditions and this is further supported by the results of Kiely (1948), according to which, pycnidiospores were pathogenic to sweet orange fruit by just spraying a suspension of inoculum in water.

The SA-WG (Annex 3 and 7) responded that, although there may be a remote possibility of pycnidiospores being splash-dispersed onto the lower reaches of the tree, this is 'highly unlikely', due to the remoteness of this possibility and the specific stimuli required for spore germination and infection to take place. The SA-WG also noted that failure to detect ascospores in South American citrus groves (Spósito, 2003) does not imply a greater role for pycnidiospores, as it may simply have represented a lack of experience in ascospore trapping techniques.

Concerning the role of insects, the SA-WG noted (Annex 3) that the likelihood of this pathway being successful was even more remote than splashing. In Annex 3, a commentary by JM Kotzé was presented, which acknowledged the potential role of insects, birds, etc. in the dispersal of the pathogen's pycnidiospores, although noting that they had not experienced such involvement. Kotzé further considered that insects, birds etc. may be able to deposit pycnidiospores on the wounds they cause on the host, but their role was insignificant if the climatic conditions were not favourable for the further development of the pathogen.

The Panel notes that:

- There is limited information available in the literature on the dispersal of *G. citricarpa* conidia from leaf litter or fruit on the orchard floor onto susceptible plant tissues in the tree canopy (leaves, fruit), on the height or the distance over which pycnidiospores of *G. citricarpa* can be splash-dispersed or on the potential role of insects, birds, etc in the dispersal of the pathogen's pycnidiospores.
- According to Kotzé (1981 and 2000), pycnidiospores of the pathogen produced on leaf litter or fruit on the orchard floor may reach susceptible fruit by rain splashing. Similarly, Agostini *et al.* (2006) reported that pycnidiospores produced on infected fruit would require the presence of susceptible tissue in close proximity to the inoculum source in order to be splash-dispersed.
- The greatest number of diseased fruit observed in the lower part of the citrus canopy (Schinor *et al.*, 2002; Spósito *et al.*, 2008) is indirect evidence that splash-dispersed pycnidiospores have an important role in increasing the disease severity within the canopy.
- According to Whiteside (1967), pycnidiospores are very important as inoculum sources, following the introduction of the disease into a new area.
- Nozaki (2007) demonstrated a higher rate of pycnidiospores germination in presence of citric acid or orange juice in vitro and on detached leaves, but germination occurred also in water.

Furthermore, the Panel found general information on the splash-dispersal of fungal conidia, according to which:

- An indicator of conidia adapted for splash-dispersal is a layer of mucilage around each spore, which prevents dispersal by wind (Fitt *et al.*, 1989; Fournet, 1969).
- In still air, splash-dispersed pathogens are usually dispersed up to a height of 50 cm above the inoculum source or up to a distance of 1 m from the host, with the number of conidia deposited on the plant surfaces decreasing steeply with increasing height or distance from the source (Fitt *et al.*, 1989). However the dispersal of conidia depends on a number of factors, such as the size and velocity of the incident drop, the size of conidia and the occurrence of air currents. Rain tower experiments have shown that in still air one splash may disperse many thousands of conidia, but most are carried in the largest droplets (diameter >1 mm) and very few in droplets <100 µm in diameter (Fitt *et al.*, 1989). Moreover, the largest drops spread the conidia over shorter distances when compared with the smaller drops. Generally, raindrops that reach the ground are 0.2-5 mm in diameter, since smaller drops evaporate rapidly unless relative humidity is near to 100%, and larger drops break up when they fall at speeds approaching their terminal velocities. Nevertheless, rain with many large drops will be most effective in dispersal of these pathogens (Fitt *et al.*, 1989).
- In the presence of wind, inoculum carried in small splash droplets may also become airborne as an aerosol of fine spray (Fitt *et al.*, 1989). The significance of wind in the dispersal of pathogens removed from the inoculum source in splash droplets becomes greater as the size of the inoculum particles becomes smaller. For example, conidia of *Phoma exigua* var. *foveata* (Foister) Boerema, which are considerably smaller (7 × 3 µm) than those of *Oculimacula yallundae* (Wallwork & Spooner) Crous & W. Gams (syn. *Pseudocercospora herpotrichoides*) (50 × 2 µm) or *Phaeosphaeria nodorum* (E. Müll.) Hedjar (syn. *Septoria nodorum*) (25 × 3 µm), are more likely to be carried in small splash droplets that become airborne (Fitt *et al.*, 1989). In field studies, conidia of *O. yallundae* and *P. nodorum* have been collected 70 cm and 1 m, respectively, above infected debris (Faulkner and Colhoun, 1976; Wale and Colhoun, 1979; Fitt and Bainbridge, 1984). In rain tower/wind tunnel experiments with wind speeds of ca. 2.5 m sec⁻², droplets carrying conidia of *O. yallundae* and *P. nodorum* have been collected at heights between 0.5 m and 4 m from the sources (Fitt and Nijman, 1983; Brennan *et al.*, 1985).
- Drops formed on the leaves due to fog, dew, mist or overhead irrigation may cause drip-splash of inoculum under canopies. This may be as important as direct rain-splash. These drip drops may be larger than 5 mm diameter because they fall only short distances and are less likely to break up compared to raindrops. Thus, they may have sufficient impact force for the dispersal of conidia in splash droplets (Fitt *et al.*, 1989).

The Panel also notes that:

- *G. citricarpa* pycnidiospores are adapted for splash dispersal, as they are covered with a gelatinous sheath (van der Aa, 1973) which, as with other pycnidiospore-producing fungi, prevents their dispersal by wind.
- The distance above ground of low hanging citrus fruit and foliage can vary from 0 to 80 cm, depending on the cultivation techniques. There is also a trend for shorter citrus trees by grafting onto dwarfing rootstocks (Colombo, 2004; Regione Puglia, 2007).
- Rain events which may trigger the splash dispersal mechanism in the citrus growing regions of the PRA area occur in the period of import of citrus fruit from South Africa (April to November) (see section 2.1.3.2., section 2.3.4.3. and section 2.3.5.3.).

- In the absence of rain, dew, mist (commonly occurring during the night in the coastal citrus-growing areas), sprinkler/overhead irrigation or pesticide sprays using high pressure equipment may cause direct- and/or drip-splash of the pycnidiospores produced on infected fruit/peel discarded on the orchard floor (see section 3.2.3, Cultural practices and control measures).
- Citrus trees grown in residential areas are usually irrigated with a water hose, a practice which may also promote the splash-dispersal of pycnidiospores from discarded fruit or peel onto the tree canopy.
- Under windy conditions, the small-sized *G. citricarpa* pycnidiospores (9.4-12.7 x 5-8.5 µm) (Baayen *et al.*, 2002) carried in small splash droplets could potentially become airborne as an aerosol of fine spray.

The Panel considers that pycnidiospores of *G. citricarpa* produced on fruit/peel discarded underneath citrus trees in the PRA area may reach susceptible tissues (young fruit, leaves, etc) in the lower reaches of the canopy by the direct- or drip-splash dispersal mechanisms, which could be triggered by rain, dew or sprinkler irrigation.

Whether imported material is to be sent to a few or many destinations within the PRA area

The distribution of the imported into the PRA area citrus fruit is not discussed in the document.

The Panel notes that:

- according to Eurostat (2008), during the period 2001-2007, citrus fruit from South Africa was imported into 25 EU-MS, including citrus-producing States (see Table 7);
- citrus fruit are imported into the PRA area during the period of the year, from April to November (see Fig. 38), when local produce is unavailable;
- citrus fruit are imported mainly through ports in both southern and northern Europe (Europhyt, 2008), however once fruit has entered the EU common market, it may move freely between MS according to regional demands;
- imported citrus fruit is mainly used for fresh consumption. On average, Europeans consumed 23kg *per capita* of fresh citrus in 2003 (FAOSTAT, 2008).

Table 7. Annual quantity (tonnes) of citrus fruit imported by the EU-Member States (EU-MS) directly from South Africa.

Importing EU-MS	Year						
	2001	2002	2003	2004	2005	2006	2007
Austria	1,7201	2,290	2,210	130	520	60	20
Belgium and Luxembourg	63,270	52,390	30,440	26,420	35,940	38,560	20,480
Bulgaria	-	-	-	-	-	-	1,910
Cyprus	-	-	-			50	20
Czech Republic	-	-	-	330		20	160
Germany	2,850	3,180	940	1,000	3,100	5,200	5,460
Denmark	850	910	900	1,240	1,550	1,900	1,980
Spain	46,140	43,490	54,880	65,920	65,340	44,260	85,080
Finland	950	930	800	850	985	860	1,270

France	21,130	12,080	14,825	18,970	16,350	11,980	13,810
United Kingdom	137,550	135,300	134,860	132,890	145,200	140,580	159,520
Greece	2,160	2,010	5,290	13,790	2,720	6,040	15,760
Ireland	810	70	260	800	2,865	4,670	9,460
Italy	26,020	29,150	38,880	39,990	50,220	38,060	48,670
Lithuania	-	-	-	430	60	1,700	3,780
Latvia	-	-	-		20		285
Malta	-	-	-	730	540	950	800
Netherlands	153,755	151,720	159,470	94,055	202,600	157,840	250,540
Poland	-	-	-	170			160
Portugal	1,230	960	680	1,210	2,445	1,040	8,920
Romania	-	-	-	-	-	-	2,630
Sweden	40	100	780	1,340	2,440	7,560	8,380
Slovenia	-	-	-	1,770	1,000	350	365
Slovakia	-	-	-	20			
Total	458,475	434,580	445,215	402,055	533,895	461,680	639,460

Source: Eurostat, 2008.

The Panel considers that, as citrus fruit are imported from South Africa into both citrus- and non-citrus-producing EU-MS during the period where local produce is unavailable, these fruit are widely distributed within the PRA area.

Proximity of entry, transit and destination points to suitable hosts

The proximity of entry, transit and destination points to suitable hosts was not discussed in the document. However the EU-WG (Annex 2) highlighted the widespread distribution of CBS-susceptible citrus species within the PRA area.

The Panel notes that:

- Citrus fruit consignments are imported into both citrus- and non-citrus producing EU-MS mainly through ports.
- Citrus species susceptible to CBS are widely distributed within the southern EU-MS (see Fig. 10-13) with the citrus orchards being located mainly in coastal areas and in close proximity to the ports. The document did not take into account the potential risk posed by citrus grown in private and public gardens or as ornamentals along the streets in urban and rural regions of the PRA area.
- Citrus fruit consignments are imported from South Africa into the EU when there is no local produce available and therefore, they are widely distributed within the PRA area (see above).

The Panel, based on the above, considers that the entry and the destination points of the citrus fruit consignments imported from South Africa into the citrus-producing EU-MS are likely to be in close proximity to CBS susceptible citrus species.

Time of year when imports take place

According to the document, fruit may enter the PRA area from April to September.

The Panel notes that:

- Citrus fruit from South Africa are imported into the PRA area during the period April to November (Europhyt; 2008).
- Citrus fruit are susceptible to infection by *G. citricarpa* for 4–6 months from fruit set (Baldassari *et al.*, 2006; Kotzé, 1981 and 2000; McOnie, 1964b; Reis *et al.*, 2003). Lemon leaves are susceptible for 10 months following their emergence (Truter *et al.*, 2004; 2007).
- Under EU conditions, the main citrus flowering period is from early April to early May with the exception of lemon which has one or two additional flowering periods from July to September (Colombo, 2004). For most of the citrus species/varieties grown in the PRA area the main leaf flush occurs in spring (April-May) and coincides with the flowering period (Guardiola, 1997; Krezdorn, 1986). Lemons have usually 2-3 additional leaf flushes until September, which correspond to summer flowering periods.

The Panel considers that the period of imports of South African citrus fruit into the PRA area coincides with the susceptible period for infection of citrus trees grown in the PRA area.

Intended use of commodity

The Panel, in agreement with the document, considers that the intended use of citrus fruit imported into the PRA area from South Africa is primarily for the fresh fruit market.

Risk from by-products and waste

The risk from by-products and waste was not discussed in the document.

The Panel notes that the risk with citrus fruit is mainly associated with the waste (e.g. domestic waste, waste from packinghouses, fruit markets, etc) and its management, as:

- Citrus fruit/peel waste disposal can not be sufficiently controlled in any part of the citrus-producing EU-MS to prevent the escape of the pathogen to the environment.
- Symptomatic citrus fruit/peel can be a source of viable pycnidiospore inoculum for at least 40 days (Agostini *et al.*, 2006) or until decomposed by other organisms, as the sporogenous layers in pycnidia are regenerative and numerous crops of pycnidiospores can be produced following regular wetting of the fruit although viability declines with time (Agostini *et al.*, 2006; Kiely, 1948; Truter *et al.*, 2007; Wager, 1952).
- Pycnidiospores taken from a slowly mummified and dried up CBS-infected orange fruit survive for up to four or, in some cases, five months (Wager, 1949).
- Pycnidiospores produced on CBS-infected fruit are able to infect attached young fruit and green citrus leaves (Kiely, 1948; McOnie, 1967; Wager, 1952).
- Citrus trees are widespread in the PRA area in commercial orchards and nurseries, in private gardens for family consumption, in public gardens and on the roadsides as ornamentals both in urban and rural areas.
- Citrus packinghouses are generally located within citrus growing areas. For example, in Spain, according to the list of collective stock-houses and shipping centers in the zones of citrus production (personal communication by Cobos JM, Subdirección General de Sanidad de la Producción Primaria, Ministerio de Medio Ambiente y Medio Rural y Marino, 15/12/2008), 78 establishments are located in the Comunidad Valenciana, 167 in the province of Murcia, 44 in Andalucía and 1 in Catalunya (see Appendix .Table 16).

The Panel considers that waste of infected citrus fruit/peel, discarded underneath or in close proximity to citrus trees in the PRA area, could be a source of pycnidiospores for the transfer of the pathogen by splash dispersal onto susceptible citrus tissues (leaves, young fruit, etc) hanging close to the orchard floor.

Conclusion on the probability of transfer to a suitable host

The Panel considers that the document did not provide enough evidence that it is “highly unlikely” for *G. citricarpa* pycnidiospores to be splash-dispersed onto the lower reaches of citrus trees grown in the PRA area.

The Panel, after taking into consideration that:

- pycnidiospores of the pathogen are adapted for splash dispersal;
- the period of imports of South African citrus fruit coincides with the susceptible period for infection of citrus trees grown in the PRA area and imported citrus fruit is widely distributed within the PRA;
- CBS infected fruit and fruit peel discarded by processors or consumers in proximity to citrus trees could be a source of pycnidiospores for transfer of the pathogen by splash dispersal onto the tree canopy;
- the splash dispersal mechanism can be triggered mainly by rain events occurring during the period of imports of citrus fruit from South Africa into the PRA area and occasionally by dew or sprinkler irrigation;
- fruit and foliage of citrus trees in the PRA area can hang close to soil level and may be reached by splash dispersed pycnidiospores produced on fruit waste, and

concludes that CBS infected fruit / peel can be discarded underneath or in close proximity to susceptible hosts in the PRA area, although the frequency of this event is not known. Furthermore, the pathogen could be transferred from this material to citrus trees by splash dispersal.

3.1.6. Conclusion on the probability of entry

The Panel notes that the SA-WG concluded in Annex 3 that the pathogen could enter the PRA area. However it is unclear if this assessment includes also the likelihood of the pathogen to be transferred from infected citrus fruit/ peel placed under a citrus tree onto susceptible tissues in the tree canopy, as this component of the analysis is first mentioned in the document in the section dealing with establishment. Notwithstanding this, the Panel after having determined that the organism:

- is associated with the citrus fruit in South Africa;
- will survive transport and storage;
- will survive existing pest management procedures, especially in the form of quiescent infections and inconspicuous symptoms;
- may be transferred to suitable hosts by means of splash dispersal from CBS infected fruit and peel.

concludes that *G. citricarpa* may enter the PRA area with infected citrus fruit.

3.2. Probability of establishment

3.2.1. Availability of suitable hosts, alternate hosts and vectors in the PRA area

The document states that citrus are susceptible to CBS with ‘Valencia’ and ‘Navel’ sweet orange varieties, grapefruit and lemons being the most susceptible. However no data was provided on the availability and distribution of susceptible citrus in the PRA area. Paul *et al.* (2003) (Annex 4) listed the citrus-growing areas within the EU, namely France, Greece, Italy, Portugal and Spain, but provided no data on the distribution of the citrus trees within those areas.

The EU-WG reported that citrus species are grown almost everywhere in the citrus- producing regions of the PRA area (Annex 2). It also noted that CBS outbreaks always begin in lemon orchards, where inoculum builds up gradually until all adjacent citrus orchards become infected (Kotzé, 1981). The EU-WG further reported that lemon and other susceptible citrus species are also grown in private gardens even in the major towns within the PRA area (Annex 2) and provided some data on the distribution of various *Citrus* spp. in Greece and Italy and on the age of the trees (Annex 6).

The Panel notes that:

- Susceptible citrus species (sweet oranges, lemons, mandarins, grapefruit, etc.) are extensively grown in commercial orchards and nurseries, in private gardens for family consumption, as ornamentals in public gardens and are naturalised throughout the citrus-growing regions of the PRA area. The total area grown with commercial citrus species in 2005 was 566,800 ha of which 82,000 ha were planted with lemon (Eurostat, 2008). Areas cultivated with citrus species and with lemon in EU MS and regions are presented in Appendix in tables 17 and 18.
- Late-maturing sweet orange varieties (e.g. ‘Valencia’) were considered in the past to be more susceptible than early maturing varieties (Kotzé, 1981). However it has been recently shown that some early-, mid- and late-maturing sweet orange varieties are equally susceptible to infection by *G. citricarpa* (Baldassari *et al.*, 2006; Schinor *et al.*, 2002).
- Citrus fruit are susceptible to CBS for 4-6 months after fruit set (Baldassari *et al.*, 2001; Kellerman and Kotzé, 1977; Kotzé, 1981; Spósito *et al.*, 2008) and lemon leaves are susceptible from their emergence up to 10 months later (Truter *et al.*, 2004; 2007). In the EU citrus-growing areas, the main flowering period for most citrus species/varieties is in spring from early-April to early-May. However, with lemons, one or two additional flowering periods may occur in summer (July-September) (Agustí, 2000; CORERAS, 2007; Cutuli *et al.*, 1985). The seasonal leaf-flush pattern of citrus may vary depending on the variety, the cultural practices (e.g. pruning, fertilisation, irrigation, etc.), the rootstock, etc. For most of the citrus species/varieties grown in the PRA area the main leaf flush occurs in spring (April-May) and coincides with the flowering period (Guardiola, 1997; Krezdorn, 1986). Lemons have usually 2-3 additional leaf flushes until September, which correspond to summer flowering periods.
- The co-existence of mature symptomatic fruit from the previous year’s crop with young fruit from the new fruit set on trees of certain citrus species (e.g. lemons) and/or varieties (e.g. ‘Valencia’ sweet oranges) extends the period during which susceptible tissues are exposed to inoculum and subsequently increases the disease level in an orchard (Kiely, 1948; Kotzé, 1981; Spósito *et al.*, 2008).

- The role of insects, birds, etc in the epidemiology of the disease has not been documented. However Kotzé (Annex 3) and Kiely (1948) considered that insects, birds etc. may be able to deposit pycnidiospores on the wounds they cause on the host, although, according to Kotzé (Annex 3), their role is insignificant if the climatic conditions are not favourable for the further development of the pathogen.

The Panel concludes that susceptible hosts of *G. citricarpa* are widely distributed within the citrus-growing regions of the PRA area in commercial orchards, nurseries, private and public gardens and along the streets in urban and rural regions. Among citrus species, lemon, which is considered to be the most susceptible and therefore, the first to be infected by the pathogen in a new area, is widely distributed in both rural and urban areas of the citrus-growing EU-MS.

3.2.2. Suitability of environment

The document states that, based on the distribution of the pathogen in South Africa and Australia, the disease occurs in the summer-rainfall northern parts of these areas and has never established in the southern regions that experience a Mediterranean climate, characterised by dry summers. The SA-WG (Annex 3) also noted that the organism would “have to behave as it never has before in any part of the world” for the disease to become established in a Mediterranean climate. The SA-WG supported its statement by referring to the work of Paul *et al.* (2005) on climate matching between South Africa, Australia and the PRA area.

The EU-WG (Annex 2) noted that the term ‘Mediterranean climate’ lacks specificity and that the organism has already established in areas with various climatic conditions. It also noted that over-reliance on climate matching techniques, when the underlying data is insufficient, may lead to underestimation of the risk of establishment of the target organism (Annex 6) and supported this statement with Kotzé (1981), according to which, past over-reliance on a climatic barrier for the establishment of CBS in a new area was proven unsatisfactory.

The EU-WG also noted that according to the literature (Kotzé, 1981), climate influences the rate of CBS spread, but not necessarily CBS establishment. It further pointed out that the effect of microclimate on disease development is more considerable in semi-arid areas, where the canopy microclimate can provide favourable conditions even though the macroclimate is unfavourable (Palti and Rotem, 1973). In support, the EU-WG (Annex 6) presented the results of Vicent and García -Jiménez (2008), according to which, in Spain, rainfall and rain days were not positively correlated with citrus canopy wetness, due to the formation of dew.

Based on (a) the evaluation of the application of CLIMEX Paul *et al.* (2005), (b) the limitations of CLIMEX in predicting the potential distribution of pathogens such as *G. citricarpa*, (c) the relative climatic similarities between locations where CBS occurs in Eastern Cape Province and where citrus is grown in the EU and (d) the results of the application of a generic infection model for foliar fungal pathogens, the Panel cannot agree with the conclusion by Paul *et al.* (2005) that the climate of the EU is unsuitable for the establishment of *G. citricarpa* (see section 2.4. ‘General conclusions on climate suitability’).

3.2.3. Cultural practices and control measures

The document refers to the use of pre-harvest sprays with protectant and curative fungicides (dithiocarbamates, benzimidazoles) for the control of CBS in South Africa.

The EU-WG (Annex 2) commented on the occurrence of benzimidazoles resistant strains of *G. citricarpa* in South African citrus orchards (Herbert and Grech, 1985). It further pointed out the

importance of cultural methods, especially irrigation, which is widely used in the EU, on the disease establishment and spread.

The SA-WG (Annex 3) concurred on the importance of irrigation, but only in relation to ascospore release after CBS establishment and noted that there would be considerable differences between the effects of drip- and micro-jet irrigation.

The Panel notes that no information is provided in the document, or in Annex 3, 4, 5 and 7, on the cultural practices (irrigation, weed control, pruning, harvest, etc) applied in South Africa during the cultivation and production of citrus.

The Panel found the following additional information on the cultural practices and control measures applied in the South African citrus-producing areas:

- The flowering and fruiting periods are uniform due to irrigation and cooler temperatures, which lead to few out-of-season fruit (Kotzé, 1981; Spósito *et al.*, 2008).
- In citrus orchards, where fruit is destined for export, out-of-season or late-hanging fruit are removed before the new flowering period. This practice reduces pycnidiospores as a source of inoculum (Kotzé, 1981).
- Wager (1949) speculated that in some areas of South Africa (e.g. in parts of the Western Cape Province), the dense weed or cover crop in citrus orchards during the wet winter season might have inhibited ascospore production on leaf litter.
- Chemicals are applied to leaf litter immediately after leaf drop and before pseudothecia develop in order to reduce ascospore inoculum (Kotzé, 1981).
- Dithiocarbamates, copper, benzimidazoles and strobilurins are registered for the control of CBS in South Africa (Schutte *et al.*, 2003). Spray application of protective chemicals, such as copper-based fungicides and dithiocarbamates, have to be carefully timed to coincide with the critical infection period (Kotzé, 2000). The number of sprays required in a protective programme depends largely on tree age and vigor, citrus cultivar, and environmental conditions. In heavily infected orchards (e.g. old 'Valencia' orchards), up to five protective sprays may be required during the 4-5 months of fruit susceptibility period (Kotzé, 2000). Until 1981, when resistant to benzimidazoles strains of *G. citricarpa* were detected, one spray with benomyl plus oil applied toward the end of the critical infection period provided effective disease control. Recently, strobilurins, which have been shown to have fungicidal activity against the pathogen and particularly against strains resistant to benomyl, are registered in South Africa for the control of CBS (Kotzé, 2000; Shutte *et al.*, 2003).

The Panel found that, in the citrus-growing EU-MS:

- Citrus orchards are generally irrigated during the dry periods (Eurostat, 2008). Irrigation systems used are drip- or mini-sprinkler (ARI, 2008; Colombo, 2004) and these irrigation methods may affect ascocarp maturation on leaf litter.
- The removal of out-of-season or late-hanging fruit is uncommon.
- Multiple flowering and leaf flushing periods occur for lemons from early April to late September.
- Fungicides registered for citrus in the PRA area are generally protectant fungicides, such as copper-based or dithiocarbamates. No strobilurins are registered for citrus and benzimidazoles are forbidden in the EU (DGADR, 2008; E-phy, 2008; Greek Ministry of

Rural Development and Food, 2008; Ministerio de Medio Ambiente y Medio Rural y Marino, 2008; Ministero del Lavoro, della Salute e delle Politiche Sociali, 2008; Ministry of Agriculture, Natural Resources & Environment, Department of Agriculture – Cyprus, 2008).

- Generally few fungicide treatments are currently applied in the EU citrus-producing areas against other citrus pathogens, such as *Alternaria alternata* pv. *citri*, *Phytophthora* spp., *Phoma tracheiphila* and *Pseudomonas syringae* pv. *syringae* (MIPAAF, 2008).
- Cultural and control practices are not likely to be undertaken for citrus grown in private gardens for family consumption and as ornamentals in public gardens or along the streets in urban and rural regions of the PRA area.

Finally, the Panel notes that the flowering and fruiting pattern of the citrus species/varieties seem to influence the epidemiology of the disease. More specifically, studies in Brazil and Argentina (Ortiz *et al.*, 2004; Reis, 2002; Spósito *et al.*, 2008), have suggested that due to irregular flowering of the cultivated citrus varieties and the subsequent overlapping of mature and young fruit, the role of pycnidiospores in the epidemiology of the disease is different than that in South Africa and Australia, where epidemics have been exclusively attributed to ascospores (Kiely, 1948; McOnie, 1964b, 1964c and 1965). In the latter countries the uniform flowering and fruiting of the cultivated varieties due to irrigation and the cooler temperatures lead to few off-season fruit (Kotzé, 1981).

The Panel concludes that the cultural practices and control measures applied in commercial citrus orchards in the PRA area cannot prevent the establishment of the pathogen.

3.2.4. Other characteristics

The occurrence of fungicide resistance was mentioned in the document; however, its relationship to genetic adaptability was not discussed.

The EU-WG (Annex 2) noted the occurrence of CBS in a range of different climates and suggested that this indicates that the organism could eventually establish in all citrus growing areas. The EU-WG also noted that *G. citricarpa* has a sexual stage, increasing its ability to adapt. The EU-WG repeated this argument in Annex 6 and referred to the occurrence of benzimidazole resistance isolates of *G. citricarpa* as further evidence of its genetic adaptability.

The SA-WG in Annex 3 stated that the occurrence of a sexual stage for this organism was not a significant factor, on the basis that it would complicate the analysis of pest establishment and so potentially undermine trade. The SA-WG further considered that fungicide resistance as an indicator of genetic adaptability was an academic and improbable argument (Annex 7).

Concerning minimum populations needed for establishment, the EU-WG suggested that a single conidium could lead to pest establishment given optimal environmental conditions. The SA-WG dismissed this contention, referring to various inconsistencies within the article on which the EU statement was based (Smith, 1996). The EU-WG (Annex 6) concurred that the likelihood of a single conidium leading to pest establishment was more theoretical than practical.

The Panel considers that factors such as the genetic adaptability of an organism are relevant to determining the ability of an organism to adapt to a new environment but concludes that

insufficient evidence is available to allow the genetic adaptability potential for *G. citricarpa* to be determined.

3.2.5. Conclusion on the probability of establishment

The Panel notes that the document did not provide a specific conclusion on the probability of establishment. However, following the study of the document and the subsequent Annexes, the Panel determined that the SA-WG considered that the organism cannot establish in the PRA area. The Panel, after taking into consideration that:

- susceptible citrus, especially lemons, are widely distributed within the citrus-growing region of the PRA area,
- cultural practices and control measures applied in the PRA area can not prevent the establishment of the organism, and
- parts of the PRA area have a climate which can be favourable for CBS establishment,

concludes that the probability of *G. citricarpa* establishing in the PRA area is greater than that indicated in the South African document.

3.3. Uncertainty

The Panel has identified the following areas of uncertainty concerning entry and establishment:

- environmental conditions that favour the infection by pycnidiospores and the response of the pathogen to interrupted leaf wetness;
- the prevalence of CBS infected fruit imported into the EU from South Africa;
- the frequency of infected fruit/peel being discarded under or in close proximity to a citrus tree;
- the epidemiological role of inoculum sources other than leaf litter and fruit (e.g. twigs, pedicels, etc);
- the degree of survival of the organism in soil;
- the role of insects and birds in inoculum dispersal.

but it considers that these uncertainties will not affect the conclusions reached by the Panel.

4. Evaluation of management options to prevent introduction of citrus black spot into the Community

4.1. Methodology used for identification and selection of appropriate risk management options

The Panel is requested to provide a scientific opinion on the appropriateness of the level of protection under the existing management options listed in Annex IV, Part A, Section I, point 16.4 of Council Directive 2000/29/EC. EFSA is also requested to identify whether effective options, alternative to those already present in Directive 2000/29/EC, could be suggested to prevent introduction of citrus black spot into the Community. Following the terms of reference, the management options examined are limited to the citrus fruit pathway. The import of citrus planting material into EU is forbidden in Directive 2000/29/EC Annex II, Part A, Section 1, point c 11.

The review was based on the principles of the International Standard on Phytosanitary Measures ISPM No. 11 and 14. Appropriate measures should be chosen based on their effectiveness in reducing the probability of introduction of the pest. The choice should be based on the following considerations, which include several of the Principles of plant quarantine as related to international trade (ISPM No. 1):

- Phytosanitary measures shown to be cost-effective and feasible -

The cost-effectiveness can not be analysed because the potential economic consequences have not been assessed in the documents provided. Therefore, only the effectiveness will be considered.

- Principle of “minimal impact” -

Measures should not be more trade restrictive than necessary. Measures should be applied to the minimum area necessary for the effective protection of the endangered area.

- Re-assessment of previous requirements -

No additional measures should be imposed if existing measures are effective.

- Principle of “equivalence” -

If different phytosanitary measures with the same effect are identified, they should be accepted as alternatives. (This principle is currently satisfied in Directive 2000/29.)

-Principle of “non-discrimination”

This aspect is not relevant as the organism *Guignardia citricarpa* is absent from the EU.

The quarantine regulations implemented by other citrus producing countries to prevent the introduction and spread of *G. citricarpa* on citrus fruit are presented. For the EU current phytosanitary measures, the interceptions of consignments with *G. citricarpa* from South Africa are analysed.

The Panel also collected and analysed information on interceptions of infected citrus fruit imported in to Europe from different official databases and selected Europhyt as the most complete data source.

4.2. Elements of quarantine regulation of major non-EU citrus producing countries for *G. citricarpa*

The Panel examined the quarantine regulations for *G. citricarpa* of non-EU citrus producing countries, where the disease is absent or present with limited distribution.

Australia. According to the Annual Operational Plan 2006-2007, defined by Plant Health Australia (PHA), citrus black spot (*G. citricarpa*) is present in Queensland and Northern Territory under official control (HAL, 2006) but it is not categorised as an “emergency plant pest” (DAFF, 2008). The state of South Australia has specific regulations related to CBS (PIRSA, 2006). According to this standard, citrus fruit and plant material may not be imported or introduced into South Australia, unless inspected and found free of specific pests and diseases, including CBS. Other states including Victoria, Western Australia and the Australian Capital Territory do not regulate *G. citricarpa*.

Brazil. *G. citricarpa* is a category A2 quarantine pests in Brazil, with potential economic importance. It is already present in the country but not widely distributed (CAB International, 2007). An official control programme exists, determined jointly with the States in which the organism has been detected (Rio de Janeiro and São Paulo). There is a prohibition on entry, transit and commerce in the Mato Grosso State of citrus fruit from the States of Rio de Janeiro and São Paulo, unless accompanied by an invoice or producer bill and transit permit based on the phytosanitary certificate at the origin (INDEA/MT, 2008). Import of citrus fruit into the State of Bahia is also prohibited from other Brazilian states, where *G. citricarpa* has been reported (SEAGRI, 1998).

India. According to Plant Quarantine (Regulation of Import into India) Order (DAC, 2003), an additional declaration is required from countries exporting *Citrus* spp. (lemon, lime, orange, grapefruit, mandarins, etc. and other Rutaceae) that the fresh fruit for consumption is free from *G. citricarpa*.

Mexico. Mexico is presently not importing citrus fruit from countries where *G. citricarpa* is present (personal communication from: M.C. José Abel López Buenfil, Subdirector de Armonización y Evaluación Internacional, Dirección de Regulación Fitosanitaria, Dirección General de Sanidad Vegetal (México) date: from Mon 10/11/2008 to Wed 19/11/2008). In the Diario (SAGARPA, 1995), which is currently under revision, the possibility of importing mandarins from China with additional declarations on the origin and treatments of the consignment is given.

New Zealand. Citrus crops are among the approved commodities for importation in to New Zealand from Australia, where *G. citricarpa* is present. According to the Biosecurity Standard (MAF, 2001) *G. citricarpa* in the fresh fruit consignments is categorised as a “Risk Group 2” pest together with *Xanthomonas axonopodis* pv *citri*. In the case of fresh fruit import that can be infected by Risk Group 2 pests, an additional declaration should attest that the consignment: has undergone appropriate pest control activities that are effective against *G. citricarpa* and *X. axonopodis* pv *citri* OR has been sourced from an area free (verified by an official detection survey) from *G. citricarpa* and *X. axonopodis* pv. *citri*.

US. According to the Work Plan for USDA, Preclearance Inspection and Cold Treatment of South African Citrus Fruit Designated for the Export to USA (APHIS, 2006) is being carried out in cooperation by the United States Department of Agriculture Animal and Plant Health Inspection Services (USDA-APHIS) and South African Department of Agriculture, Directorate

Agricultural Products Inspection Services and Directorate Plant Health (DoA). DoA in cooperation with USDA-APHIS will monitor all orchards, packhouses and inspection deposits. A phytosanitary certificate issued by the South African Department of Agriculture must accompany each shipment of citrus fruit. The phytosanitary certificate must contain the following Additional Declaration: “*The citrus fruit in this consignment was grown in and packed in South Africa in the Western Cape Province and in the Northern Cape Province in the districts of Hartswater and Warrenton.*” and shipped from the Western Cape Province of South Africa. The designated citrus black spot free areas in the Western Cape of South Africa are as follows: the magisterial districts of Bellville, Bredasdorp, Caledon, Cape, Ceres, Clanwilliam, Goodwood, Grabouw, Heidelberg, Hermanus, Hopefield, Kuilsrivier, Ladismith, Malmesbury, Mitchell’s Plain, Montagu, Moorreesburg, Paarl, Piketberg, Robertson, Somerset-West, Stellenbosch, Strand, Swellendam, Tulbagh, Villiersdorp, Vredenburg, Wellington, Worcester and Wynberg. The designated citrus black spot free areas in the Northern Cape of South Africa are as follows: the magisterial districts of Hartswater and Warrenton. South African citrus is subject to product inspection by the USDA-APHIS. Only orchards of packers approved by USDA/DoA will be eligible for export.

The Panel notes that *G. citricarpa* is presently considered as a quarantine organism by many citrus producing countries. The measures applied to citrus fruit import vary, requiring additional declaration stating pest free area of origin, appropriate treatment or a pest free consignment.

4.3. Evaluation of the current EU measures

The directive 2000/29/EC gives protective measures against the introduction into the community of organisms harmful to plants or plant products and against their spread within the community. Paragraph 16.4 of Annex IV Part A Section I in the directive describes special requirements which must be laid down by all EU-MS, for the introduction and movement of fruits of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, other than fruits of *Citrus aurantium* L., originating in third countries. Four alternative options are provided:

16.4 (a) “the fruits originate in a country recognised as being free from *Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*), in accordance with the procedure referred to in Article 18(2)”;

16.4 (b) “the fruits originate in an area recognised as being free from *Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*), in accordance with the procedure referred to in Article 18(2), and mentioned on the certificates referred to in Articles 7 or 8 of this Directive”;

16.4 (c) “no symptoms of *Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*), have been observed in the field of production and in its immediate vicinity since the beginning of the last cycle of vegetation, and none of the fruits harvested in the field of production has shown, in appropriate official examination, symptoms of this organism”;

16.4 (d) “the fruits originate in a field of production subjected to appropriate treatments against *Guignardia citricarpa* Kiely (all strains pathogenic to *Citrus*), and none of the fruits harvested in the field of production has shown, in appropriate official examination, symptoms of this organism”.

The Panel notes that, theoretically, the four options are equivalent: each of the options, if fully met by the exporting country, would effectively prevent the introduction of *G. citricarpa* into the EU. Hence, the IPPC principle of ‘equivalence’ is met for the alternative options listed in 2000/29/EC.

However the Panel observes that a considerable number of citrus fresh fruit consignments sent from South Africa to EU MS has been intercepted at the entry border stations because of presence of *G. citricarpa* (Fig. 41 and 42, based on data from Europhyt, 2008). The number of interceptions for the whole EU was increasing in the latest 3 years mainly due to an increase of interceptions by the Netherlands, as illustrated in table 9, for a comparison of NL and UK, the two main citrus importer countries in EU (see table 7). Adding to a growing alertness, the improvement of diagnostic methodology (EPPO, 2003) may contribute to explaining the increase of the number of interceptions. These interceptions demonstrate the high risk of entry of the pathogen into Europe by citrus fresh fruit import from South Africa.

Table 9. **Number of interception per year because of *G. citricarpa* on citrus fruit consignments imported from South Africa into the Netherlands and the United Kingdom (Europhyt, 2008).**

Number of interceptions	2006	2007	2008 (until October)
NL	19	38	61
UK	2	4	4

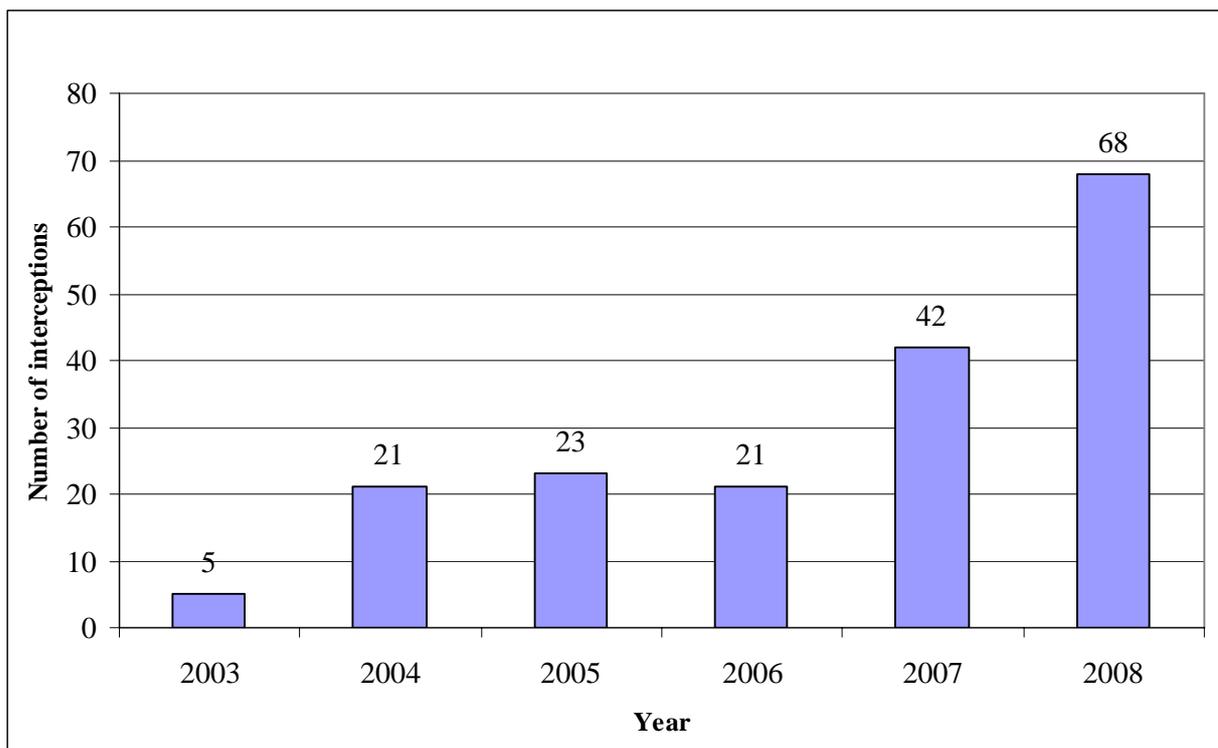


Figure 41. **Total number of EU interceptions of citrus fruit consignments from South Africa for *G. citricarpa* along the period 2003-2008 (Europhyt, 2008).**

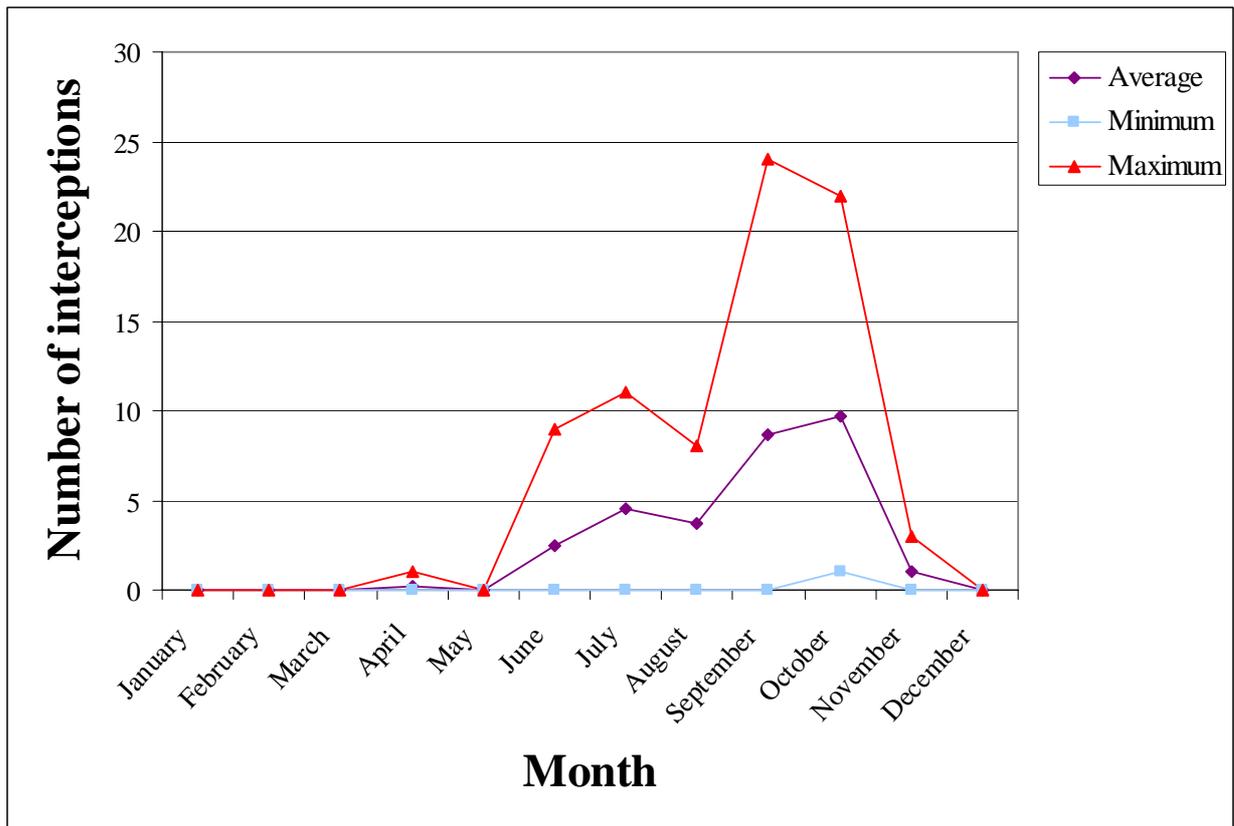


Figure 42. Monthly trend of EU interceptions of citrus fruit from South Africa because of *G. citricarpa* in the period 2003-2008.

The Panel analysed in more detail the possible practical limitations to the effectiveness of each of the four options.

Option 16.4 (a): The Panel notes that this option is effective but can not apply to South Africa as the organism is present in South Africa.

Option 16.4 (b): The EU Commission recognised that in South Africa, Western Cape and the magisterial districts of Hartswater and Warrenton in Northern Cape are free from *G. citricarpa* Kiely pathogenic to citrus (Commission Decision 2006/473/EC). The CBS-free status of the above provinces as given in the Commission Decision is based on official statement of South Africa. The Panel notes that the effectiveness of this option is determined by the accuracy of annual monitoring and surveillance programmes performed by South Africa for the demarcation of the pest free area. Details concerning this statement and the underlying observations were not available to the Panel. The Panel notes that the pest free area districts in South Africa in the Work Plan for USDA Preclearance Inspection and Cold Treatment of South African Citrus Fruit Designated for the Export to USA (APHIS, 2006) are more detailed and on a smaller scale than in the EU regulation (Commission Decision 2006/473/EC).

Option 16.4 (c): The effectiveness of this option depends on the intensity of procedures for sampling and inspection in the field. The specification of methods and level of accuracy of these procedures is not required according to 2000/29/EC. The term ‘immediate vicinity’ is not specified and it should be related to the epidemiology of *G. citricarpa*. In case of low incidence of *G. citricarpa* in a field or its ‘immediate vicinity’, the disease could go unrecognised in the field. Symptomless infected fruit could develop symptoms after harvest (Kotzè, 1981) and during transport (see probability of entry 3.1.3.1 and Agostini *et al.*, 2006). Symptomatic fruit

could escape culling in the packing house and CBS symptoms could be confused with those caused by other citrus pathogens, mechanical or insect damage (see probability of entry 3.1.2.5.). Therefore, the Panel concludes that the effectiveness of this option in practice is limited.

Option 16.4 (d): This measure regards pre-harvest treatments in the production field but it does not include post harvest treatments.

A number of protective and systemic fungicides, such as copper products, dithiocarbamates, benzimidazoles and strobilurins are effective in reducing citrus black spot severity and incidence in the field. Some examples from experiments from Argentina, Brazil and South Africa are reported below.

The field spray programme for lemons in Misiones, Argentina, in 2002 (one application of carbendazim, and one application of trifloxistrobin+copperhydroxide) reduced CBS incidence in fruit from 41.4% (untreated control) to 6.8 % (Agositini *et al.*, 2006a).

In experiments in Brazil, combining different fungicides, always with the addition of mineral oil, and different timing of applications, four treatments of copper hydroxide followed by carbendazim plus copper hydroxide reduced the disease incidence on fruit from 92.75% (untreated control) to 53.0%. Similar results were obtained with other combinations and timings, which included also piraclostrobin (Spósito, 2003). Timing of sprays was shown to be important in experiments with copper oxychloride plus mineral oil repeated applications in Mogi-Guaçu, São Paulo, Brazil (Reis *et al.*, 2003).

In South Africa (Nelspruit region), during 2004 and 2005, a field spray programme for Valencia oranges showed that the alternation of various tank mix combinations (azoxystrobin, copper oxychloride, benomyl, mancozeb, carbendazim plus oil) resulted in 92-98% fruit with no CBS lesion, compared to 31% in untreated fruit. In this example, the spray programme included anti-resistance strategies to benzimidazoles and strobilurins (Schutte, 2006).

On the basis of these examples and additional literature (Kellerman and Kotzé, 1977; Miles *et al.*, 2004; Schutte, 2002 and 2006; Schutte *et al.*, 1996 and 2003), the Panel concludes that fungicide field treatments do not completely eliminate fruit infections or quiescent infections.

Moreover, side-effects of fungicide treatments need to be considered: copper fungicides may cause stippling of fruit tissue due to direct copper injury, (Schutte *et al.*, 1997) and negative environmental effects, like high toxicity to aquatic organisms, and food safety effects i.e. residues in the fruit adding to the overall copper exposure in diet (EFSA, 2008). *G. citricarpa* may develop resistance to some fungicides (CAB International 2007; Herbert and Grech, 1985; Kotzé, 1981; Schutte, 2006; Schutte *et al.*, 2003).

When combining fungicide treatments in the field with cultural measures, a complete elimination of fruit infection has also not been demonstrated (see section 3.2.3.). Examples are the removal of sources of inoculum (mature fruit and leaf litter) from the orchards before the new crop sets and the maintenance of tree vigour (Kotzé, 1963; Kotzé, 1981; Paul, 2006; Spósito, 2003). In São Paulo State, Brazil, intercropping in a sweet orange grove with coastcross, *Cynodon dactylon*, compared to control (cutting down weeds and deposit them on soil under canopy), significantly reduced the number of ascospores (3.21 vs 14.36 ascospores/week) and increased the percentage of fruit commercially acceptable (less than 5% of peel with lesions, i.e. up to 3 on the scale 0-6 proposed by Spósito, 2003) from 77.3% of the control to over 90%. The effects on disease index were less satisfactory, the best treatment being the treatment with copper sprays of trees, which reduced significantly (19.1% reduction) this parameter (Bellotte, 2006). Application of urea, calcium nitrate, dolomite lime, experimental mixtures of organisms, and commercial mixtures of enzymes and microorganisms on the fallen leaves in a 'Siciliano' lemon orchard reduced the disease index by 48-59% (Bellotte, 2006).

The Panel observes uncertainties from sampling and inspection procedures, which are part of various management options, and from insufficient data on the effectiveness of mitigation methods, when applied alone or in a systems approach.

4.4. Evaluation of alternatives measures

4.4.1. Options for the consignments

Measures may include any combinations of the following:

4.4.1.1. Inspection or testing for freedom from a pest

Pest freedom of consignment is part of options 16.4 c and d of Annex 4 of Directive 2000/29 EU and has to be confirmed by inspection of each consignment (Directive 2000/29/EC art 6). Inspections are based on sampling and the sample size should be adequate to give an acceptable probability of detecting *G. citricarpa*. In the EU sampling schemes and sample sizes are set by inspection services of the EU-MS. The ISPM No. 31 and the EPPO Phytosanitary procedure Standard PM 3/65(1) describe the general procedure for sampling of phytosanitary inspection of consignments. For citrus, special sampling procedures are described in the Work Plan for USDA Preclearance Inspection and Cold Treatment of South African Citrus Fruit Designated for the Export to USA (APHIS, 2006).

The Panel recommends the establishment of a pest specific sampling scheme and an inspection methodology with a known probability to detect a certain infestation level for *G. citricarpa*.

4.4.1.2. Prohibition of parts of the host.

The Panel notes that citrus plant and plant parts, excluded fruits, are already forbidden by 2000/29/EC Annex II, Part A, Section 1, point c no. 11 and thus not relevant to the terms of reference.

4.4.1.3. A pre-entry or post-entry quarantine system

A pre-entry or post-entry quarantine system can be considered to be the most intensive form of inspection or testing where suitable facilities and resources are available, and may be the only option for certain pests not detectable on entry.

Pre-entry or post-entry quarantine system measures are currently not considered for *G. citricarpa* in Council Directive 2000/29/EC.

Methods are available in literature for accelerating symptom development on fruit, which could be used in pre- and post-entry quarantine. As infected fruit can be symptomless during storage (Brodrick and Rabie, 1970; see section 3.1.3.2.), specific treatments of samples of citrus fruit before inspections either at exporting country (pre-entry) or entry point (post-entry) can be considered.

Light and temperature, for example, influence the development rate of symptoms (see introduction, Brodrick and Rabie, 1970; Kotzé, 1981). To force symptom development, fruit samples may be held at high temperature (27 °C) and under continuous light prior to entering the packing house (Brodrick and Rabie, 1970). In one study under these conditions, CBS lesions developed within 5-7 days on naturally-infected Valencia oranges (Korf, 1998).

Regarding pre-harvest inspections, Baldassari *et al.* (2007) have shown that treating asymptomatic fruit of orange 'Pêra-Rio', aged between 20 and 28 weeks after flowering, by immersion in a solution of ethephon (2.10 g/l, 1 min) induced precocious symptom expression (assessing: 28-35 days after treatment) of *G. citricarpa* in proportions equivalent to those observed in fruit matured on trees. This allows a prediction to be made on the presence and severity of the disease in advance of 105 days of the harvest. This type of treatment is included in the Brazilian criteria and proceedings for risk management of *G. citricarpa* (Instrução normativa nº 3, 08/01/2008, Ministro de estado da agricultura, pecuária e abastecimento).

The Panel considers that these methods for sampling, inspection and testing could be used in a pre-entry quarantine system, as a supplemental requirement to the existing options.

Post-entry quarantine of citrus fruit would not be practical since if the consignment proved to be infected it would be necessary to destroy or reject the consignment after the minimum 5-7 day storage period for symptom development.

4.4.1.4. Specified conditions of preparation of the consignment (e.g. handling to prevent infestation or re-infestation)

According to literature the susceptibility period is terminated when fruit is fully mature (see introduction) and low storage and shipping temperature makes new fruit infections improbable (Korf, 2001). The Panel therefore considers that re-infestation of consignments is unlikely.

4.4.1.5. Specified treatment of the consignment

Post harvest treatments against *G. citricarpa* are currently not considered in Council Directive 2000/29/EC. Post-harvest treatments for citrus fruit may include chemical, thermal or other physical methods. In the Republic of South Africa, citrus fruit in packing houses is routinely subjected to the following steps (Villiers and Joubert, 2006):

- Washing line, employing a chlorine bath or dry brushing
- Removal of under- and over-sized fruits
- High-pressure spray (chlorinate)
- Optional hot water bath
- Grading the fruit, to remove fruit with obvious injuries and blemishes
- Fungicide application
- Waxing
- Packing

Wax treatments have shown to reduce the number of fruits with severe black spot (Seberry *et al.* 1967; Wild, 1981). Korf *et al.* (2001) applied various treatments (warm water bath, storage at 4.5 °C and 25 °C, waxing, high-pressure spraying, chlorine bath, chemical tank dip with guazatin, imazalil sulphate and 2,4-D sodium salt) and all treatments combined in two packing house experiments. Individual treatments showed some reduction of re-isolation, the combination of all treatments showed a much larger reduction of re-isolation but none of the treatments, individual or combined, eliminated the pathogen completely. Agostini *et al.* (2006) also showed that the pathogen remains viable on infected fruit receiving a wide range of pre-and/or post-harvest treatments.

The Panel did not find evidence for post-harvest treatments that completely eliminate *G. citricarpa* from infested consignments. However the application of such measures, in

combination with the field treatments required under option 16.4(d), may further reduce the risk of introduction.

4.4.1.6. Restrictions on end use, distribution and periods of entry of the commodity.

If the endangered area can be demarcated, specific restriction on end use, distribution and periods of entry of citrus fruit could be established for the endangered and the non-endangered areas.

The endangered area should cover all types of citrus production, including groves, field and greenhouse production of ornamental citrus and plant propagation material. The simplest way would be to designate the endangered area as citrus producing by EU MS. A more detailed approach would consider regions or provinces within these EU-MS, suitable for the establishment of *G. citricarpa*. This demarcation of the endangered area would employ the concept of protected zones, as defined in the Directive 2000/29/EC. The following options are separated for endangered and non endangered areas.

- Options for non endangered areas

Citrus fruit does not constitute a risk to the non endangered area. Therefore the import and trade into and within this area could be possible under the condition that the fruit and fruit waste is not moved into the endangered area. By default, this condition is absent in the EU where free movement of goods is possible within the EU territory. To prevent movement of citrus fruit and waste from the non endangered area into the endangered area of the EU, specific import conditions for the non endangered area may be specified in relation to the type of end use of the imported citrus fruit consignments. Two types of end use are distinguished by the Panel: processing and fresh market. For processing, the import conditions could be specified by a derogation laying down the responsibilities of the processing factories. These conditions for processing and for waste disposal should guarantee that any citrus fruit and fruit waste potentially carrying the pathogen, do not reach the endangered areas.

For fresh market, conditions for retailers could be formulated to prevent the transport of any citrus fruit and fruit waste potentially carrying the pathogen to the endangered areas, in relation to traceability requirements specified in Regulation 178/2002 article 18. Restrictions on end use of imported fresh products have been laid down in legislation before: for ware potatoes originating from Egypt, EU-MS are obliged to lay down appropriate labelling requirements with the aim of preventing the potatoes from being planted and appropriate measures for the disposal of waste after packaging or processing of the potatoes (2004/4/EC). However the Panel notes the novelty for such possible restrictions on import of citrus fruit, where the entry of possibly infested material would be permitted.

- Options for endangered area

In the endangered area, plants with tissues susceptible to *G. citricarpa* infection (leaves and/or fruit) are present all year around. This aspect, in combination with the prolonged viability of mycelium of *G. citricarpa* during cold storage of citrus fruit, does not allow for designation of a safe period of entry.

For restrictions on the type of end use, import conditions may be specified in relation to the type of end use (e.g. processing, fresh market) and its location. Regarding the use of citrus fruit for processing, the responsibility of factories can be specified in a derogation. The conditions for processing and for waste disposal should guarantee that any citrus fruit and fruit waste potentially carrying the pathogen, do not escape in to the environment. Regarding the use of citrus fruit for fresh market, the Panel considers that the conditions for waste disposal can not

be sufficiently controlled in any parts of the endangered area to prevent the escape of *G. citricarpa* to the environment.

4.4.2. Options preventing or reducing infestation in the crop

Measures may include:

4.4.2.1. Treatment of the crop, field, or place of production.

The Panel notes that this is considered in Directive 2000/29 EC option d. For details, see above in current measures.

4.4.2.2. Restriction of the composition of a consignment (resistant or less susceptible species)

The Panel notes that, apart from sour oranges and Tahiti limes, all citrus varieties currently cultivated for fruit production are susceptible (Aguilar-Vildoso *et al.*, 2002; Chung *et al.*, 2005; Paul 2006; see also section 1.2).

4.4.2.3. Growing plants under specially protected conditions (glasshouse, isolation)

No measures of this kind are envisaged by the Panel.

4.4.2.4. Harvesting of plants at a certain age or a specified time of year

No measures of this kind are envisaged by the Panel.

4.4.2.5. Production in a certification scheme.

As the organism is also airborne, the Panel considers that certification schemes are not sufficient to mitigate the risk in infected areas.

4.4.3. Options ensuring that the area, place or site of production or crop is free from the pest

Measures may include:

4.4.3.1. Pest-free area.

The requirements for pest-free area status are described in ISPM No. 4: *Requirements for the establishment of pest free areas*.

4.4.3.2. Pest-free place of production or pest-free production site.

The requirements are described in ISPM No. 10: *Requirements for the establishment of pest free places of production and pest-free production sites*.

4.4.3.3. Inspection of crop to confirm pest freedom.

The Panel notes that these measures are already considered in the current Council Directive 2000/29/EC, Annex IV part A, sect. 1, par.16.4. Additional requirements on the accuracy of field inspection and area surveillance programs, specifying the required level of confidence to detect a specified incidence level may be specified for a consistently reliable determination of pest free areas and pest free places and fields of production.

4.4.4. Options for other types of pathways

The Panel notes that according to the terms of reference only the fruit pathway should be assessed.

4.4.5. Options within the importing country

According to ISPM No. 11 measures applied within the importing country may also be used. These could include careful surveillance to try and detect the entry of the pest as early as possible, eradication programmes to eliminate foci of infestation and/or containment action to limit spread.

For *G. citricarpa*, field surveillance alone is not reasonable considering that the pathogen may also be present in asymptomatic citrus fruit as latent mycelium and it is documented that there is a long lag phase between the first infection and the symptoms development (see introduction). Therefore, the Panel concludes that surveillance may not detect timely the pest introduction.

4.4.6. Prohibition of commodities

According to ISPM No. 11, if no satisfactory measure to reduce risk to an acceptable level can be found, the final option may be to prohibit importation of relevant commodities. This should be viewed as a measure of last resort and should be considered in light of the anticipated efficacy, especially in instances where the incentives for illegal import may be significant.

The Panel concludes that this option would be effective but more restrictive than the present measures.

4.4.7. Phytosanitary certificates and other compliance measures

A phytosanitary certificate is required for import of fresh citrus fruit from third countries under the current regulation (Council Directive 2000/29/EC, art. 13).

4.5. Conclusions on pest risk management options

4.5.1. Existing EU measures

The Panel concludes on the four current EU options for phytosanitary measures against the introduction of *G. citricarpa*, described in EU Directive 2000/29/EC Annex IV part A section I point 16.4, that theoretically the four existing management options are effective and in line with the IPPC principle of 'equivalence', but practical limitations may reduce their effectiveness. Option 16.4 (a) (pest free country) would be effective, but does not apply to South Africa. Option 16.4 (b) (pest free area) is effective in principle, but requires intensive continuous monitoring to maintain an accurate delimitation of this area. Information on such a monitoring programme was not provided to the Panel, so the effectiveness of this option could not be evaluated specifically for citrus fruit originating from SA. The Panel considered option 16.4 (c) (no symptoms in the field of production) to be not fully effective: the effectiveness depends on the intensity of the field inspection procedures in the field of production. Specification of the methods and level of accuracy of these inspection procedures is not required according to Directive 2000/29/EC, and they are not specified by South Africa. The Panel considered option 16.4 (d) (appropriate field treatments) insufficiently effective, since no mitigating measure in the field, or combination of field treatments has been shown to fully prevent or eliminate fruit infections.

Observing the frequent interceptions of consignments of citrus fruit infested with *G. citricarpa*, originating from South Africa, the Panel concludes that the existing risk management options are not sufficient to prevent the entry of *G. citricarpa*.

The Panel observes that existing measures apply to the whole territory of the European Community, where the movement of consignments of citrus fruit is not restricted. The Panel concludes that phytosanitary inspections and interceptions at all points of entry to the Community are appropriate in order to protect the citrus fruit growing areas. Therefore, the existing measures are in line with the IPPC principle of minimal impact.

4.5.2. Alternative measures

The Panel observes that post-harvest treatments of fruit are currently not listed as risk management options in Annex IV, Part A, Section I, point 16.4, of Council Directive 2000/29/EC. The combination of pre-harvest (field) treatments with post harvest treatments would further reduce, although not eliminate, the risk of introduction. The Panel suggests including effective post-harvest treatments in option 16.4 (d).

The Panel notes that, despite routine applications in South Africa of pre- and post-harvest treatments, frequent interceptions of infested consignments occur at the European Community points of entry. The Panel suggests an investigation of the exact causes for infested consignments to arrive at the EU border despite applied mitigation measures in South Africa.

Among the options to be applied at the country of origin, the Panel suggests that methods to accelerate citrus black spot symptoms development (such as high temperature and continuous light or ethephon dipping), combined with a standardised sampling scheme, could be applied in a pre-entry quarantine system, to improve the detection of infested consignments before shipping.

For the European Community, the Panel suggests that demarcation of endangered and non-endangered areas could be combined with distinctive measures regarding the end use and distribution of citrus fruit, that are less trade-restrictive.

4.5.3. Uncertainties

Uncertainties associated with estimating the effectiveness of risk management options, which do not affect the Panel conclusions, may arise from lack of information on the sampling procedures that are part of various management options, and from insufficient data on the effectiveness of mitigation methods when applied alone or in a systems approach.

CONCLUSIONS AND RECOMMENDATIONS

The Panel, has studied the pest risk assessment and additional supporting evidence supplied by South Africa, as well as new information collected by the Panel, and concludes the following.

1. With regard to the suitability of the EU citrus fruit producing areas for establishment of CBS in terms of their climatic conditions:

Based on (a) the evaluation of the application of CLIMEX (Paul *et al.*, 2005), (b) the limitations of CLIMEX in predicting the potential distribution of pathogens such as *G. citricarpa*, (c) the relative climatic similarities between locations where CBS occurs in Eastern Cape Province and some locations where citrus is grown in the EU and (d) the results of the application of a generic infection model for foliar fungal pathogens, the Panel cannot agree with the conclusion by Paul *et al.* (2005) that the climate of the EU is unsuitable for the establishment of *G. citricarpa*.

2. With regard to the likelihood of an introduction, leading to an establishment, of CBS to the EU citrus fruit producing areas on CBS infected citrus fruit:

The Panel considers that *G. citricarpa* is associated with the citrus fruit in South Africa, is able to survive transport, storage and existing pest management procedures, especially in the form of quiescent infections and inconspicuous symptoms, and may be transferred to suitable hosts by means of splash dispersal from citrus black spot infected citrus fruit and peel. The Panel therefore concludes that *G. citricarpa* may enter the PRA area with infected citrus fruit. The Panel has also determined that, given the widespread distribution of susceptible hosts within the PRA area, cultural and climatic factors will not prevent the establishment of the pathogen.

The Panel concludes therefore that entry of *G. citricarpa*, leading to establishment in the EU citrus fruit production areas, on CBS infected citrus fruit is possible. The South African documents do not provide sufficient evidence to demonstrate that import of citrus fruit from infested areas is a very unlikely pathway for the introduction of *G. citricarpa* into these areas.

3. With regard to the appropriateness of the level of protection under the existing risk management options listed in Annex IV, Part A, Section I, point 16.4 of Council Directive 2000/29/EC:

Theoretically, the four existing risk management options are effective and in line with the IPPC principle of 'equivalence', but practical limitations may reduce their effectiveness. Option 16.4 (a) (pest free country) would be effective, but does not apply to South Africa. Option 16.4 (b) (pest free area) is effective in principle, but requires intensive continuous monitoring to maintain an accurate delimitation of this area. Information on such a monitoring programme was not provided to the Panel, so the effectiveness of this option could not be evaluated specifically for citrus fruit originating from South Africa. The Panel considered option 16.4 (c) (no symptoms in the field of production) to be not fully effective: the effectiveness depends on the intensity of the field inspection procedures in the field of production. Specification of the methods and level of accuracy of these inspection procedures is not required according to 2000/29/EC, and they are not specified by South Africa. The Panel considered option 16.4 (d) (appropriate field treatments) insufficiently effective, since no mitigating measure in the field, or combination of field treatments has been shown to fully prevent or eliminate fruit infections.

Observing the frequent interceptions of consignments of citrus fruit infested with *G. citricarpa*, originating from South Africa, the Panel concludes that the existing risk management options are not sufficient to prevent the entry of *G. citricarpa*.

The Panel observes that the existing measures apply to the whole territory of the European Community, where the movement of consignments of citrus fruit is not restricted. The Panel concludes that phytosanitary inspections and interceptions at all points of entry to the Community are appropriate in order to protect the citrus fruit growing areas. Therefore, the existing measures are in line with the IPPC principle of minimal impact.

Uncertainties associated with estimating the effectiveness of risk management options, which do not affect the Panel conclusions, may arise from lack of information on the sampling procedures that are part of various management options, and from insufficient data on the effectiveness of mitigation methods.

4. With regard to the identification of effective options, alternative to those already present in Directive 2000/29/EC, to prevent introduction of citrus black spot into the Community:

The Panel observes that post-harvest treatments of fruit are currently not listed as risk management options in Annex IV, Part A, Section I, point 16.4 of Council Directive 2000/29/EC. The combination of pre-harvest (field) treatments with post harvest treatments would further reduce, but not eliminate the risk of introduction. The Panel suggests including effective post-harvest treatments in option 16.4 (d). It is noted that, despite routine application of post-harvest treatment of citrus fruit by South Africa, frequent interceptions of infested consignments occur at the Community points of entry. The Panel suggests an investigation of the exact causes for infested consignments to arrive at the EU border despite applied mitigation measures in South Africa.

For South Africa, as the country of origin, the Panel suggests that methods to accelerate citrus black spot symptoms development, combined with a standardised sampling scheme, could be applied in a pre-entry quarantine system to improve the detection of infested consignments before shipping.

For the European Community, the Panel suggests that demarcation of endangered and non-endangered areas could be combined with distinctive measures regarding end use and distribution of citrus fruit, that are less trade-restrictive.

DOCUMENTATION PROVIDED TO EFSA

1. Letter, dated 8 April 2008 with ref. SANCO E/1/RV/al D (2008) 510189 from P. Testori Coggi to C. Geslain-Lanéelle.
2. Hattingh *et al.* (2000) Citrus Black Spot: Pest Risk Assessment document for the review of current phytosanitary regulations pertaining to the export of fresh citrus fruit from the Republic of South Africa to the EU. May 2000. (Annex 1)
3. Report of the Commission Working Group on evaluation of the Pest Risk Assessment (PRA) prepared by South Africa on Citrus Black Spot (CBS). October 2001. (Annex 2)
4. Response from South Africa on the Report (dated 24/10/2001) of the EC Working Group (EC WG) relating to the WG's evaluation of the Pest Risk Assessment (PRA) by South Africa on Citrus Black Spot (CBS). September 2002. (Annex 3)
5. Further Data Relating to the PRA for Citrus Black Spot and the Export of Fresh Citrus Fruit from South Africa to the EU. September 2003. (Annex 4)
6. Further Data Relating to the PRA for Citrus Black Spot and the Export of Fresh Citrus Fruit from South Africa to the EU – Research Report on Potential Transmission from Fruit to Leaf litter. July 2004. (Annex 5)
7. Report of the Commission Working Group on evaluation of the Pest Risk Assessment prepared by South Africa on Citrus Black Spot (caused by *Guignardia citricarpa* Kiely). June 2006. (Annex 6)
8. Report of the South African CBS Expert Working Group on evaluation of the Pest Risk Analysis for Citrus Black Spot (*Guignardia citricarpa*) on fresh citrus fruit from South Africa to the European Union. June 2007. (Annex 7)

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⁴ All references marked by (*) refer to citations in the Appendix.

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APPENDIX¹

1. APPENDIX: CLIMEX analysis with parameters from Paul *et al.* (2005)

Table 1. CLIMEX results for 29 weather stations having complete data for the period 1990-2007 from the JRC-MARS meteorological database (EI=ecoclimatic index; GI=growth index; CS=cold stress index; HS=heat stress index; WS=wet stress index)

Country	Station name	Longitude	Latitude	Altitude	EI	GI	CS	HS	WS
Cyprus	Larnaca	33.63	34.88	2	1	1	18	0	0
France	Bastia	9.48	42.55	12	3	8	62	0	0
France	Ajaccio	8.8	41.92	9	2	5	66	0	0
Greece	Kerkyra	19.92	39.62	4	4	9	50	0	0
Greece	Rhodos	28.08	36.4	11	4	6	31	0	0
Greece	Athens	23.73	37.9	15	0	1	45	0	0
Greece	Andravida	21.28	37.92	14	2	4	51	0	0
Greece	Larissa	22.42	39.63	74	0	1	78	0	0
Greece	Thessaloniki/Mikra	22.97	40.52	4	0	1	70	0	0
Italy	Catania/Fontanarossa	15.05	37.47	17	2	3	39	0	0
Italy	Florence	11.2	43.8	38	2	7	68	0	0
Italy	Lamezia Terme	16.25	38.9	16	4	9	53	0	0
Italy	Grosseto	11.05	42.73	7	1	4	71	0	0
Italy	Cozzo Spadaro	15.13	36.68	51	7	10	32	0	0
Italy	Napoli/Capodichino	14.3	40.85	72	5	10	52	0	0
Italy	Trapani/Birgi	12.5	37.92	9	4	7	39	0	0
Italy	Palermo/Punta Raisi	13.1	38.18	34	6	9	33	0	0
Italy	Messina	15.55	38.2	54	10	15	35	0	0
Italy	Foggia Amendola	15.72	41.53	60	0	1	63	0	0
Italy	Marina Di Ginosa	16.88	40.43	12	1	3	53	0	0
Italy	Gela	14.22	37.08	65	3	4	34	0	0
Malta	Luqa	14.48	35.85	91	8	11	27	0	0
Portugal	Faro	-7.97	37.02	8	3	4	26	0	0
Spain	Menorca/Mahon	4.23	39.87	86	3	6	47	0	0
Spain	Jerez De La Frontera	-6.07	36.75	28	4	5	27	0	0
Spain	Sevilla/San Pablo	-5.9	37.42	31	4	5	21	0	0
Spain	Murcia/Alcantarilla	-1.23	37.95	75	0	0	30	0	0
Spain	Reus	1.17	41.15	76	2	4	54	0	0
Spain	Murcia	-1.17	38	62	0	0	27	0	0

¹ The references cited in this appendix are marked by (*) in the References list of the Scientific opinion.

Table 2. CLIMEX results for all 64 weather stations with complete and incomplete data for the period 1990-2007 from the JRC-MARS meteorological database (EI=ecoclimatic index; GI=growth index; CS=cold stress index)

Country	Station name	Longitude	Latitude	Altitude	No. years with data	EI	GI	CS
Bulgaria	Achtopol	27.85	42.1	19	10	0	2	100
Cyprus	Larnaca	33.63	34.88	2	18	1	1	18
France	Bastia	9.48	42.55	12	18	3	8	62
France	Ajaccio	8.8	41.92	9	18	2	5	66
France	Calvi	8.78	42.52	58	14	2	5	59
France	Figari	9.1	41.52	23	10	2	4	54
France	Cap Corse	9.35	43	80	9	4	8	52
Greece	Larissa	22.42	39.63	74	18	0	1	78
Greece	Thessaloniki/Mikra	22.97	40.52	4	18	0	1	70
Greece	Rhodos	28.08	36.4	11	18	4	6	31
Greece	Athens	23.73	37.9	15	18	0	1	45
Greece	Kerkyra	19.92	39.62	4	18	4	9	50
Greece	Andravidia	21.28	37.92	14	18	2	4	51
Greece	Naxos	25.38	37.1	9	16	1	2	42
Italy	Trapani/Birgi	12.5	37.92	9	18	4	7	39
Italy	Lamezia Terme	16.25	38.9	16	18	4	9	53
Italy	Napoli/Capodichino	14.3	40.85	72	18	5	10	52
Italy	Cozzo Spadaro	15.13	36.68	51	18	7	10	32
Italy	Messina	15.55	38.2	54	18	10	15	35
Italy	Palermo/Punta Raisi	13.1	38.18	34	18	6	9	33
Italy	Marina Di Ginosa	16.88	40.43	12	18	1	3	53
Italy	Grosseto	11.05	42.73	7	18	1	4	71
Italy	Foggia Amendola	15.72	41.53	60	18	0	1	63
Italy	Catania/Fontanarossa	15.05	37.47	17	18	2	3	39
Italy	Florence	11.2	43.8	38	18	2	7	68
Italy	Gela	14.22	37.08	65	18	3	4	34
Italy	Pescara	14.2	42.43	11	16	1	5	82
Italy	Reggio Calabria	15.65	38.07	21	12	3	3	27
Italy	Catania/Sigonella	14.92	37.4	29	10	1	2	37
Italy	Siracusa	15.28	37.07	2	2	1	3	84
Italy	Pontecagnano	14.92	40.62	39	2	9	12	26
Italy	Firenze	11.22	43.78	18	1	3	8	62
Malta	Luqa	14.48	35.85	91	18	8	11	27
Portugal	Faro	-7.97	37.02	8	18	3	4	26
Portugal	Viana Do Castelo	-8.8	41.7	18	12	2	6	59
Portugal	Sagres	-8.95	37	25	9	2	3	39
Portugal	Cabo Carvoeiro	-9.4	39.35	34	9	1	3	68
Portugal	Montijo	-9.03	38.7	11	8	3	5	35

Portugal	Lisboa/Geof	-9.15	38.72	95	7	4	5	31
Spain	Murcia	-1.17	38	62	18	0	0	27
Spain	Sevilla/San Pablo	-5.9	37.42	31	18	4	5	21
Spain	Menorca/Mahon	4.23	39.87	86	18	3	6	47
Spain	Jerez De La Frontera	-6.07	36.75	28	18	4	5	27
Spain	Murcia/Alcantarilla	-1.23	37.95	75	18	0	0	30
Spain	Reus	1.17	41.15	76	18	2	4	54
Spain	Alicante/El Altet	-0.55	38.28	31	16	0	0	28
Spain	Valencia Aeropuerto	-0.47	39.5	62	16	2	4	33
Spain	Murcia/San Javier	-0.8	37.78	3	13	0	0	32
Spain	Alicante	-0.5	38.37	82	12	1	1	14
Spain	Valencia	-0.38	39.48	11	12	5	6	15
Spain	Castellon	-0.07	39.95	35	12	1	2	31
Spain	Aero Tablada	-6	37.37	5	3	7	10	25
Spain	Castello d'Empuries	3.1	42.23	4	2	0	0	68
Spain	Ayamonte	-7.33	37.18	2	1	4	5	27
Spain	Adra	-3.02	36.75	10	1	0	0	15
Spain	Oliva	-0.1	38.93	5	1	6	8	26
Spain	Almoradi	-0.72	38.08	60	1	1	1	29
Spain	Tarragona.Univer.	1.23	41.12	48	1	1	1	46
Spain	Javea	0.17	38.78	15	1	6	9	35
Spain	Cadiz	-6.27	36.5	9	1	5	7	31
Spain	Polinya	-0.37	39.2	12	1	5	7	31
Spain	San Fernando	-6.2	36.47	30	1	4	6	36
Spain	Cartagena	-0.98	37.6	17	1	1	1	30
Spain	Aguilas	-1.58	37.42	26	1	0	0	19

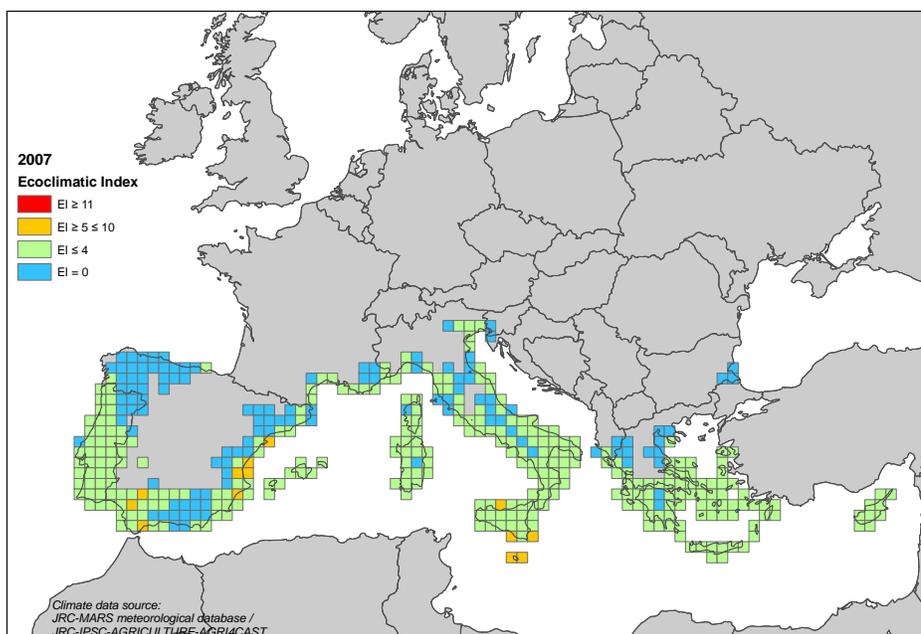


Figure 1 CLIMEX ecoclimatic index for CBS based on climatic data for 2007 from the JRC-MARS meteorological database interpolated to 418 grids at 50 km resolution representing the area of citrus production in the EU year period.

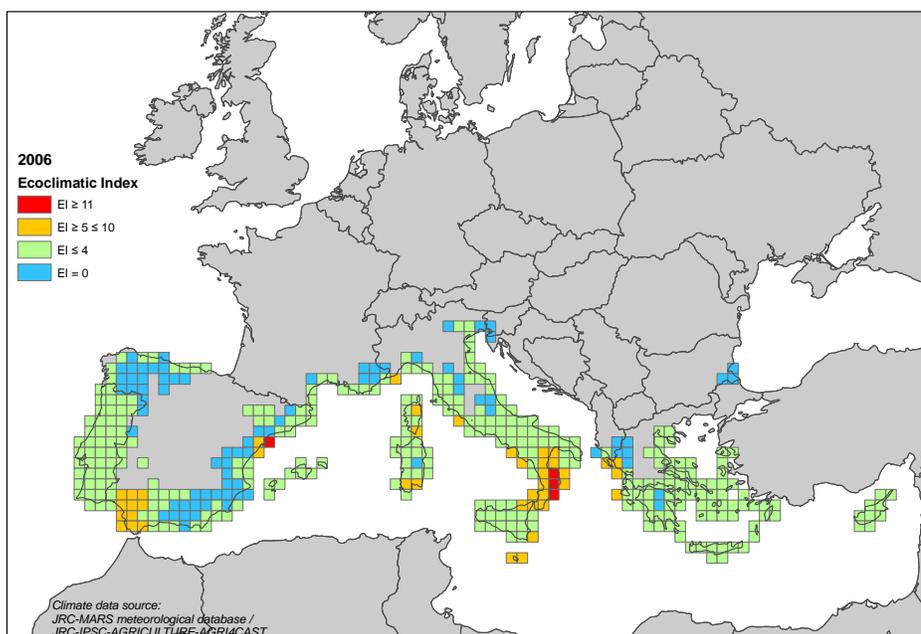


Figure 2 CLIMEX ecoclimatic index for CBS based on climatic data for 2006 from the JRC-MARS meteorological database interpolated to 418 grids at 50 km resolution representing the area of citrus production in the EU year period.

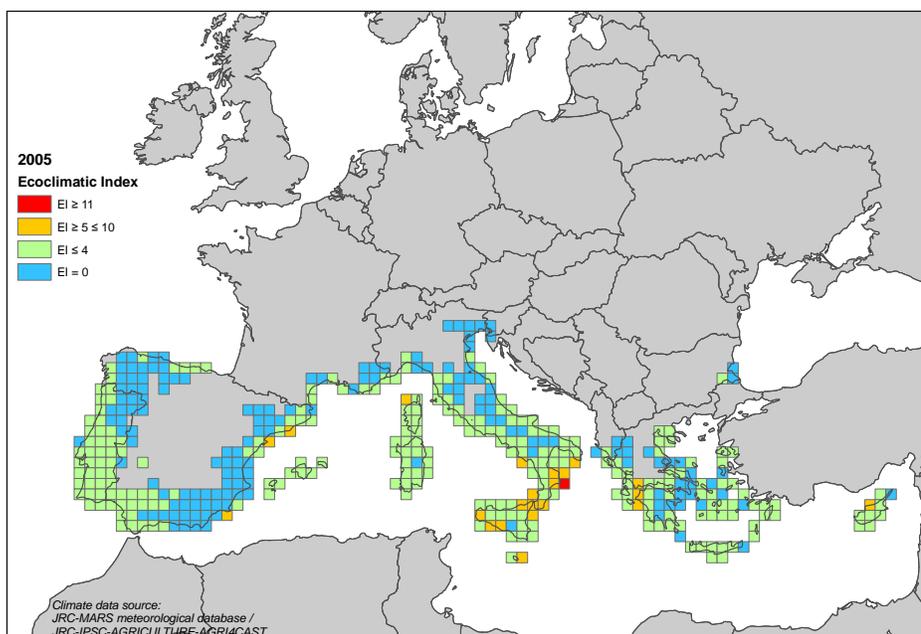


Figure 3. CLIMEX ecoclimatic index for CBS based on climatic data for 2005 from the JRC-MARS meteorological database interpolated to 418 grids at 50 km resolution representing the area of citrus production in the EU year period.

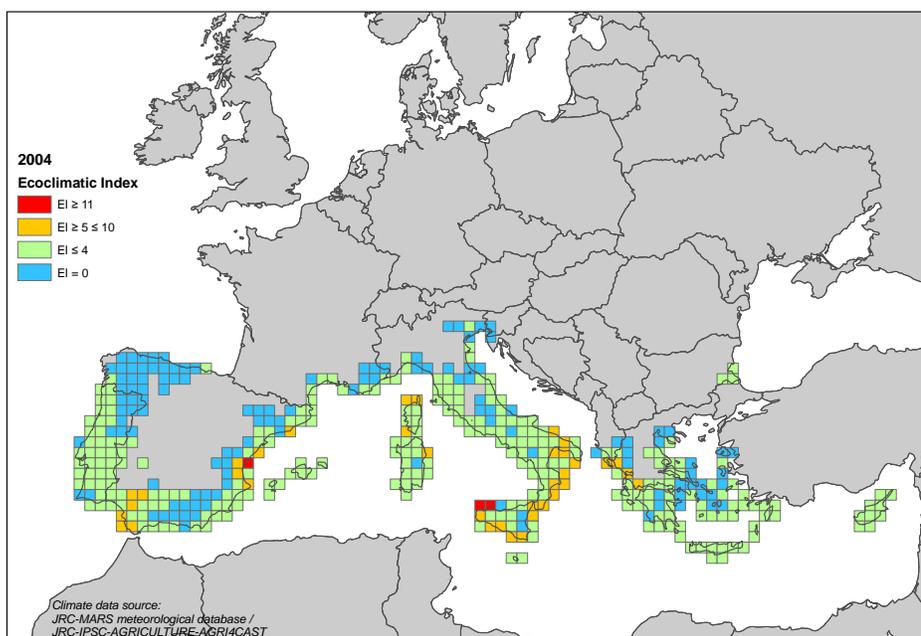


Figure 4. CLIMEX ecoclimatic index for CBS based on climatic data for 2004 from the JRC-MARS meteorological database interpolated to 418 grids at 50 km resolution representing the area of citrus production in the EU year period.

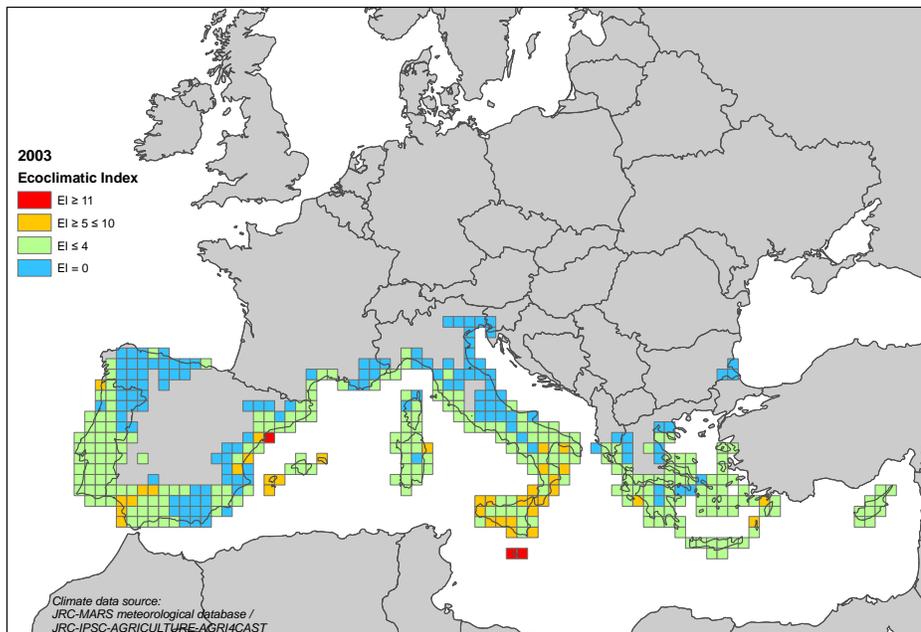


Figure 5. CLIMEX ecoclimatic index for CBS based on climatic data for 2003 from the JRC-MARS meteorological database interpolated to 418 grids at 50 km resolution representing the area of citrus production in the EU year period.

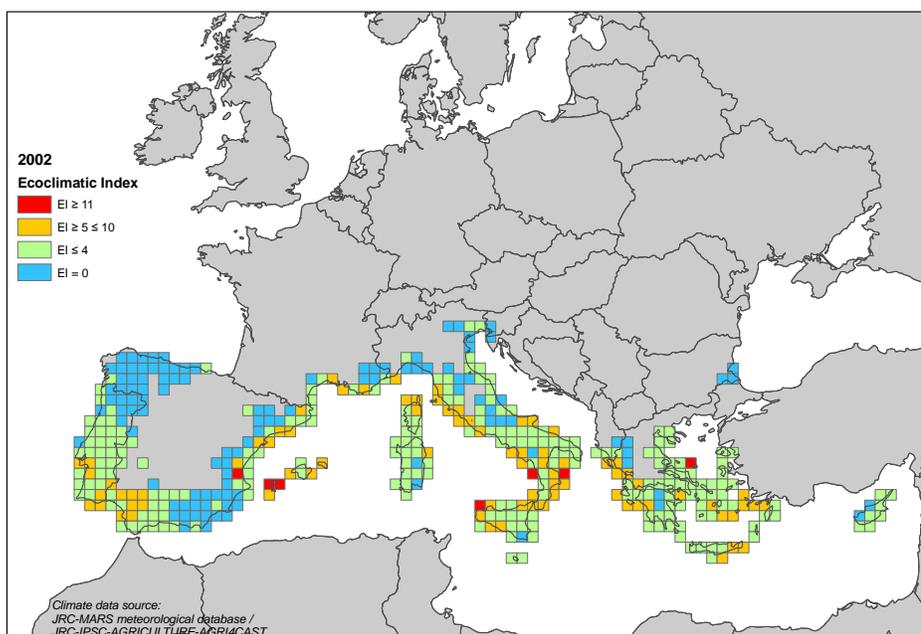


Figure 6. CLIMEX ecoclimatic index for CBS based on climatic data for 2002 from the JRC-MARS meteorological database interpolated to 418 grids at 50 km resolution representing the area of citrus production in the EU year period.

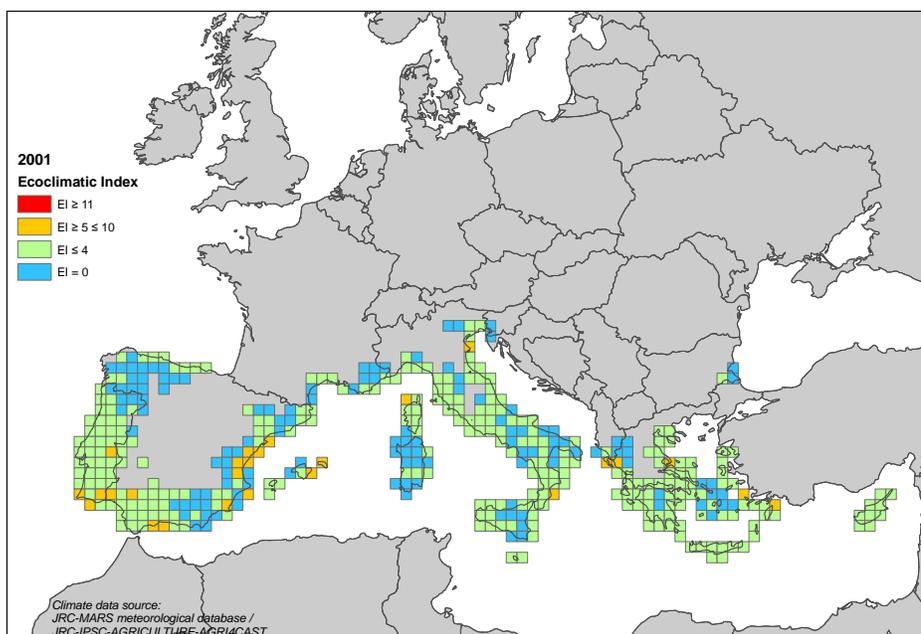


Figure 7. CLIMEX ecoclimatic index for CBS based on climatic data for 2001 from the JRC-MARS meteorological database interpolated to 418 grids at 50 km resolution representing the area of citrus production in the EU year period.

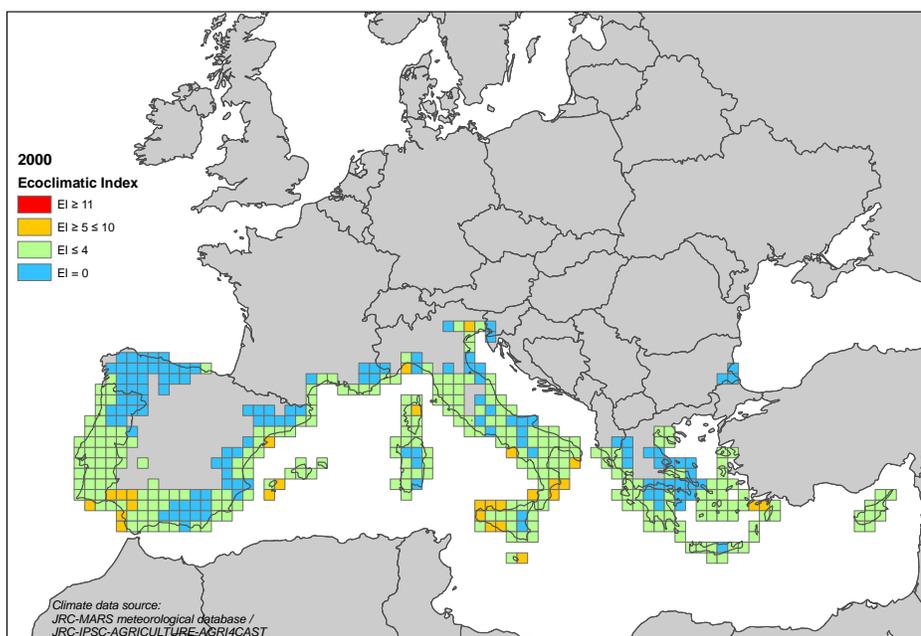


Figure 8. CLIMEX ecoclimatic index for CBS based on climatic data for 2000 from the JRC-MARS meteorological database interpolated to 418 grids at 50 km resolution representing the area of citrus production in the EU year period.

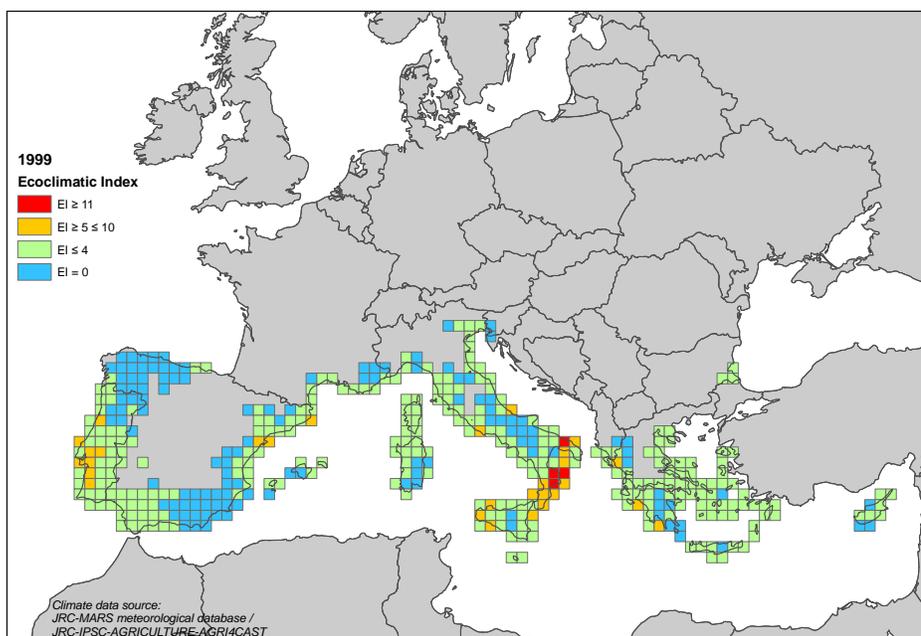


Figure 9. CLIMEX ecoclimatic index for CBS based on climatic data for 1999 from the JRC-MARS meteorological database interpolated to 418 grids at 50 km resolution representing the area of citrus production in the EU year period.

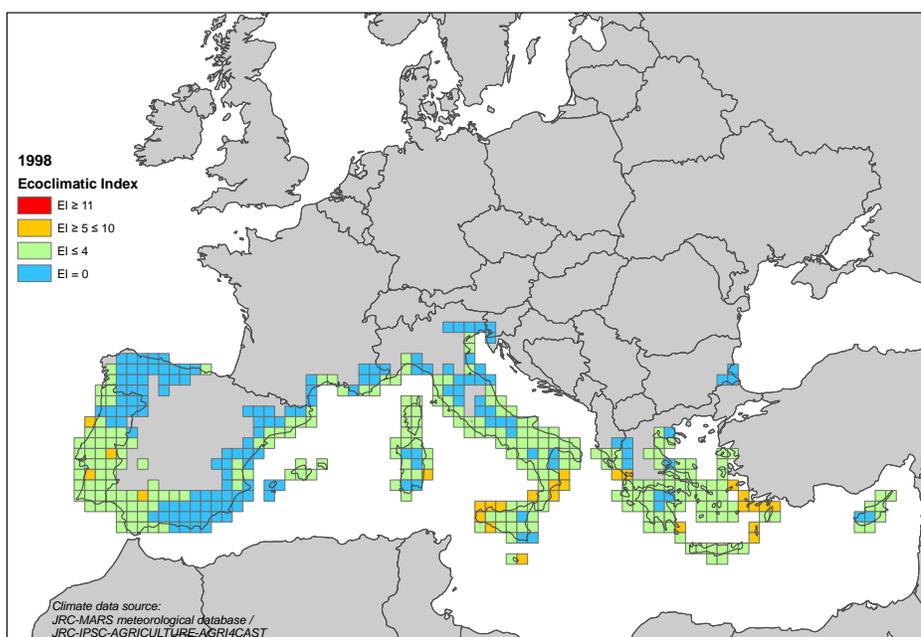


Figure 10. CLIMEX ecoclimatic index for CBS based on climatic data for 1998 from the JRC-MARS meteorological database interpolated to 418 grids at 50 km resolution representing the area of citrus production in the EU year period.

2. APPENDIX: Modelling climate suitability for *G. citricarpa* infection with generated/interpolated meteorological data

2.1. Generation of daily meteorological data by the grids.

Daily data of the period 1998-2007 were used in this analysis to derive hourly weather data and subsequently to use such data as inputs of a generic infection model (Magarey *et al.*, 2005), as described in the coming paragraphs. Data were derived for the EU grid cells, where there is cultivation of citrus (fig. 11).

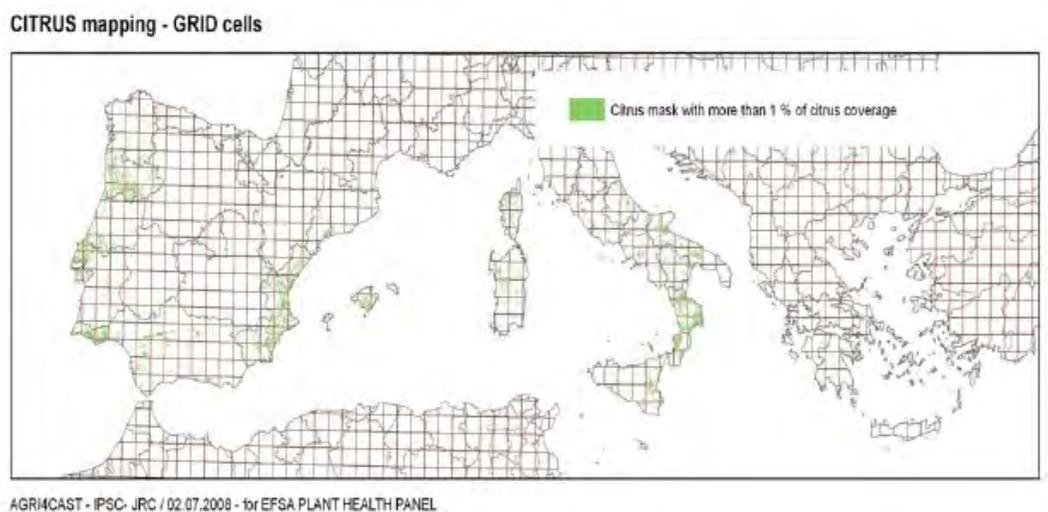


Figure 11. EU citrus mask with grids of more than 1% of citrus coverage. The dots indicate the density of 1 km x 1 km grids with more than 1 % citrus coverage. The light green squares are the 50 Km x 50 Km grids where areas with more than 1 % citrus coverage is present. The grey grey squares are additional grids selected by the Panel for analysis.

The Joint Research Centre, IPSC – AGRI4CAST maintains a data set of weather and crop data at a grid of 50 x 50 km. For each of the cells, daily weather data are interpolated from neighboring stations, making available a weather daily data series since 1975. Because actual weather stations are scattered irregularly in the EU-27 member states, the grid of interpolated data is a weather data source uniformly spaced and consistently processed using data quality procedures. The grid of actual weather stations and the interpolated grid are shown in fig. 12.

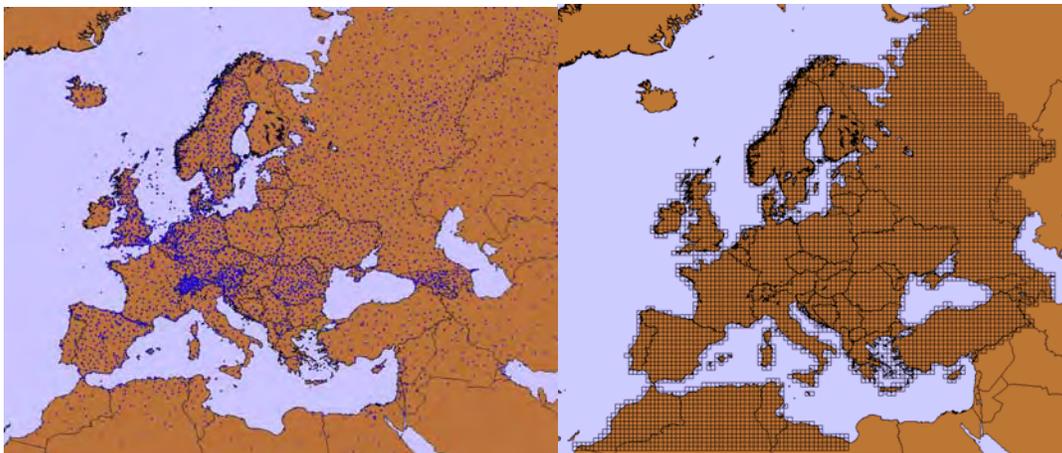


Fig. 12. The network of weather stations and the grid derived using several interpolation procedures.

2.2. Generation of hourly meteorological data.

The establishment in EU of organisms harmful to plant and plant products originating from other geographical areas needs, as a prerequisite, the suitability of weather conditions in the variety of environments of EU Member States. For fungal pathogens, the evaluation of potential infections requires, as key inputs, series of weather data in the areas which can be identified as at risk for a specific crop or perennial. Some simulations, as the generic infection model developed by Magarey *et al.* (2005), require a finer temporal resolution for weather data, for instance hourly values.

The derivation of the hourly values and the estimation of derived meteorological variables (e.g. vapour pressure deficit, leaf wetness, simple and composite weather indices) were done by the JRC using the CLIMA libraries (Donatelli *et al.*, 2005).

2.2.1. The CLIMA libraries

CLIMA is a synthetic weather estimator/generator built as a component library with 5 main subcomponents, including estimation/generation functions for each relevant weather variable. These basic model subcomponents are: *AirT*, air temperature generation; *ET*, reference evapotranspiration estimation; *GSRad*: solar radiation estimation/generation; *Rain*: precipitation generation; *Wind*: wind speed generation.

These key weather variables are modelled on either daily or sub-daily time steps. Most of the weather models implemented have been extracted from peer-reviewed sources which are the most widely adopted concepts on weather generation and estimation. The relevant information was collected, interlinked and uniformly formatted into navigable structures to grant easy access to readers (web site <http://www.apesimulator.org/help.aspx>). Each of the components offers a wide range of alternative methods to perform estimates and data generation. The main components of the CLIMA weather generator are shown in Figure 13. The components are used to estimate / generate the variables used by the LeafWetness library listed in Table 3, with the reference to the particular method (“strategy”) used.

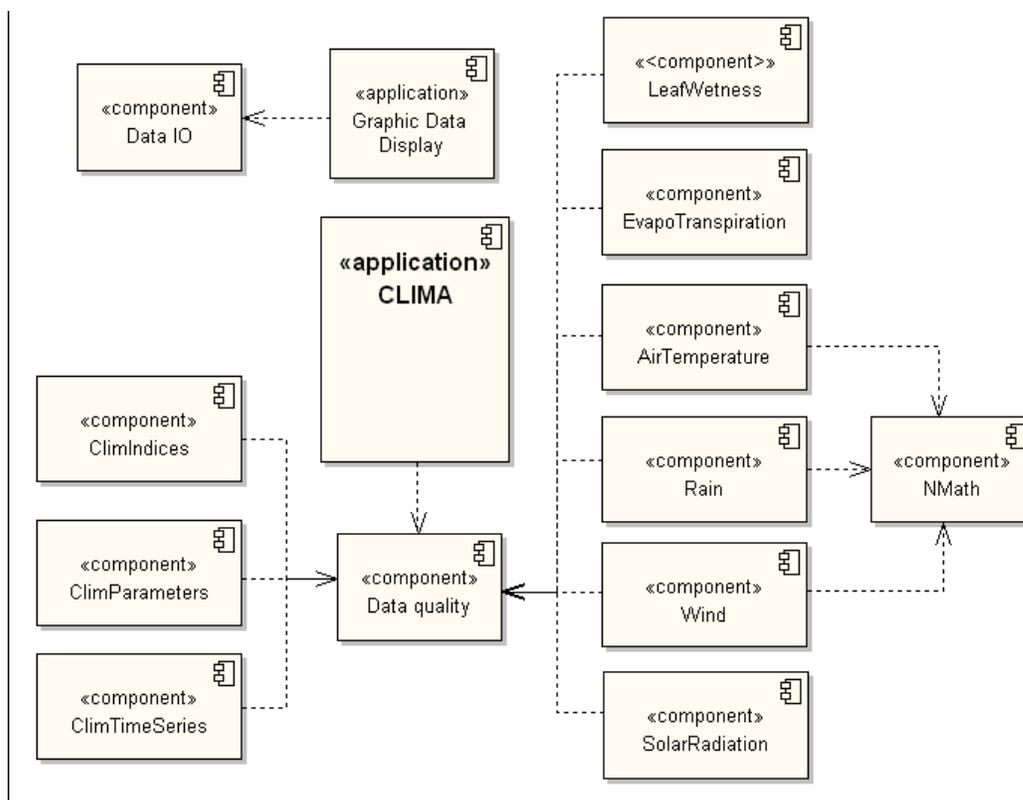


Figure 13. The main components of the CLIMA weather generator.

Table 3. Models to estimate/generate hourly weather data from daily values. Rainfall and wind hourly values are generated from daily values (stochastic component), other variables are estimated.

Name of the model (class)	Name of the variable
HAirAndDewTemperature	HourlyAirTemperature
	HourlyDewPointTemperature
HAirRelativeHumidity	HourlyRelativeHumidity
HVPDFAO	HourlyActualVaporPressure
	HourlyVaporPressureDeficit
HSVPDAsae	HourlySlopeVaporPressure
HLHVHarrison	HourlySaturationVaporPressure
	HourlyLatentHeatOfVaporization
HADAsae	HourlyAtmosphericDensity
HGSRGlobalSolarRadiationHourly	HourlyGlobalSolarRadiation
HNRFAO	HourlyNetRadiation
	HourlySoilHeatFlux
HWMitchell	HourlyWindSpeed
HRMetetest	HourlyRain
HPsychrometricConstant	HourlyPsychrometricConstant
HARFAO	HourlyAerodynamicResistance

2.2.2. Generation of air relative humidity

One of the most important input required by the *LeafWetness* component, in particular by the models SWEB, CART and ET (that will be briefly described in the following paragraphs), is hourly air relative humidity, because it is directly related to the presence of water on the leaf blade. Consequently, to estimate leaf wetness duration, air relative humidity must be accurately estimated.

Because of the lack of daily and hourly values of air relative humidity from the grid weather data time series, we have decided to use a strategy of the CLIMA component called *HairRelativeHumidity*, that is a set of methods for the generation of these variables from hourly air temperature and hourly dew point temperature. These inputs are also generated by a method available in the CLIMA libraries, *HAirAndDewTemperature*, as summarized in Table 2,

This model needs a parameter called *EphratDewPointTemperatureMax* to estimate hourly dew point temperature. This parameter represents the maximum temperature at which dew formation can happen. The method using this parameter is very sensitive to its value, hence impacting on the estimate of hourly values of dew point temperature. The author of the method describes this parameter as site specific. Given that it could not be calibrated against actual data (not available for all sites and not available in the grid weather data series), a simple regressive model was fit to estimate the parameter using data from weather data of sites where dew temperature hourly was available. The regressors chosen were: monthly average of air minimum temperature, monthly average of air temperature range, and monthly average of rainy days. These regressors were computed over 10 years (1998-2007 of the grid weather data). Two different regressions were fit, one for the months of March, April and October, and one for the months of May, June, July, August and September. The results of these multiple regression analysis are shown in Tables 4 and 5.

Table 4. Results of the regression analysis performed using *EphratDewPointTemperatureMax* as dependent variable and average monthly minimum air temperature, monthly range between average maximum air temperature and average minimum air temperature and monthly rainy days as predictors, for the month of March, April and October.

March, April, October	
Multiple R	0.98
R-Squared	0.96
Correct R-Squared	0.94
Standard Error	0.86
Number of observations	12

March, April, October	Coefficients	Standard error	Stat t	Significativity level
Intercept	-4.70	3.08	-1.52	0.166
AverageMonthlyMinimumTemperature	0.95	0.09	10.02	0.00001
AverageMonthlyRangeOfTemperature	0.41	0.19	2.11	0.06
AverageMonthlyRainDays	0.22	0.10	2.33	0.05

Table 5. Results of the regression analysis performed using *EphratDewPointTemperatureMax* as dependent variable and average monthly minimum air temperature and monthly range between average maximum air temperature and average minimum air temperature as predictors, for the month of May, June, July, August and September.

May, June ,July, August, September	
Multiple R	0.96
R-Squared	0.92
Correct R-Squared	0.91
Standard Error	0.88
Number of observations	21

May, June ,July, August, September	Coefficients	Standard error	Stat t	Significativity level
Intercept	17.025	1.632	10.434	0.000000005
AverageMonthlyMinimumTemperature	0.686	0.076	8.981	0.000000045
AverageMonthlyRangeOfTemperature	-0.996	0.084	11.819	0.000000001

These regressions were used to estimate the values of the parameter *EphratDewPointTemperatureMax*, which were then used to estimate hourly value of dew temperature for each grid cell.

2.3. Simulation of leaf wetness

2.3.1. Importance of leaf wetness in plant disease epidemiology

Leaf wetness is recognized as a very important agrometeorological variable for plant disease epidemiology (Pedro and Gillespie, 1982; Huber and Gillespie, 1992; Gleason et al., 1994; Kim et al., 2002). For this reason, the time when free water remains on the surface of plant tissues (leaves, fruit, flowers, etc.), termed leaf wetness duration (LWD), is a driving variable for the forecasting of plant disease epidemics because it strongly influences many processes such as the start of fungal pathogens active life cycle, the penetration of them through the leaves, the occurrence of primary and secondary infections. The model developed by Magarey *et al.* (2005) used in chapter 2 to estimate the potential infection requires this parameter.

2.3.2. Modeling leaf wetness

Because leaf wetness duration is not widely measured, several methods have been developed to estimate it from weather data. These methods, which are adopted by the existing leaf wetness models, can be divided into two broad categories, empirical (Gleason et al., 1994; Rao et al., 1998; Kim et al., 2002) and process-based (Pedro and Gillespie, 1982; Luo and Goudriaan, 2000; Magarey et al., 2006; Sentelhas et al., 2006). The latter considers physical principles of dew formation and dew and/or rain evaporation. They have shown good portability and sufficiently accurate results, but their complexity is a disadvantage for operational use. Alternatively, empirical models simulate leaf wetness duration by using simple relationships of

this variable with parameters measured at standard agro-meteorological stations. Their success depends on the accuracy of the weather data used as inputs and they are more site-specific than physical models (Huber and Gillespie, 1992; Kim *et al.*, 2005).

2.3.3. The LeafWetness component

In this context, a reusable and extensible software component, called *LeafWetness*, was developed in the frame of CLIMA components, implementing in the current version 5 models to estimate hourly values of LWD. The software component is available for download, inclusive of documentation and sample projects to illustrate re-use in custom developed applications. The models vary from process based to fully empirical, as described in the previous paragraph, in order to allow the best estimation of LWD according to the available data and the condition of application.

All the models were applied with the parameterisation proposed by the Authors. The models implemented are:

- SWEB (Surface Wetness Energy Balance) (Magarey *et al.*, 2006),
- LWR (Leaf Wetness Reference) (Sentelhas *et al.*, 2006),
- DP (Dew Parametrization) (Garratt and Segal, 1988),
- CART (Classification And Regression Tree) (Kim *et al.*, 2002)
- ET (Extended Threshold) (Witchink Kruit *et al.*, 2004).

A brief description of these models follows.

SWEB

SWEB is the most mechanistic model of the LeafWetness library because of its representation of the inner processes related to leaf wetness. In particular, in this implementation it presents five modules, they are:

- WindSpeed: that implements a calculation of the wind speed at the height of the canopy with two different methods,
- NetRadiation: that calculates the fraction of net radiation intercepted by the canopy for the simulation of dew fall,
- WaterBudget: that considers the fraction of rain intercepted by the canopy and the condensation of water as dew and their contribution to leaf wetness,
- CanopyEvaporation: that simulates the latent heat flux density from the canopy and so the negative term for the water balance of the canopy,
- WaterBalance: that calculates the actual wet area of the canopy and estimates if there is leaf wetness.

It was already applied in epidemiological and modelling studies (Dalla Marta *et al.*, 2005).

LWR

LWR implements a Penman-Monteith approach for the calculation of leaf wetness using data measured in a standard weather station to provide a simple 'reference' LWD.

It assumes that air temperature measured at a given height above turf grass at a standard weather station is equivalent to temperature at the same height above the top of a crop canopy, and that adding a resistance item to the model is enough to account for the air layer from measurement height, above the canopy, to the level of the leaves.

The model simply treated the rain interception using measured rainfall amount and a fixed maximum amount of water in the rain reservoir (0.6 mm).

DP

This method to predict LWD is the dew parameterisation by Garratt and Segal (1988) based on earlier work by Penman and Monteith. It predicts dew amounts as well as dew duration.

CART

CART uses an empirical approach for the simulation of leaf wetness. It utilizes dew point depression, wind speed (with a correction factor) and relative humidity as inputs. The *CART* model assigns hourly data to one of four categories according to threshold values of 3.7°C for DPD, 2.5 m/s for wind speed (Wind), and 87.8% for RH. Hours are classified as dry if either $DPD \geq 3.7^\circ\text{C}$ (category 1) or $RH < 87.8\%$ and $Wind \geq 2.5 \text{ m/s}$ (category 4).

Hours in categories 2 and 3 are classified as either dry or wet by subsequent SLD analysis.

The model implements a calculation of wind speed at canopy height from measured values of wind speed. The estimations of wind speed at 10 m height were corrected to canopy height using a logarithmic wind profile equation.

ET

ET is the simplest model of the LeafWetness library. It implements a very empirical approach for the calculation of leaf wetness: the hours in which relative humidity is under 70% are considered as dry. This method uses a base RH threshold of 87%, and wetness is extended to lower humidity ranges depending on the rate of change in RH. For periods with RH between 70% and 87%, leaves are assumed to be wet if average RH increases more than 3% in 30 min, and leaves are assumed to become dry if average RH decreases more than 2% in 30 min. During periods with average $RH < 70\%$ leaves are assumed to be dry, and during periods with average $RH > 87\%$ leaves are assumed to be wet.

2.3.4. Interaction between CLIMA and LeafWetness

The only daily inputs needed by *CLIMA* for the generation of all the hourly inputs used by *LeafWetness* are air minimum and maximum temperature, rain, global solar radiation, and wind. A complete list of hourly values of the variables generated by *CLIMA* and used by the *LeafWetness* component is shown in table 1, with the name of the modelling approach (implemented as a class) used.

The hourly values of the variables generated are then used as input by the different models of the *LeafWetness* library, as shown in table 6.

Table 6. Input requirements of leaf wetness models.

	CART	SWEB	DP	ET	LWR
HourlyAirTemperature	X	X			
HourlyDewPointTemperature	X				
HourlyWindSpeed	X	X			X
HourlyNetRadiation		X	X		X
HourlySlopeVaporPressureCurve		X	X		X
HourlyLatentHeatOfVaporization		X	X		
RainSixtyMinutes		X			X
HourlyAtmosphericDensity		X	X		
HourlyRelativeAirHumidity	X	X		X	
HourlySaturationVaporPressure		X	X		X
HourlySpecificHeatOfAir		X	X		
HourlyAerodynamicResistance			X		X
HourlyActualVaporPressure			X		X
HourlyPsychrometricConstant			X		X
HourlySoilHeatFlux			X		

Starting from these inputs, each model of the *LeafWetness* library estimates leaf wetness status using an hourly time step: true if the canopy is considered wet and false if it is considered dry. The output of the models are expressed in qualitative terms, describing the dry or wet conditions during the hour that represent the input of the CBS infection model.

2.3.5. Selecting the leaf wetness model

The first statistical analysis was performed to compare the LWD as measured by sensors and calculated by the five LWD models. To compare the number of hours correctly classified as wet and dry, a dichotomous categorical verification was applied (Wilks, 1995). In particular the indices used were probability of detection (POD), probability of null event (PNE), and BIAS.

	ESTIMATED YES	ESTIMATED NO
OBSERVED YES	HITS	MISSES
OBSERVED NO	FALSE ALARMS	CORRECT NEGATIVES

$$P_o = \frac{\text{hits}}{\text{hits} + \text{misses}}$$

$$P_n = 1 - \frac{\text{false alarms}}{\text{false alarms} + \text{correct negatives}}$$

$$B = \frac{\text{hits} + \text{false alarms}}{\text{hits} + \text{misses}}$$

Where: P_o = POD, P_n = PNE, B = BIAS

The first two range between 0 and 1 and represent respectively the probability that an occurred event (wetness) and an occurred non-event (dryness) were correctly simulated, while the BIAS shows the tendency of the model to under or over-estimate the simulated phenomenon; if the BIAS is greater than 1 it means that the simulated events are more than the real ones.

To test the reliability of sensors, the measured and the simulated observed total duration of leaf wetness were compared and mean absolute error (MAE), root mean square error (RMSE) and mean real error (MRE) were calculated.

$$M_r = \frac{\sum_0^n (v_o - v_s)}{n}$$

$$M_a = \frac{\sum_0^n (|v_o - v_s|)}{n}$$

$$R_m = \left(\frac{\sum_0^n (v_o - v_s)^2}{n} \right)^{0.5}$$

Where:

M_r = MRE, M_a = MAE, R_m = RMSE;

n = number of data (observed – simulated pairs), v_o = observed values, v_s = estimated values

Moreover the number of wet hours measured by the sensors was plotted with the number of wet hours simulated by the model for each day and the coefficient of determination (R^2) was calculated.

Model selection was performed using data collected from some locations of Sicily and Calabria (Italy) (table 7), due to the availability of the majority of input variables and the quality of LWD data. Moreover the number of stations and the length of the measurement period allows to obtain a general indication of LWD models efficacy.

Table 7. Characteristics of agrometeorological stations. Between brackets the provincial administrations (SR=Siracusa; PA=Palermo; CT=Catania; ME=Messina; AG=Agrigento; CS=Cosenza; RC=Reggio Calabria).

Location	Y UTM	X UTM	Elevation (m)	Available years
Siracusa (SR)	4101770	514148	90	03, 04, 06
Misilmeri (PA)	4210500	363375	160	04, 05, 06
Mineo (CT)	4130521	475760	200	03, 05, 06
Caronia (ME)	4209150	455207	50	04
Paternò (CT)	4152071	487181	100	03, 04, 05
Ribera (AG)	4144951	346639	30	03, 04, 05
San Marco (CS)	4378412	596228	400	06, 07
Gioia Tauro (RC)	4252800	578565	20	05, 06, 07

2.3.6. Results

The general analysis of LWD (Fig. 14) shows a reduction of monthly LW hours during the summer months, whereas late spring and the beginning of fall showed the higher duration of LW. The variability among the height stations is quite relevant, presenting in many cases a difference higher than 100%. Caronia showed the lower LWD, but only one year of data is available.

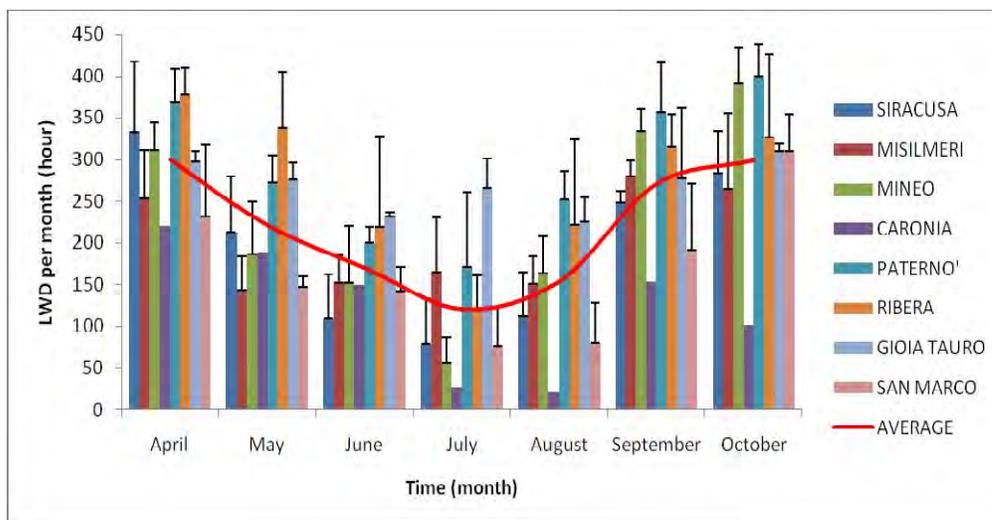


Figure 14. Average leaf wetness duration (LWD) for the 8 locations during monitoring period (bars represent standard deviation).

To select the best LWD models, the first analysis was performed by comparing simulation and observation data, for the whole data sets and expressing LWD in number of hours per month (Fig. 14). All the used error indices showed that SWEB simulated more precisely the real LWD. MRE indicated a very little tendency to underestimate LWD, while MAE and RMSE showed an average error of about 40-50 hour per month, representing a percentage ranging between 10 and 20% in comparing to the total number of LW hours per month. All the other tested models showed worst performances, particularly for empirical models CART and mechanistic model LWR (Tab.8).

Table 8. Comparison between simulations and observations for the whole data sets (errors are expressed in hours per month).

	MRE	MAE	RMSE	R2
SWEB	-7.69	40.17	53.33	0.76
CART	45.68	103.33	122.84	0.16
DP	-72.82	99.95	121.19	0.17
ET	21.59	63.03	80.85	0.45
LWR	45.85	112.94	134.33	0.10

To complete the analysis, dichotomous categorical verification was applied. In table 9 examples are presented for several months and agrometeorological stations. It is very clear that SWEB provided the best simulation of LWD. All the used indices showed better values for this model (on average POD = 0.64, PNE = 0.81, BIAS = 0.91. The latter confirmed the tendency to underestimate LWD data, but globally POD and PNE values are really satisfactory.

Table 9. Comparison between simulations and observations LWD data.

	SWEB	CART	DP	ET	LWR
Paternò 2005					
pod	0.60	0.29	0.50	0.50	0.50
pne	0.86	0.85	0.52	0.75	0.73
bias	0.74	0.60	2.87	1.17	1.84
San Marco 2006					
pod	0.67	0.89	0.93	0.93	0.93
pne	0.87	0.14	0.68	0.44	0.66
bias	0.97	2.60	2.32	2.89	2.50
Mineo 2005					
pod	0.73	0.51	0.73	0.71	0.72
pne	0.82	0.76	0.52	0.74	0.72
bias	0.96	1.04	3.09	1.59	2.16
Ribera 2003					
pod	0.49	0.08	0.10	0.11	0.11
pne	0.85	0.96	0.46	0.78	0.72
bias	0.59	0.16	1.82	0.57	1.08
Siracusa 2003					
pod	0.75	0.62	0.83	0.82	0.83

pne	0.69	0.68	0.54	0.61	0.71
Bias	1.23	1.31	2.82	1.91	1.99

On the basis of these results, SWEB model was chosen to simulate LWD and to provide the input data for the infection model. To have a more detailed view of SWEB performances, simulations were analysed in order to evaluate spatial and temporal variability.

Table 10. Analysis of SWEB performances in the studied agrometeorological stations (data analysed in hours per month).

Agrometeorological station	MRE	MAE	RMSE	R²
Siracusa	-28	35	50	0.88
Misilmeri	-36	53	70	0.44
Mineo	3	36	43	0.72
Caronia	-2	32	40	0.79
Paternò	29	41	56	0.69
Ribera	9	43	59	0.86
Gioia tauro	-3	37	47	0.48
San marco	-28	37	48	0.86

Table 11. Comparison between SWEB simulations and observations in the months analysed (data analysed in hours per day).

	MRE	MAE	RMSE	R2
April	-0.3	0.9	1.2	0.78
May	-0.4	1.2	1.6	0.66
June	-0.6	1.0	1.3	0.67
July	0.4	1.3	1.8	0.57
August	0.3	1.4	1.8	0.49
September	0.2	1.6	1.8	0.33
October	-1.3	2.1	2.6	0.12

The analysis of spatial variability showed a good stability of SWEB performances (Tab. 10). The lower values of are obtained in Misilmeri and Goia Tauro stations, but the correlations is still statistically significant ($p < 0.05$).

As concerning the temporal variability, excellent results were obtained during the spring and summer months (Tab. 11). Lower quality of results was obtained during fall period, but only October was characterised by a very low level of the correlation between simulations and observations. However this month is characterised by a very high duration of LW (about 12 hours) and the average level of MAE (2.1 hours) is a clear signal that SWEB outputs are able to describe in a good way the trend LWD during this important epidemiological period for CBS infections.

3. APPENDIX: Modelling climate suitability for *G. citricarpa* infection with data from agro-meteorological stations, including sensitivity analysis

Table 12. Algorithm to determine an hour with suitable weather conditions for a successful start of a potential infection with Ascospores

Input variables:					
t =	Hourly time scale [h]: 1,...,N				
End _{Seq} (t) =	Indicator for end of sequence in the time line [yes/no]				
LW _{Ind} (t) =	Indicator of leaf wetness in hour t [yes/no]				
T(t) =	Mean temperature in hour t [°C]				
Parameters:		low	medium	high	best choice
T _{min} =	Minimal temperature for development [°C]	12	15		15
T _{opt} =	Optimal temperature for development [°C]		27	29.5	27
T _{max} =	Maximal temperature for development [°C]		35		35
W _{min} =	Minimal duration of wetness for infection [h]		15		15
W _{max} =	Maximal duration of wetness for infection [h]		38	46	38
D ₅₀ =	Maximal time of interrupted wetness under development	0	3	14	0; 3; 14

[h]

Interim variables:

- Durat_{Wet} = Duration of wetness (only moderate temperatures) [h]
- Durat_{Dry} = Duration of interrupted wetness [h]
- T_{cum} = Cumulative temperature of wetness period (only moderate temperatures) [°C]
- s = Time step in future [h]
- Stop = Indicator for end of loop [yes/no]
- T_{mean} = Average Temperature of wetness period (only moderate temperatures) [°C]
- F = Non-linear transformation of temperature [-], see formula for function
- W_{need} = Necessary length of wet periods with moderate temperature for infection [h]

$$f(T | T_{min}, T_{opt}, T_{max}) = \left(\frac{T_{max} - T}{T_{max} - T_{opt}} \right) \cdot \left(\frac{T - T_{min}}{T_{opt} - T_{min}} \right)^{\frac{(T_{opt} - T_{min})}{(T_{max} - T_{opt})}}$$

Output:

Infect_{Ind}(t) = Indicator for potential infection [yes/no/unknown]

Do for each hour t=1 to N

(Is leaf wet: LW_{Ind}(t) = 'yes') AND (Is temperature moderate: T_{min} < T(t) < T_{max}) ?

no

yes

	No infection:	Duration of Wetness: $Durat_{Wet} \leftarrow 0$			
	Infect _{ind} (t) \leftarrow 'no'	Duration of Dryness: $Durat_{Dry} \leftarrow 0$			
		Cumulate Temperature of Wetness: $T_{cum} \leftarrow 0$			
		Future time step: $s \leftarrow t$			
		End of the loop: $Stop \leftarrow$ 'no'			
		Is leaf wet: $LW_{ind}(s) =$ 'yes'?			
		no	yes		
		Duration of dryness: $Durat_{Dry} \leftarrow Durat_{Dry} + 1$	Duration of dryness: $Durat_{Dry} \leftarrow 0$		
		Is the dryness period too long: $Durat_{Dry} > D_{50}$?		Is temperature moderate: $T_{min} < T(s) < T_{max}$?	
		no	yes	no	yes
			No infection: Infect _{ind} (t) \leftarrow 'no'		Duration of wetness: $Durat_{Wet} \leftarrow Durat_{Wet} + 1$
			End of the loop: $Stop \leftarrow$ 'yes'		Cumulative Temperature: $T_{cum} \leftarrow T_{cum} + T(s)$
				Mean temperature: $T_{mean} \leftarrow T_{cum} / Durat_{Wet}$	
				Transformation: $F \leftarrow f(T_{mean} T_{min}, T_{opt}, T_{max})$	
				Wetness needs: $W_{need} \leftarrow MIN(W_{min}/f; W_{max})$	
				Is wetness period long enough: $W_{need} \leq Durat_{Wet}$?	
			no	yes	

						Infection possible: $\text{Infect}_{\text{Ind}}(t) \leftarrow \text{'yes'}$	
						End of the loop: $\text{Stop} \leftarrow \text{'yes'}$	
						(Is s end of time sequence: $\text{End}_{\text{Seq}}(s) = \text{'yes'}$) AND (Is no result reached: $\text{Stop} = \text{'no'}$)?	
						no	yes
						Next future time step: $s \leftarrow s + 1$	No more information: $\text{Infect}_{\text{Ind}} \leftarrow \text{'unknown'}$
			End of the loop: $\text{Stop} \leftarrow \text{'yes'}$				
Repeat the loop until Stop = 'yes'							

2

3 Table 13. Results for ascospores potential infections by month (data from agro-meteorological stations).

Month			Part of hours with suitable weather conditions for a successful start of a potential infection with Ascospores [%]												
			Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Place	D50	other parameter	1	2	3	4	5	6	7	8	9	10	11	12	
Spain: Tivenys (Tarragona)	0	best	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.76	0.00	0.00	0.12
		min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		max	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.93	0.00	0.00	0.14
	3	best	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.79	0.00	0.00	0.12
		min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		max	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.96	0.00	0.00	0.15
	14	best	0.00	0.00	0.00	0.00	9.81	0.00	0.00	5.72	7.22	6.66	0.00	0.00	3.21
		min	0.00	0.00	0.00	0.00	7.66	0.00	0.00	0.00	6.11	5.09	0.00	0.00	2.10
		max	0.00	0.00	0.00	0.00	27.55	0.28	0.00	6.68	7.64	9.29	0.00	0.00	5.52
Spain: Amposta (Tarragona)	0	best	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	1.26	0.00	0.00	0.20
		min	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		max	0.00	0.00		0.00	1.08	0.00	0.00	0.00	0.00	1.36	0.00	0.00	0.32
	3	best	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.28	1.36	0.00	0.00	0.24

		min	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		max	0.00	0.00		0.00	1.08	0.00	0.00	0.00	0.97	1.45	0.00	0.00	0.44
	14	best	0.00	0.00		0.00	13.98	9.44	18.75	33.24	30.69	10.09	0.00	0.00	11.56
		min	0.00	0.00		0.00	10.75	7.92	1.60	23.02	26.94	8.66	0.00	0.00	8.48
		max	0.00	2.83		0.00	33.47	9.44	21.17	33.65	36.67	20.09	0.00	0.00	16.25
Spain: Burriana (Castellon)	0	best	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.33	5.85	0.00	0.00	1.60
		min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.33	2.49	0.00	0.00	1.02
		max	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.44	8.33	0.00	0.00	2.04
	3	best	0.00	0.00	0.00	0.00	0.00	0.00	0.54	3.49	5.33	6.72	0.00	0.00	2.10
		min	0.00	0.00	0.00	0.00	0.00	0.00	0.54	1.34	5.33	3.02	0.00	0.00	1.28
		max	0.00	0.43	0.00	0.00	0.00	0.99	0.54	3.49	5.44	10.89	0.00	0.00	2.94
	14	best	0.00	0.00	0.00	0.00	15.19	18.57	29.30	34.27	19.38	24.66	0.00	0.00	14.71
		min	0.00	0.00	0.00	0.00	14.11	13.71	23.52	29.70	15.08	22.51	0.00	0.00	12.48
		max	0.00	9.63	1.21	3.90	29.17	30.57	30.51	34.68	21.74	39.52	0.80	0.00	21.11
Spain: Xilxes (Castellon)	0	best	0.00	0.00	0.00	0.00	0.00	0.00	3.76	0.00	0.57	0.00	0.00	0.00	0.20
		min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		max	0.00	0.00	0.00	0.00	0.67	0.00	4.15	0.00	0.57	0.00	0.00	0.00	0.28

	3	best	0.00	0.00	0.00	0.00	0.00	0.00	12.93	1.18	2.79	8.10	0.00	0.00	1.50
		min	0.00	0.00	0.00	0.00	0.00	0.00	8.14	0.00	0.15	5.36	0.00	0.00	0.76
		max	0.00	0.00	0.00	0.00	0.67	0.00	13.72	1.18	2.97	8.79	0.00	0.00	1.66
	14	best	0.00	0.00	0.00	0.00	8.33	17.13	33.33	18.29	23.63	18.35	0.00	0.00	7.93
		min	0.00	0.00	0.00	0.00	6.18	14.17	24.87	5.52	17.82	16.23	0.00	0.00	5.53
		max	0.00	0.00	0.00	0.28	29.30	30.48	34.26	21.39	31.78	27.73	0.00	0.00	12.74
Spain: Chiva (Valencia)	0	best	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		max	0.00	0.00	0.00	0.00	0.74	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06
	3	best	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.34	0.00	0.00	0.00	0.03
		min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		max	0.00	0.00	0.00	0.00	1.04	0.00	0.00	0.00	0.51	0.00	0.00	0.00	0.13
	14	best	0.00	0.00	0.00	0.00	0.71	6.11	7.02	4.65	13.39	4.81	0.00	0.00	2.90
		min	0.00	0.00	0.00	0.00	0.31	4.14	2.16	1.45	11.19	3.88	0.00	0.00	1.81
		max	0.00	0.00	0.00	0.32	8.36	8.10	7.43	4.81	16.88	9.57	3.11	0.00	4.70
			Part of hours with suitable weather conditions for a successful start of a potential infection with Ascospores [%]												
Month			Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total

Place	D50	other parameter	1	2	3	4	5	6	7	8	9	10	11	12	
Spain: Tivenys (Tarragona)	0	best	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.76	0.00	0.00	0.12
		min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		max	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.93	0.00	0.00	0.14
	3	best	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.79	0.00	0.00	0.12
		min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		max	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.96	0.00	0.00	0.15
	14	best	0.00	0.00	0.00	0.00	9.81	0.00	0.00	5.72	7.22	6.66	0.00	0.00	3.21
		min	0.00	0.00	0.00	0.00	7.66	0.00	0.00	0.00	6.11	5.09	0.00	0.00	2.10
		max	0.00	0.00	0.00	0.00	27.55	0.28	0.00	6.68	7.64	9.29	0.00	0.00	5.52
Spain: Amposta (Tarragona)	0	best	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	1.26	0.00	0.00	0.20
		min	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		max	0.00	0.00		0.00	1.08	0.00	0.00	0.00	0.00	1.36	0.00	0.00	0.32
	3	best	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.28	1.36	0.00	0.00	0.24
		min	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		max	0.00	0.00		0.00	1.08	0.00	0.00	0.00	0.97	1.45	0.00	0.00	0.44
	14	best	0.00	0.00		0.00	13.98	9.44	18.75	33.24	30.69	10.09	0.00	0.00	11.56

		min	0.00	0.00		0.00	10.75	7.92	1.60	23.02	26.94	8.66	0.00	0.00	8.48
		max	0.00	2.83		0.00	33.47	9.44	21.17	33.65	36.67	20.09	0.00	0.00	16.25
Spain: Burriana (Castellon)	0	best	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.33	5.85	0.00	0.00	1.60
		min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.33	2.49	0.00	0.00	1.02
		max	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	5.44	8.33	0.00	0.00	2.04
	3	best	0.00	0.00	0.00	0.00	0.00	0.00	0.54	3.49	5.33	6.72	0.00	0.00	2.10
		min	0.00	0.00	0.00	0.00	0.00	0.00	0.54	1.34	5.33	3.02	0.00	0.00	1.28
		max	0.00	0.43	0.00	0.00	0.00	0.99	0.54	3.49	5.44	10.89	0.00	0.00	2.94
	14	best	0.00	0.00	0.00	0.00	15.19	18.57	29.30	34.27	19.38	24.66	0.00	0.00	14.71
		min	0.00	0.00	0.00	0.00	14.11	13.71	23.52	29.70	15.08	22.51	0.00	0.00	12.48
		max	0.00	9.63	1.21	3.90	29.17	30.57	30.51	34.68	21.74	39.52	0.80	0.00	21.11
Spain: Xilxes (Castellon)	0	best	0.00	0.00	0.00	0.00	0.00	0.00	3.76	0.00	0.57	0.00	0.00	0.00	0.20
		min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		max	0.00	0.00	0.00	0.00	0.67	0.00	4.15	0.00	0.57	0.00	0.00	0.00	0.28
	3	best	0.00	0.00	0.00	0.00	0.00	0.00	12.93	1.18	2.79	8.10	0.00	0.00	1.50
		min	0.00	0.00	0.00	0.00	0.00	0.00	8.14	0.00	0.15	5.36	0.00	0.00	0.76
		max	0.00	0.00	0.00	0.00	0.67	0.00	13.72	1.18	2.97	8.79	0.00	0.00	1.66
	14	best	0.00	0.00	0.00	0.00	8.33	17.13	33.33	18.29	23.63	18.35	0.00	0.00	7.93

		min	0.00	0.00	0.00	0.00	6.18	14.17	24.87	5.52	17.82	16.23	0.00	0.00	5.53
		max	0.00	0.00	0.00	0.28	29.30	30.48	34.26	21.39	31.78	27.73	0.00	0.00	12.74
Spain: Chiva (Valencia)	0	best	0.00	0.00	0.00	0.00	0.00								
		min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		max	0.00	0.00	0.00	0.00	0.74	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.06
	3	best	0.00	0.34	0.00	0.00	0.00	0.03							
		min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		max	0.00	0.00	0.00	0.00	1.04	0.00	0.00	0.00	0.51	0.00	0.00	0.00	0.13
	14	best	0.00	0.00	0.00	0.00	0.71	6.11	7.02	4.65	13.39	4.81	0.00	0.00	2.90
		min	0.00	0.00	0.00	0.00	0.31	4.14	2.16	1.45	11.19	3.88	0.00	0.00	1.81
		max	0.00	0.00	0.00	0.32	8.36	8.10	7.43	4.81	16.88	9.57	3.11	0.00	4.70

Table 14. Algorithm to determine an hour with suitable weather conditions for a successful start of a potential infection with Pycnidiospores

Input variables:					
t =	Hourly time scale [h]: 1,...,N				
End _{Seq} (t) =	Indicator for end of sequence in the time line [yes/no]				
LW _{Ind} (t) =	Indicator of leaf wetness in hour t [yes/no]				
T(t) =	Mean temperature in hour t [°C]				
RNF(t) =	Total rainfall in hour t [mm]				
Parameters:		low	medium	high	best choice
T _{min} =	Minimal temperature for development [°C]		10	15	10
T _{opt} =	Optimal temperature for development [°C]		25	30	25
T _{max} =	Maximal temperature for development [°C]		35		35
W _{min} =	Minimal duration of wetness for infection [h]		12	14	12
W _{max} =	Maximal duration of wetness for infection [h]		35	48	35
D ₅₀ =	Maximal time of interrupted wetness under development [h]	0	3	14	0; 3; 14

Interim variables:

- Durat_{Wet} = Duration of wetness (only moderate temperatures) [h]
- Durat_{Dry} = Duration of interrupted wetness [h]
- T_{cum} = Cumulative temperature of wetness period (only moderate temperatures) [°C]
- s = Time step in future [h]
- Stop = Indicator for end of loop [yes/no]
- T_{mean} = Average Temperature of wetness period (only moderate temperatures) [°C]
- F = Non-linear transformation of temperature [-], see formula for function
- W_{need} = Necessary length of wet periods with moderate temperature for infection [h]

$$f(T | T_{min}, T_{opt}, T_{max}) = \left(\frac{T_{max} - T}{T_{max} - T_{opt}} \right) \cdot \left(\frac{T - T_{min}}{T_{opt} - T_{min}} \right)^{\frac{(T_{opt} - T_{min})}{(T_{max} - T_{opt})}}$$

Output:

- Infect_{Ind}(t) = Indicator for potential infection [yes/no/unknown]

Do for each hour t=1 to N

(Is leaf wet: LW _{Ind} (t) = 'yes') AND (Is temperature moderate: T _{min} < T(t) < T _{max}) AND (Is a rain event: RNF(t) > 0) ?	
no	yes
No infection:	Duration of Wetness: Durat _{Wet} ← 0
Infect _{Ind} (t) ←	Duration of Dryness: Durat _{Dry} ← 0

	'no'				
		Cumulate Temperature of Wetness: $T_{cum} \leftarrow 0$			
		Future time step: $s \leftarrow t$			
		End of the loop: Stop \leftarrow 'no'			
		Is leaf wet: $LW_{Ind}(s) = \text{'yes'}$?			
		no		yes	
		Duration of dryness: $Durat_{Dry} \leftarrow Durat_{Dry} + 1$		Duration of dryness: $Durat_{Dry} = 0$	
		Is the dryness period too long: $Durat_{Dry} > D_{50}$?		Is temperature moderate: $T_{min} < T(s) < T_{max}$?	
		no	yes	no	yes
			No infection: $Infect_{Ind}(t) \leftarrow \text{'no'}$		Duration of wetness: $Durat_{Wet} \leftarrow Durat_{Wet} + 1$
		End of the loop: Stop \leftarrow 'yes'		Cumulative Temperature: $T_{cum} \leftarrow T_{cum} + T(s)$	
				Mean temperature: $T_{mean} \leftarrow T_{cum} / Durat_{Wet}$	
				Transformation: $F \leftarrow f(T_{mean} T_{min}, T_{opt}, T_{max})$	
				Wetness needs: $W_{need} \leftarrow \text{MIN}(W_{min}/f, W_{max})$	
				Is wetness period long enough: $W_{need} \leq Durat_{Wet}$?	
			no	yes	
				Infection possible: $Infect_{Ind}(t) \leftarrow \text{'yes'}$	

				End of the loop: Stop \leftarrow 'yes'	
		(Is s end of time sequence: End _{Seq} (s) = 'yes') AND (Is no result reached: Stop = 'no')?			
		no		yes	
		Next future time step:	$s \leftarrow s + 1$	No more information:	Infect _{ind} \leftarrow 'unknown'
				End of the loop:	Stop \leftarrow 'yes'
Repeat the loop until Stop = 'yes'					

7 Table 15. Results for pycnidiospores potential infections by month (data from agro-meteorological stations).

			Part of hours with suitable weather conditions for a successful start of a potential infection with Pycnidiospores [%]												
Month			Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Place	D50	other parameter	1	2	3	4	5	6	7	8	9	10	11	12	
Spain: Tivenys (Tarragona)	0	best	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.42	0.00	0.00	0.08
		min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		max	0.00	0.00	0.00	0.00	0.13	0.00	0.00	0.00	0.00	0.42	0.00	0.00	0.08
	3	best	0.00	0.00	0.00	0.00	1.21	0.00	0.00	0.00	0.00	1.11	0.00	0.00	0.29
		min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		max	0.00	0.00	0.00	0.00	1.21	0.00	0.00	0.00	0.00	1.11	0.00	0.00	0.29
	14	best	0.00	0.00	0.00	0.00	12.63	0.42	0.00	0.00	0.56	1.62	0.00	0.00	1.58
		min	0.00	0.00	0.00	0.00	2.96	0.00	0.00	0.00	0.00	0.26	0.00	0.00	0.33
		max	0.00	0.00	0.00	0.00	12.63	0.42	0.00	0.00	0.56	1.62	0.00	0.00	1.58
Spain: Amposta (Tarragona)	0	best	0.00	0.00		0.00	0.81	0.00	0.00	0.00	0.00	0.36	0.00	0.00	0.14
		min	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		max	0.00	0.00		0.00	0.81	0.00	0.00	0.00	0.00	0.36	0.00	0.00	0.14

	3	best	0.00	0.00		0.00	0.81	0.00	0.00	0.00	0.83	0.54	0.00	0.00	0.25
		min	0.00	0.00		0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		max	0.00	0.00		0.00	0.81	0.00	0.00	0.00	0.83	0.54	0.00	0.00	0.25
	14	best	0.00	5.18		0.00	9.54	0.28	0.48	0.00	3.47	1.46	0.00	0.00	2.09
		min	0.00	0.00		0.00	2.15	0.00	0.00	0.00	0.69	0.82	0.00	0.00	0.42
		max	0.00	5.18		0.00	9.54	0.28	0.48	0.00	3.47	1.46	0.00	0.00	2.09
Spain: Burriana (Castellon)	0	best	0.00	0.31	0.07	0.00	0.00	0.05							
		min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		max	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.31	0.07	0.00	0.00	0.05
	3	best	0.00	0.00	0.00	0.00	0.00	2.09	0.00	0.00	0.72	0.34	0.00	0.00	0.31
		min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.07	0.00	0.00	0.01
		max	0.00	0.00	0.00	0.00	0.00	2.09	0.00	0.00	0.72	0.34	0.00	0.00	0.31
	14	best	0.00	0.14	0.00	0.14	2.42	4.59	0.13	0.27	1.23	1.95	0.35	0.00	1.13
		min	0.00	0.00	0.00	0.00	0.13	0.83	0.00	0.13	0.10	1.68	0.00	0.00	0.39
		max	0.00	0.14	0.00	0.14	2.42	4.59	0.13	0.27	1.23	1.95	0.35	0.00	1.13
Spain: Xilxes (Castellon)	0	best	0.00	3.31	0.00	0.00	0.00	0.00	0.00	0.00	0.57	0.82	0.00	0.00	0.42
		min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		max	0.00	3.31	0.00	0.00	0.00	0.00	0.00	0.00	0.57	0.82	0.00	0.00	0.42

	3	best	0.00	3.31	0.00	0.00	0.00	0.37	0.00	0.46	0.57	2.18	0.00	0.00	0.61
		min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	1.22	0.00	0.00	0.11
		max	0.00	3.31	0.00	0.00	0.00	0.37	0.00	0.46	0.57	2.18	0.00	0.00	0.61
	14	best	0.00	5.76	0.00	0.00	6.32	4.27	0.00	0.76	2.02	5.13	0.00	2.04	2.29
		min	0.00	0.00	0.00	0.00	0.54	0.00	0.00	0.00	0.29	2.19	0.00	0.00	0.28
		max	0.00	5.76	0.00	0.00	6.32	4.27	0.00	0.76	2.02	5.13	0.00	2.04	2.29
Spain: Chiva (Valencia)	0	best	0.00	0.00	0.00	0.00	1.87	0.00	0.10	0.00	0.11	0.00	0.00	0.00	0.18
		min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		max	0.00	0.00	0.00	0.00	1.87	0.00	0.10	0.00	0.11	0.00	0.00	0.00	0.18
	3	best	0.00	0.00	0.06	0.00	2.17	0.00	0.10	0.05	0.56	1.10	0.00	0.00	0.34
		min	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		max	0.00	0.00	0.06	0.00	2.17	0.00	0.10	0.05	0.56	1.10	0.00	0.00	0.34
	14	best	0.00	0.00	0.17	0.69	4.00	0.20	0.41	0.47	1.40	2.06	0.49	0.00	0.82
		min	0.00	0.00	0.00	0.00	0.00	0.10	0.00	0.16	0.78	0.10	0.00	0.00	0.09
		max	0.00	0.00	0.17	0.69	4.00	0.20	0.41	0.47	1.40	2.06	0.49	0.00	0.82

9 **4. APPENDIX: Likelihood of an introduction, leading to an establishment, of *G.***
 10 ***citricarpa* to the EU citrus growing areas on citrus black spot infected citrus fruits**
 11

12 **Table 16. Citrus cooperative stockhouses and shipping centers in zones of citrus**
 13 **production in Spain (Cobos, 2008).**

Autonomous Community	Province	Municipality	Number of stockhouses/shipping centers
ANDALUCIA	ALMERIA	Roquetas	1
		Vera	1
		TOTAL	2
	CÁDIZ	Algeciras	1
		Chipiona	3
		Conil de la Frontera	1
		Jimena de la Frontera	3
		San Roque	1
		TOTAL	9
		GRANADA	Lecrín
Santa Fe	1		
TOTAL	2		
HUELVA	Campillo	1	
	Cartaya	1	
	Gibraleón	1	
	Lepe	3	
	Moguer	1	
	San Juan del Puerto	2	
	Villarrasa	1	
	TOTAL	10	
MÁLAGA	Alhaurín de la Torre	3	
	Benamargosa	1	
	Cartama	3	

		Casares	1
		Estepona	1
		Málaga	1
		Pizarra	3
		Vélez-Málaga	2
		TOTAL	15
	SEVILLA	Brenes	1
		Cantillana	1
		Mairena del Alcor	1
		Tocina	1
		Villaverde del Río	1
		Viso del Alcor	1
		TOTAL	6
CATALUÑA	TARRAGONA	Ulldecona	1
		TOTAL	1
MURCIA	-	Abanilla	1
		Abarán	8
		Alguazas	1
		Alhama	6
		Archena	4
		Beniel	17
		Blanca	13
		Bullas	1
		Calasparra	2
		Cartagena	3
		Ceutí	1
		Cieza	7
		Librilla	2
		Lorca	3
		Lorquí	1
		Mazarrón	1

		Molina de Segura	4
		Mula	2
		Murcia	70
		San Javier	1
		San Pedro del Pinatar	3
		Santomera	9
		Torre Pacheco	3
		Totana	2
		Ulea	3
		Villanueva del Rio Segura	1
		TOTAL	167
COMUNIDAD VALENCIANA	ALICANTE	Alicante	2
		Altea	1
		Bigastro	1
		Pilar de la Horadada	2
		San Juan	1
		TOTAL	7
	CASTELLÓN	Almassora	1
		Almenara	3
		Alqueries del Niño Perdido	1
		Betxí	5
		Burriana	4
		Castellón	1
		La Llosa	1
		Nules	1
		Xilxes	2
		Vall d'Uixó	1
		Vila-Real	2
		TOTAL	22

	VALENCIA	Albat dels Sorells	1
		Albuixech	1
		Alcacer	1
		Alqueria de la Comtesa	1
		Alzira	1
		Benifairó de la Vallidigna	4
		Canals	1
		Carlet	2
		Carcaixent	1
		Corbera	2
		Cullera	1
		Estubeny	1
		Faura	1
		La Pobla del Duc	1
		Miramar	1
		Museros	1
		Picanya	1
		Piles	1
		Puçol	1
		Puig	1
		Quartell	1
		Oliva	2
		Silla	1
		Tavernes de la Vallidigna	1
		Valencia	17
		Villanueva de Castelló	2

		TOTAL	49
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14 Table 17. Area of citrus plantations in the EU member states and regions from Eurostat
 15 (2008).

Country name	Region name	Area (ha) of citrus plantations in 2005
Cyprus		4,540
	Cyprus	4,540
Greece		52,450
	Peloponnisos	25,750
	Dytiki Ellada	9,470
	Ipeiros	7,100
	Kriti	5,590
	Notio Aigaio	1,130
	Attiki	1,050
	Ionia Nisia	890
	Stereia Ellada	630
	Voreio Aigaio	730
	Thessalia	80
	Kentriki Makedonia	30
France		3,800
	Corse	1,760
	Guyane	970
	Martinique	350
	Guadeloupe	340
	Reunion	330
	Provence-Alpes-Côte d'Azur	40
	Languedoc-Roussillon	20
Italy		121,940
	Sicilia	63,460
	Calabria	33,350
	Basilicata	10,400
	Puglia	7,270
	Sardegna	4,250
	Campania	2,510
	Lazio	640
	Liguria	60
Malta		80
	Malta	80
Portugal		19,910
	Algarve	13,100
	Alentejo	2,850
	Lisboa	1,190
	Norte	990
	Centro	970
	Região Autónoma dos Açores	740
	Região Autónoma da Madeira	70
Spain		283,130
	Comunidad Valenciana	173,900
	Andalucia	63,840
	Región de Murcia	34,320
	Cataluña	8,150

	Illes Balears	1,920
	Canarias	800
	Extremadura	110
	Galicia	40
	Castilla y León	30
	Castilla-la Mancha	10
	Principado de Asturias	10

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17 Table 18. Area of lemon plantations in the EU member states and regions.

Country name	Region name	Area (ha) of lemon plantations	Year	Source
Greece		7,470	2005	Greek Ministry of Rural Development and Food
	Corinthia	3,300		
	Achaea	1,456		
	Illia	700		
	Preveza	500		
	Piraeus	430		
	Aetolia-Acarmania	353		
	Argolis	140		
	Messenia	135		
	Laconia	130		
	Dodecanese	124		
	Chania	80		
	Arta	50		
Chios	42			
Thesprotia	30			
Spain		45,171	2005	Ministerio de Medio Ambiente y Medio Rural y Marino, Gobierno de España (ES)
	Región de Murcia	24,223		
	Comunidad Valenciana	11,958		
	Andalucía	8,388		
	Illes Balears	240		
	Canarias (ES)	230		
	Galicia	81		
	Cataluña	37		
	Cantabria	12		
	Pais Vasco	1		
	Castilla y León	1		
	Principado de Asturias	-		
	Extremadura	-		
France		23	2005	EUROSTAT
	Corse	17		
	Provence-Alpes-Côte d'Azur	5		
	Languedoc-Roussillon	-		

Italy		32,362	2001-04	ISTAT
	Sicilia	28,634		
	Calabria	1,503		
	Campania	1,354		
	Puglia	280		
	Sardegna	473		
	Others (Liguria, Lazio, Toscana)	62		
	Basilicata	55		
Cyprus		665	2005	EUROSTAT
	Cyprus			
Portugal		494	2005	EUROSTAT
	Algarve	207		
	Lisboa e Vale do Tejo (NUTS95)	197		
	Norte	53		
	Centro(PT) (NUTS95)	27		
	Alentejo (NUTS95)	11		
	Região Autónoma dos Açores (PT)	-		

**South African Citrus Black Spot Expert Working Group Position Document –
Comments on the European Food Safety Authority’s Opinion on CBS, New
Information and Implications for the Pest Risk Assessment.**

October 2009

Members of the South African Citrus Black Spot Expert Working Group

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Summary

Relevant scientific evidence was consolidated in 2000 into a Pest Risk Assessment for *Guignardia citricarpa* pertaining to fresh citrus fruit exports from South Africa to the European Union. Considerable additional relevant scientific data has subsequently been generated and been the subject of numerous subsequent bilateral exchanges between SA and the EU. Both SA and EU previously made use of Expert Working Groups (SA WG and EU WG) to provide scientific input. Notably the SA WG consisted of several plant pathologists with extensive field and laboratory research experience with CBS, whereas the EU WG did not in any instance involve a CBS expert. The SA WG has maintained that the fruit pathway does not constitute risk of *G. citricarpa* potentially establishing in the EU and that the current EU phytosanitary regulations are unduly restrictive. The EU WG has maintained that it does consider the fruit pathway to pose risk and that the current phytosanitary regulations are appropriate. In 2008 EFSA published an opinion on the data exchanged and the PRA and concluded that it was in agreement with the previous EU position and suggested intensified measures. EFSA criticised aspects of the data previously provided by SA and provided additional information.

Additional studies were recently undertaken in relation to aspects of EFSA's comments and data. The SA WG considered EFSA's opinion and the recent additional studies. The SA WG identified many serious problems with the EFSA opinion and information provided by EFSA. The SA WG concluded that no evidence has been presented by the EU (including the EFSA Opinion) which supports deviation from the conclusion of the original PRA. The SA WG considered, on evaluation of currently available information, that there is now considerably stronger support for the original conclusion of the PRA. The SA WG concluded that the current EU phytosanitary regulations pertaining to *G. citricarpa* on fresh citrus fruit imported into the EU are without adequate technical justification and are unnecessarily restrictive and disruptive to trade relevant to risk. In accordance with the IPPC principles of "technical justification" and "minimal impact", failure to rescind these phytosanitary regulations pertaining to *G. citricarpa* on fresh citrus fruit imports would constitute an unjustified technical barrier to trade, especially considering that 22 of the 27 Member States do not commercially produce citrus.

The SA WG proposed that whereas the current EU phytosanitary regulations pertaining to *G. citricarpa* in association with the fresh fruit pathway should be removed, a suitable quality standard for *G. citricarpa* infected fruit would be appropriate. The SA WG further recommended that should the EU decline to accept the PRA outcome, suitable international third party intervention be pursued.

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1. BACKGROUND

SA has exported fresh citrus fruit to Europe since 1908. With the exception of lemons, SA supplies the bulk of the citrus on the European summer market. Historically, the traditional European market for SA citrus fruit has been northern Europe. Prior to harmonisation of EU phytosanitary regulations, these citrus fruit exports were not subject to phytosanitary regulations pertaining to *G. citricarpa*. However, the maximum permissible level of *G. citricarpa* infected fruit entering the market was always regulated through appropriate quality standards which permitted up to three lesions per fruit. In 1992, during the drafting of the harmonised EU phytosanitary regulations, SA advised the EU that the proposed harmonised EU phytosanitary regulations, pertaining to CBS on fresh citrus fruit for export to all EU Member States, were unduly restrictive relative to the associated risk.

SA consolidated relevant available scientific data into a PRA and submitted it to the EU in 2000 in support of the SA request for relaxation of the relevant EU regulations. There were several subsequent exchanges of data between SA and the EU, including reports on additional research conducted by SA, in response to requests from the EU to clarify certain aspects of the PRA (Annexure 1). In 2007, SA provided the EU with additional information and indicated that the data exchanged should be sufficient to resolve the issue and called upon the EU to conclude or third party involvement to be pursued. In 2008, the EU subjected the data to internal review by the European Food Safety Authority (EFSA). EFSA concluded its assessment of the data in December 2008 and published its opinion in early 2009.

Prior to involvement of EFSA, the EU had used an European CBS Working Group to consider inputs from SA and to formulate EU technical positions on the matter. Likewise, the technical positions of SA have been established on the basis of inputs made by a SA CBS Working Group. The SA CBS Working Group was again convened on 19 October 2009 to assess the EFSA opinion, the additional information provided by EFSA and reports on recent additional studies. The SA CBS Working Group's position is recorded in this document.

2. COMMENTS ON THE EUROPEAN UNION FOOD SAFETY AUTHORITY OPINION ON CBS

For the sake of convenience, each specific comment is made with reference to the relevant numbering within the EFSA (2008) text.

P. 1, Panel Members; P. 9, Acknowledgements, First Section; and P. 8, Terms of Reference.

Whereas the list of panel members and invited experts is extensive, and the wide ranging expertise of the panel is recognised, it is noteworthy that the list does not include any names of pathologists with well recognised expertise that is specific to *G. citricarpa*. The list does not include the names of any citrus pathologist that has extensive familiarity with the organism under practical field conditions. The documents supplied to EFSA by the European Commission are all exchanges between South Africa and the European Union regarding the Pest Risk Assessment of CBS. In evaluating this information, the EFSA panel invited inputs from the Spanish plant pathologist who was instrumental (as a member of the EU CBS Working Group) in formulating an earlier EU position. Conversely, no well recognized CBS specialist, or member of the SA CBS Working Group, was invited to participate in the EFSA review process. Accordingly, the EFSA Opinion is viewed as an EU internal process and not as review by an independent third party, as was proposed by SA in 2007.

P. 7, last paragraph. EFSA acknowledged the SA position regarding the sequential hurdle nature of the PRA. However, when EFSA presents its opinion on the outcome of the PRA, this aspect receives little apparent attention and the EFSA opinion seems to be based on the assessment of each step in the PRA in isolation. This is an issue raised previously by the SA WG in criticism of the earlier approach taken by the EU WG, but EFSA has perpetuated the EU WG's failure to adequately consider this aspect. This is a serious deficiency that compromises the validity of EFSA opinion on the overall PRA conclusion.

P. 12, paragraph 2, second last sentence. EFSA quoted Whiteside (1967) as providing evidence that pycnidiospores were the most important inoculum source at the initial stages of the disease in "Rhodesia".

The following extracts from Whiteside (1967) are relevant to demonstrating that it is inappropriate to refer to this work as evidence of the importance of pycnidiospores: (a) Whiteside had a "**suspicion** pycnidia may have developed from dead twigs and old fruit stems", but this was not proven; (b) On p. 89, Whiteside stated that: "As an additional check and to ensure accuracy of identification, duplicate samples from some of the orchards were sent to Dr. McOnie who very kindly carried out confirmatory tests and compared samples from South African material. The results of this new survey showed that all the previous cases of latent infection detected in Rhodesian orchards were actually due to the non-virulent *Guignardia sp.* and not to *G. citricarpa*". The probable misidentification by Whiteside was also reported by McOnie (1964a).

Furthermore, given that lesions with which pycnidiospores are associated are visually obvious, it is understandable that Whiteside made erroneous assumptions about the role of pycnidiospores, at a time prior to both the development of a good understanding of the role of ascospores and the development of reliable ascospore trapping techniques. The invalidity of Whiteside's assumption was made evident in later years through research that resulted in a better understanding of the disease under field conditions (Kotzé, 1981). The SA WG previously pointed out to the EC WG that they had made the same erroneous misinterpretation of this situation, but this seems to have been disregarded by EFSA in its evaluation of the earlier exchanges of information.

P. 12, paragraph 2, last sentence. EFSA referred to studies conducted in Brazil to substantiate their argument that pycnidiospores are more important as an inoculum source than has been put forward by the SA WG. However, the quoted studies in Brazil, were conducted in areas where the climatic conditions are vastly different from those that occur anywhere in Europe (Annexure 2). Consequently, this information was quoted out of context and erroneously applied in the EFSA argument. As with the above point, the same error was previously made by the EC WG, the SA WG highlighted this in previous exchanges (Annexure 1), but EFSA seems to have disregarded this.

P. 12, paragraph 3, lines 5 to 7. EFSA referred to Lee & Huang (1973) to indicate that it is not only the winter drop of leaves that is important in the disease cycle, but also spring and summer leaf drop. This may well be so in a region where conditions that are suitable for ascospore maturation, spore release and infection, occur within an appropriate window of time after leaf drop, as is the case in Taiwan. However, later leaf drop will not lead to infection in regions where this does not coincide with suitable conditions. Field experience in South Africa has shown this to be the case in regions with hot dry summers due to inadequate rainfall sufficiently soon after leaf drop. One such region in South Africa is the Western Cape Province, where a Mediterranean type climate occurs with hot dry summers.

P. 12, paragraph 3, lines 14 & 15. EFSA quoted Kotzé (1963) as follows: "Environmental conditions required for ascospore germination varied from 15 to 29.5°C and 15 to 38 hours of wetness". However, the reported work was conducted on the organism in culture in petri dishes under laboratory conditions (p.32 in J.M Kotzé's thesis) and not under field conditions. This was therefore not a reliable reference to requisite field conditions.

P. 12, paragraph 4, lines 2 to 4. EFSA referred to "splash dispersal" and wash-off and quoted five references. However, the occurrence of "splash dispersal" as opposed to wash-off was not

established in the work reported in any of these publications. Furthermore, we are not aware of any work that reliably establishes that “splash dispersal” of *Guignardia citricarpa* pycnidiospores does occur, whereas evidence of wash-off is apparent.

P. 12, paragraph 4, lines 5 to 7. EFSA contended that a pycnidium can continue producing viable pycnidiospores for several months. However, the studies that EFSA quoted in support of this statement did not provide evidence for the duration of spore production from individual fruiting bodies and none reported on the viability of pycnidiospores under field conditions.

P. 12, paragraph 6, first sentence. EFSA stated that CBS has significant impact due to the external blemishes that make fruit unsuitable for the fresh market. This is not accurate since individual blemishes are small and relatively inconspicuous. It is true that heavy infestation resulting in multiple lesions on a fruit will render the fruit unsuitable for the fresh market. However, severity of infection is dependent on suitability of the climate for an epidemic. Apart from total absence of CBS from parts of South Africa where the climate is unsuitable for establishment of the organism, there are also regions where the climate is only marginally suitable for CBS. In such marginally suitable regions, even in the absence of control measures, infection levels are sufficiently low to have no material impact on the value of the fruit in the fresh market, from a cosmetic perspective.

P. 14 to 25, Section 2.1. EFSA criticised SA’s approach to climate using the CLIMEX model. SA previously only provided the EU with reports on the “compare locations” CLIMEX work because, as articulated in Annexure 3, that is the component of the work that is relevant to the issue at hand and the “match climates” component would not add any value. The EFSA comments on the “match climates” aspect are irrelevant. Excluding this aspect, it is acknowledged that certain aspects of the EFSA criticism are valid. Accordingly, a revision of the CLIMEX modelling work was undertaken and the report is provided as Annexure 3. The SA WG endorses this work (Annexure 3) and considers all the concerns raised by EFSA, to be adequately addressed through the combination of the work reported on Annexure 3 and this document.

In its evaluation of SA CLIMEX work, EFSA (2008) presented some illustrative graphic comparisons between aspects of the climate in selected sites in SA and Europe. This has been further investigated as reported in Annexure 4 and the SA WG also endorses this work.

In addition to Annexures 3 & 4, the following comments relate to Section 2.1 of EFSA (2008):

EFSA conducted some illustrative comparisons between aspects of the climate in five selected sites where citrus is grown. EFSA selected one site in SA where CBS is abundant and one where CBS occurs, but is far less abundant (near marginal) and three sites in the European Mediterranean region. The critical rainfall period on the adjusted time scale provided by EFSA (2008) are months 4 to 8 (Northern hemisphere scale). The graphical illustration of rainfall as in EFSA (2008) clearly reflects the difference between the highly suitable SA site (with abundant summer rainfall) and the less suitable site (with a bimodal, but low volume, predominantly summer rainfall pattern) in terms of timing and abundance of rain. The European sites, in stark contrast with the SA suitable site, show the opposite pattern.

EFSA (2008) also presented an illustrative graphical comparison of minimum and maximum temperatures for these five sites. Here the critical periods are the months 1 to 4 (pre-spore release maturation period) and 4 to 8 (infection period). It is the maximum temperatures that most clearly show the critical differences between the SA and European sites. In the maturation period, all three European sites have markedly lower maximum temperatures than the SA sites. In the infection period, it is only over the last two months that two of the three European sites reach the same maximum temperatures as the SA sites. These differences are critical to the suitability of the sites for the disease. It is therefore inappropriate for EFSA to have concluded that there is insufficient difference between these climates to prevent establishment.

The relevance of differences in maximum temperatures, in these critical periods, is further discussed in the reports on the revised CLIMEX modelling (Annexure 3) and the comprehensive temperature and rainfall comparison including the analysis of field spore trapping data (Annexure 4). It would have been valuable if EFSA had included a “negative control” (such as a site from the Western Cape Province where the organism has failed to establish) in their comparative analysis. This has been incorporated in the analysis conducted by Fourie *et al.* (2009) (Annexure 4). Inclusion of such sites in the comparison clearly illustrates that the differences between sites in SA where *G. citricarpa* occurs and European sites are greater than the difference between SA sites where *G. citricarpa* occurs and SA sites that are unsuitable for establishment.

EFSA further applied a host plant phenological time scale to the data. EFSA referred to fruit set as a measurement point and it seems that some point during bloom was considered to represent the start of fruit set. It would have been preferable to use a more specific measurement in this regard, such as 100% petal fall. EFSA plotted the occurrence of bloom

in SA against the climatic data for the month of November. This is incorrect. Petal fall in SA generally occurs around the third week of September (in the northern regions) and mid-October (in the southern regions). EFSA plotted bloom in the European Mediterranean region against climate data for May. This also seems to be somewhat late, with Full Bloom of Clementines in Spain generally being in mid-April, and is more directly comparable with the southern parts of SA (Western Cape Province). In contradiction to their choice of phenological scale, EFSA (2008) indicated on p. 78 (third bullet point) that “Under EU conditions, the main citrus flowering period is from early April to early May”. The phenological time scale used by EFSA should therefore be shifted approximately one month to six weeks earlier.

The effect of such an adjustment to the phenological scale would be to shift the maturation period (prior to spore release) earlier, into the period where there is greater separation between the climates in SA and Europe, especially in maximum temperatures. EFSA recognized the potential for these differences in temperature to delay maturation in Europe. If an inoculum source were able to survive that long (which is unlikely), this would potentially shift the window for spore release (and infection) later, into the period centred around months 6, 7 & 8. This period coincides with the peak of the dry period in the chosen European sites and was shown by EFSA’s own studies to be unsuitable for infection (EFSA, 2008, P. 44, first paragraph).

In light of the above, and the additional work presented in Annexures 3 & 4, the EFSA interpretation of the illustrative differences in critical aspects of the climate as presented in EFSA (2008) is considered erroneous. Both the data presented by EFSA (when considered in terms of appropriate critical aspects) and Annexures 3, 4 & 5, provide additional support for SA’s original position on the unsuitability of the climate in European citrus growing regions for the establishment of *G. citricarpa*.

P. 25 to 34, Section 2.2. This section is addressed by the revision of CLIMEX as reported in Annexure 3. In addition to Yonow & Hattingh (2009) the following specific point is noted:

P. 34, first paragraph, lines 7 to 9: The SA WG does not agree with this statement made by EFSA. It is widely recognized by plant pathologists with practical field experience with *G. citricarpa* that the occurrence and severity of *G. citricarpa* is sensitive to the timing of leaf drop, host susceptibility and the occurrence of suitable climatic conditions. The SA WG contends that a delay in maturation, combined with coincidence with unsuitable climatic

conditions (as discussed above) has a material impact on the suitability of the area for potential establishment of the organism.

P. 34 to 61, Section 2.3.

P. 34, Section 2.3. The use of an *infection* model to model *climate suitability* (for establishment) is conceptually flawed as it addresses only one aspect the life cycle of an organism. This is especially pertinent in the case of *G. citricarpa*, which is influenced by climatic conditions throughout its life cycle, most importantly pseudothecium maturation prior to ascospore dispersal.

P. 35, Section 2.3.2. Parameter values used in this *infection* model are based largely on *in vitro* germination studies, and in defence of this weakness, EFSA states “Spore germination is required for infection, thus environmental conditions that are not conducive for spore germination are also limiting for infection” but concede “the value of T_{opt} for spore germination and infection do not necessarily coincide”.

P. 36, Section 2.3.2, paragraph 5. The 1st percentile for daily T_{min} during 2328 monitored ascospore dispersal events was 15.1°C, which shows that in less than 1% of occasions ascospores would be present at the parameter values of 15°C and 12°C that EFSA used for T_{min} (Annexure 4). This further demonstrates the weakness of using an infection model to model climate suitability.

P. 38, Section 2.3.2, paragraph 1. The study by Reis *et al.* (2006) cannot be used as a reliable indication for estimation of leaf wetness parameter values. Firstly, this study reported weekly averages of daily leaf wetness duration values (*i.e.* crude estimation), and secondly, found leaf wetness to be poorly correlated with ascospore dispersal and disease severity ($R^2 < 0.24$). Moreover, this study is quoted out of context by EFSA when setting a D_{50} parameter of 14 hours and the relevance of such a protracted period is dubious.

P. 38, Section 2.3.2, paragraph 2. EFSA disregarded rainfall as would be required for onset of ascospore dispersal in the ascospore model.

P. 38, Section 2.3.3.2. EFSA failed to include true negative controls, *i.e.* recognised pest-free areas in countries where the disease has become established, for example Western Cape Province of South Africa or New South Wales in Australia.

P. 38, Section 2.3.3.2, paragraph 1, lines 9 to 13. EFSA acknowledged on P.12, par.1 of the Appendix that “One of the most important inputs required by the *LeafWetness* component, in

particular by the models SWEB, CART and ET ..., is **hourly** air relative humidity, because it is directly related to the presence of water on the leaf blade". However, **daily** relative humidity values had to be *estimated* from **monthly** averages for all EU sites. Moreover, **hourly** values for all weather parameters had to be *estimated* by using CLIMA. In CLIMA, certain key **hourly** parameters, such as dew temperature, were also *estimated* from **monthly** weather parameters using regression analyses of 12 and 21 observations only (Appendix 2.2.2.). CLIMA was proposed in published conference proceedings and the SA-WG questions whether this has been peer reviewed. Nonetheless, this approach casts doubt over the reliability of these estimations, especially when used for modelling in the PRA context.

The SWEB model was used to *estimate* leaf wetness, which is a critical component in the generic infection model used (as stated in EFSA Appendix 2.3.1.), from *estimated / generated* hourly values (see above). When comparing SWEB-simulated to observed leaf wetness data (only 5 datasets), the probability of correctly simulating a wet event (POD) ranged from 0.49 to 0.75, the probability of correctly simulating a null event (PNE) ranged from 0.69 to 0.86, and the bias (under- or over-estimation ratio) ranged from 0.59 to 1.23 (Appendix P.19). Spatially, the SWEB simulations generally appeared to be stable (R^2 values at 6 sites ranging from 0.69 to 0.88), although 2 of the 8 sites were poorly simulated (R^2 values of 0.44 and 0.48). Temporal variability showed fair accuracy during the months of April, May and June (R^2 values of 0.78, 0.67 and 0.66, respectively), but progressively less accurate simulations for July to October (R^2 values declined from 0.57 to 0.12). A model with such a low R^2 value cannot be considered reliable, despite EFSA having defended this poor accuracy by referring to the mean absolute error relative to the higher leaf wetness hours during these months. EFSA furthermore contended "that SWEB outputs are able to describe in a good way the **trend** LWD during this important epidemiological period for CBS infections" (EFSA Appendix P.20). These factors collectively render such analyses completely unsuitable as a basis for drawing the concrete risk conclusions that EFSA has attached to them.

P. 40 to 42, Section 2.3.3.3. The results are of little value in risk assessment as true negative sites were not included (see above). Moreover, the broad presentation of results as *number of potential infection events per year* should be viewed in context of a complete CBS disease cycle, which includes critical aspects such as inoculum availability, maturation and dispersal and host phenology, and most importantly the concurrence of inoculum availability, dispersal and susceptible host material with climate suitability.

P. 42, Section 2.3.4. This study was conducted only on European sites, and included no true positive or negative control sites and therefore relied on the previous “preliminary tests” (2.3.3.) as validation.

P. 43, Section 2.3.4.2. The reliability of this approach is questionable, as hourly parameters, including SWEB-simulated leaf wetness data, used in this model were *estimated* (see above).

P. 43 to 44, Section 2.3.4.3. EFSA demonstrated that “a limited number or no potential infection events were found” in June, July and August (*i.e.* European summer). A limited number of sites, April and May (*i.e.* European mid- to late-spring) were reported to have <10% hours suitable for infection by pycnidiospores. September and to a lesser extent October (*i.e.* European autumn), was regarded as “the most favourable month for pycnidiospores infection” with <10% hours suitable for infection at more sites than in other months. However, it should be noted that the accuracy of SWEB leaf wetness estimations (a critical parameter in the infection model used) during these months were 33% and 12%, and therefore the reliability of these model predictions is doubtful, especially in terms of “percentages of hours with potentially successful infection”.

For ascospore infection, EFSA found that June to August (EU summer) was unsuitable for infection, as was April (too cold) and May (too dry). September and October (EU autumn) were the most favourable months, although leaf wetness and/or temperature were limiting factors. For the same reasons mentioned previously, the reliability of these model predictions during these months is doubtful.

From all scientific accounts from countries where CBS has established, infections, most importantly by ascospores, occur during late-spring and summer. Thus, the EFSA results reported here, showing that conditions for ascospore are potentially favourable for infection in autumn only, support the SA-WG opinion in 2002 that “the organism would have to behave as it never has before in any part of the world for the disease to become established in a Mediterranean climate”. It should furthermore be noted that the majority of sites in the EU were shown by EFSA’s own analyses to be unsuitable for infection (<1% hours suitable for infection).

P. 50, Section 2.3.5. This study was also conducted on European sites only, and includes no true positive or negative control sites. Results can therefore only be regarded as speculative.

P. 52 to 60, Section 2.3.5.3. EFSA again demonstrated the unlikelihood of CBS infection during spring and summer, and never more than 5% and 10% hours with potential successful

pycnidiospore and ascospore infection, respectively. By using a D_{50} of 14h, which has absolutely no scientific basis, EFSA attempts to show higher percentages of time suitable for infection.

From results of the sensitivity analysis, EFSA conceded that the risk of infection by pycnidiospores is clearly low during July to August (EU summer), and that simulations for other months were “more uncertain” given the large differences observed following the sensitivity analysis. Likewise, for ascospore infection, EFSA concluded that “the simulation of ascospore infection is highly uncertain”. D_{50} and T_{min} were shown to be the two main sources of uncertainty. Both of these values were selected by EFSA in the absence of suitable supportive evidence and both relate to estimated climatic values, further compounding the unreliability of the model outputs’ use in risk assessment.

P. 60, Section 2.3.6. EFSA concluded from the studies conducted in this section that “Although the uncertainty is high, climatic conditions favourable for pycnidiospore infection occur in many of the locations where citrus is grown in the EU particularly in September and October”, and noted that, for CBS establishment following entry via the fruit pathway, “climatic conditions at any location therefore only need to remain suitable for the time required to complete one infection”. The SA WG previously advised the EC WG that it is erroneous to equate infection with establishment and persistence, but EFSA has repeated this error. EFSA has disregarded the complexity of the *G. citricarpa* life cycle, as a single infection event by no means constitutes *establishment*.

P. 61, Section 2.3.6, paragraphs 2 & 3. EFSA correctly stated that following an initial pycnidiospore infection event “for successful establishment in a given area, the prevalent climate should be suitable for ascospore infection”. However, the studies in this section showed that conditions were only marginally suitable for infection in spring and autumn, and not in summer, which severely limits the chances for synchrony between host susceptibility, available inoculum and suitable climate, as required for successful ascospore infections.

P. 61, Section 2.3.6, paragraph 4. Cold-stress has been shown to limit the chances for potential establishment (Paul *et al.*, 2005; Annexure 3). EFSA attempted to disregard these studies by quoting Agostini *et al.* (2006) and Peres *et al.* (2007). However, these studies were conducted on cold-stored fruit and *in vitro* culture preservation, respectively, and were quoted out of context.

EFSA furthermore maintained that “minimum winter temperatures in EU citrus growing locations, such as Valencia, Messina and Porto, were similar to those at Addo in Eastern Cape Province in an area where CBS has been reported”, but fail to mention the remarkable difference

in maximum and mean winter temperatures, which are markedly lower in the EU locations (EFSA report Fig. 3-4, P.19-20; also demonstrated in Annexure 4).

EFSA correctly noted that “the maturation of pseudothecia and ascospores in the leaf litter may be delayed by cool winter temperatures potentially causing asynchrony between the pathogen and the host”. Leaves fall throughout the year, but mostly during the spring flush (Erickson and Brannaman, 1950). Thus, the bulk of fallen leaves will have been exposed to the dry and hot conditions of the Mediterranean summer and it is doubtful whether these leaves would still sustain pseudothecium maturation and ascospore dispersal in the autumn months of September and October. Therefore, considering EFSA’s own analyses, during the marginally suitable climatic conditions in September and October (in limited locations and not consecutively suitable over years), the availability of inoculum and suitably susceptible host tissue will be limited, thereby decreasing the chances of successful ascospore infection. The SA-WG finds EFSA’s conclusion that “the cooler maximum temperatures in the EU citrus growing areas are unlikely to prevent establishment of *G. citricarpa*” incongruous with the results presented in the EFSA report and the arguments stated here.

P. 62, Section 2.4.

The SA-WG believes that in consideration of the arguments noted above, as well as the inherent weaknesses and uncertainties in the EFSA modelling approach, EFSA overstated the significance of their findings in this general conclusion, especially when stating “the Panel cannot agree with the conclusion by Paul *et al.* (2005) that the climate of the EU is unsuitable for the establishment of *G. citricarpa*”. This conclusion, based on the results provided, raises concern about the impartial objectivity of the EFSA opinion and this concern is strengthened by consideration of the composition of the scientific panel consulted by EFSA in formulating its opinion. At the very least, EFSA should concede that, although they might not agree with the conclusion reached by Paul *et al.* (2005), their own data analysis indicated that establishment of *G. citricarpa* in the EU, and more specifically these few localities, would be unlikely.

On the strength of the information provided previously (through bilateral exchanges between SA and the EU), the new work reported (Annexures 3, 4 & 5) and the absence of compelling contrary information, the SA WG is adamant that the climate in European citrus growing regions precludes potential establishment of *G. citricarpa*.

P. 63 to 84, Section 3.

P. 63, Section 3.1.1. The SA WG acknowledges that the fruit pathway and the relevant regulations also relate to passenger traffic.

P. 63, Section 3.1.2: Probability of pest being associated with fruit at origin.

Prevalence of pest in source area.

P. 63, bullet point 1. EFSA stated: “CBS has already established in areas with various environmental conditions such as cool, misty, dry, hot, semi-arid, sub-tropical, etc (Wager, 1952). The disease has indeed established in South Africa in areas with various environmental conditions, but all these have summer rainfall. Truter *et al.* (2007): “Although the disease has spread to most of the summer rainfall areas in South Africa since its first reported occurrence in 1929 (Doidge, 1929), it has not been able to establish in predominantly winter rainfall areas”.

EFSA (2008) quoted: “Too much hope has been placed in the past on climate as a limiting factor (Kotzé, 1981)”. This statement was made by the author with reference to the development of the CBS disease in epidemic proportions within summer rainfall areas, not to the establishment of the disease or the spread of the disease to winter rainfall regions. It is noteworthy that, in stark contrast with the context in which EFSA quotes Kotzé, the author himself (being widely recognized as the leading international authority on CBS) has repeatedly indicated that climate is indeed a significant barrier to distribution of the organism. The EC WG was previously supplied with this information through the preceding bilateral data exchanges. Kotzé emphatically affirms his position regarding the unsuitability of winter rainfall climates in Annexure 2.

P. 63, bullet point 4. EFSA did not recognise the extensive variation in prevalence of CBS across citrus production areas in SA. CBS prevalence in SA varies from abundant in more climatically suitable regions, through areas of low abundance, to areas of officially recognised low pest prevalence and pest free areas (South Africa, 1983). EFSA offered the unavailability of publications on the outcome of surveys, as justification for not having commented on the absence of CBS and CBS Pest Free Area status of the Western Cape. EFSA proceeded to omit recognition of such areas in parts of its subsequent analysis, where such information would be of particular relevance, despite the existence of other publications referring to the failure of CBS to establish in these areas (Wager, 1952; McOnie, 1964a, 1964b & 1965a; Kotzé, 1981, Truter *et al.*, 2007). The CBS pest free status of the Western Cape Province was established through official surveys, has been recognized by the EU and is utilized in the ongoing regulation of citrus exports from SA to the EU in accordance with Directive 2000/29/EC. EFSA’s omission of this information from relevant aspects of this assessment is therefore incongruous with what is apparent and materially compromises the reliability of EFSA’s assessment.

Additional official surveys have recently been undertaken by the South African Department of Agriculture, Forestry and Fisheries in the Northern Cape, Free State and Western Cape Provinces, resulting in (a) confirmation of the CBS pest free status of the Western Cape Province; (b) establishment of the pest free status of the Northern Cape Province; (c) establishment of the CBS pest free status of parts of the Free State Province and parts of the North West Province; and (d) the establishment of Pest Free Places of Production (within an Area of Low Pest Prevalence) in northern parts of the Limpopo Province.

P. 63, last paragraph. EFSA's statement that "the pest is widespread within the citrus-producing areas of South Africa with the *possible* exception of the Western Cape Province" shows disregard for the reality, credibility of data provided in support of bilateral trade agreements (European Union, 1998 & 2006) and previous scientific reports (Wager, 1952; McOnie, 1964a, 1964b & 1965a; Kotzé, 1981; Truter *et al.*, 2007).

Pest management, cultural and commercial procedures at the place of origin

P. 66, "The Panel notes that:", first bullet point. Differentiation in the context of field control is not particularly relevant to risk management since mistaking *G. mangiferae* spores for *G. citricarpa* spores will only result in additional control, not less.

Second bullet point. EFSA quoted Agostini *et al.* (2006) as support for the assertion that pre-harvest sprays were ineffective in eliminating quiescent infections in fruit. Firstly this quotation of Agostini is out of context. Agostini *et al.* (2006) stated: "The preventative field fungicide program also consistently reduced the percentage of isolation of *G. citricarpa* from affected fruit, whereas storage temperature and packinghouse fungicide treatment gave variable results" and "The most effective means to reduce postharvest development of symptoms is through preventative application of fungicides during the fruit growing season and storage of harvested fruit at cold temperatures". Pre-harvest disease management strategies have been developed over many years in SA and other parts of the world where the CBS occurs (for example Kellerman & Kotzé, 1977). These techniques are highly effective in controlling the disease if diligently applied. Likewise, practical experience has shown that packhouse grading can be effective in removing symptomatic fruit.

P. 67, Section 3.1.2: Conclusion on the probability of the pest being associated with fruit at origin.

It is apparent that the wording used by EFSA tends to place emphasis on aspects that indicate higher risk, as is reflected in concentrating on failure of the various individual measures to provide complete exclusion of the organism. It would be more informative in evaluating the

various mitigation steps, to consider both their limitations and the extent to which they can contribute to reducing risk. EFSA (2008) seems to have selectively listed considerations that can be expounded upon in a way that reflects greater risk, whereas other considerations (being the subject of earlier SA – EU technical exchanges), that support a lower risk assessment, have largely been omitted from EFSA’s discussion.

EFSA disregarded the wide variation in prevalence of the organism across different production regions. EFSA stated that large volumes of citrus fruit from South Africa enter the PRA area annually, “by which time they have already been exposed to the pathogen in the South African orchards”. This is clearly an inaccurate generalisation and an over estimation of the probability of association. EFSA stated that “infected citrus fruit from South Africa continues to be intercepted ...”. This is indeed so, but relative to the volume of fruit that is annually exported from SA to EU, the intercepted volume is an exceedingly low proportion.

EFSA’s conclusion that “the probability of the organism being associated with the citrus fruit in South Africa is high” did not take into consideration the variation in CBS-prevalence between climatically different citrus producing areas in South Africa, nor that extensive chemical control is practiced in areas where CBS occurs and that >95% clean fruit can be obtained (Schutte *et al.*, 1997, 2003). These papers were cited by EFSA, but ignored in this argument when they state “No data was provided on the efficacy of pre-harvest sprays”. EFSA rather opted to quote an Argentinean study by Agostini *et al.* (2006) that “pre-harvest sprays were ineffective in eliminating quiescent infections on fruit” (P.66), but only partly, as Agostini *et al.* (2006) concluded “Although field fungicide applications reduced incidence to low levels, they did not totally eliminate quiescent infections even when incidence was very low at harvest”. In fact, in this study, CBS incidence was reduced from 76.8, 79.6 and 69.5% to 6.8, 7.1 and 5.5% in the Murcott, Valencia and lemon experiments, respectively. Agostini *et al.* (2006) fail to specify spray application technique, but Argentinean spray application is generally poorer than that practiced in South Africa (Dr GC Schutte, *pers. comm.*) where spray volumes of up to 12,000 L/ha are used to apply CBS preventative sprays (Grout, 1997, 2003).

The SA WG does not agree with EFSA’s conclusion for this section. Given the above considerations and those previously articulated in the preceding exchanges between SA and the EU, it is apparent that it would be technically appropriate for the conclusion at this point in the PRA to be that the likelihood of association is overall low.

P. 68 to 72, Section 3.1.3: Probability of survival during transport or storage

Vulnerability of the life-stages during transport and storage.

P. 69, first paragraph. EFSA referred to Wager (1949) who reported that pycnidiospores taken from a slowly mummified and dried up CBS-infected orange fruit survived for up to four or, in some cases, five months. It must be noted that this observation by Wager was done on one fruit under laboratory conditions. Conditions in orchards are not similar. The SA WG previously advised the EU WG that the quoted laboratory observations of Wager (1949) should not be extrapolated to field conditions, but EFSA (2008) repeated the EU WG argument without recognition of this caution.

P. 69, first bullet point. The SA WG previously provided references in support of the statement that pycnidiospores are short lived. The SA WG is of the opinion that this remains the current status of scientific opinion on the matter.

P. 69, second and last bullet points. Reference to isolation of the organism's mycelium from infected fruit cannot be equated with infectivity of pycnidiospores. In the work quoted by EFSA, namely Agostini *et al.* (2006), the identity of the organism was also not verified.

P. 69. Prevalence of pest likely to be associated with a consignment. The meaning of Figure 40 is unclear. It is acknowledged that inspection on arrival is conducted on a sample basis and the statistics would have been more meaningful if they had reflected a proportion of the consignments inspected. EFSA's conclusion that "the prevalence of the pathogen in citrus fruit consignments imported from South Africa into the PRA area is not known" ignores their reference to Eurostat (2008), where it states that <1.12% (mean of 0.23% for 2005-2008; 12.22% in Slovenia during 2005 excluded) of the total volume of citrus exported were intercepted due to presence of *G. citricarpa*. The volume intercepted, as a proportion of the total volume that entered the EU, is therefore shown to be extremely small. Given the regulation that a consignment will be rejected based on the interception of one lesion on one fruit within a consignment, the actual prevalence of the pathogen on a 'per fruit' basis would be dramatically lower.

P. 72. Commercial procedures applied to the consignments in country of origin, destination, transport or storage. The EFSA opinion should be qualified to relate only to infected fruit.

P. 72. Conclusion on the probability of survival during transport or storage. The EFSA opinion should have been qualified to relate only to infected fruit. Whereas SA has never claimed that handling procedures will entirely preclude infected fruit, various commercial handling procedures do indeed reduce the likelihood of persistence of viable inoculum on infected fruit.

The practices involved and the nature of their effect have been the subject of prior submissions by SA.

P. 72 & 73, Section 3.1.4, Probability of surviving existing pest management procedures.

P.73, first bullet point. Whereas packhouse fruit sorting is unlikely to eliminate all infected fruit, it is indeed an effective means of reducing the occurrence of infected fruit.

P. 73, last paragraph. The SA WG maintains that given the inaccuracies of assumptions made by EFSA regarding the efficacy of existing pest management procedures, including disregard for the proven efficacy of intensive pre-harvest chemical control (Schutte *et al.*, 1997, 2003), it cannot agree with EFSA's conclusion. The SA WG remains of the opinion that the probability of surviving is moderate to low, as is reflected in the low incidence of interception, when considered as a proportion of the total volume exported and considering that an entire consignment is rejected when a single lesion is found on a single fruit from the consignment.

P. 73 to 79. Probability of transfer to a suitable host.

P. 73, Dispersal mechanisms.

P. 73, Section 3.1.5, second last bullet point. EFSA referred to the role of sprinkler irrigation in the spread of “various fungal and bacterial diseases” and states that it “is equivalent to rain”. The continued use in Europe of the outdated and inefficient practice of sprinkler irrigation on citrus is questioned. It is not appropriate to assume that generalizing about the role of sprinkler irrigation for various diseases is at all applicable to *G. citricarpa*. If sprinkler irrigation were to be used, it might play a similar role in dispersal of conidia from pycnidia, but cannot be considered equivalent to rain. Various climatic conditions associated with rain events that favour disease infection (such as high RH, which is conducive to longer periods of leaf wetness) will not necessarily be associated with sprinkler irrigation.

P. 73, Section 3.1.5, paragraph 8. In CBS epidemiology, the “role of insects in pycnidiospores spread” has never been mentioned in any scientific study or review. The relevance of this aspect is doubtful, as its contribution to the CBS epidemic would be minuscule in comparison with general ascospore and even pycnidiospore dispersal. *G. citricarpa* is furthermore not a wound pathogen, and conidia deposited in/on wounds would be unlikely to recognise the host surface, germinate, colonise or establish a latent infection (also refer to P.74, par.7).

P. 74, third last bullet point. EFSA stated that “The greatest number of diseased fruit observed in the lower part of the citrus canopy (Schinor *et al.*, 2002; Spósito *et al.*, 2008) is indirect evidence that splash-dispersed pycnidiospores have an important role in increasing the disease

severity within the canopy". There is an alternative and more likely explanation for the distribution of infected fruit within the tree canopy than the one cited by EFSA. Run off from infected fruit can be expected to result in a higher concentration of infection on the lower strata of the canopy, as can also be more likely be expected from closer proximity to the source of ascospores (being leaf litter). There is no compelling scientific evidence to support the hypothesis that splash dispersal is a common reality. EFSA did not refer to the following publications which reported results that were contrary to the work quoted by EFSA: McOnie (1964b) p. 65 did a trial under the heading "Vertical distribution of infections", as well as Kiely (1948) in Australia. They both found that lower fruits and upper fruits were equally infected with pycnidiospores. Mature pycnidiospores are extruded in a gelatinous mass and because of their stickiness, rely on running water for dissemination. Splash dispersal of pycnidiospores is according to McOnie (1965b) not possible.

P. 74, second last bullet point. EFSA's reference to Whiteside (1967) is misleading as was highlighted above (SA WG comment on "EFSA, P. 12, paragraph 2, second last sentence").

P. 74 (last sentence) & P. 75, first four bullet points. The speculative assumptions made about the relevance of splash dispersal of other fungi are of little relevance for *G. citricarpa*.

P. 75, Section 3.1.5, last paragraph. Whereas EFSA stated that rainfall (as a requirement for dispersal) occurs in Europe over the period April to November (overlapping with import of SA citrus fruit), this is misleading because the period in fact spans the dry season in the European Mediterranean (winter rainfall) region (EFSA, 2008, p. 18; Fourie *et al.*, 2009).

P. 76, first bullet point. The relevance of reference to the archaic practice of overhead irrigation in modern day citriculture is questionable. Furthermore, EFSA itself indicated that the types of irrigation used are "drip- and mini-sprinkler" (P. 82, fourth last bullet point).

P.76, first paragraph (after third bullet point). It is apparent that the arguments presented by the EU WG and EFSA to support their contention that probable dispersal mechanisms do exist, are highly speculative. However, despite this obvious weakness, EFSA declines to acknowledge that the likelihood is clearly extremely low.

P. 76, destinations within the PRA area. EFSA proposed that the distribution of fruit within the EU can be estimated to be proportional to population distribution. This is not appropriate. The traditional markets for SA citrus fruit are the more northern EU MS and more recently there has been strong development of the market in eastern MS. The majority of SA citrus enters the EU through northern MS ports for sale in northern MS. Of the balance of fruit that does enter the

EU through southern MS, a large proportion follows this route because of the economic benefits offered by the existence of infrastructure that exists in these MS for the movement of locally produced fruit to the rest of the EU. Consequently, much of the fruit that enters through southern MS is also distributed to the traditional northern MS markets.

P. 77, proximity to suitable hosts. Citrus is cultivated in the southern EU MS, principally in coastal regions, whereas the bulk of SA's EU exports go to northern MS. Hence the bulk of the fruit will not be anywhere near suitable hosts in the EU. It is acknowledged that fruit which is placed on the market in the southern MS will overlap with regions in which citrus is produced in the EU. However, as the SA WG has previously highlighted, the probability of somebody placing such an imported fruit in sufficiently close proximity (in direct contact or at most a few centimeters away) to a suitable host, must realistically be considered very low indeed.

P. 78, Section 3.1.5, paragraph 4. EFSA concluded that "the period of imports of South African citrus fruit into the PRA area coincides with the susceptible period for infection of citrus trees grown in the PRA area", but refrained from qualifying this statement by stating the general unsuitability of conditions to successful dispersal and infection (EFSA, Section 2.3) during this period, especially during June, July, August and November.

P. 78, Risk from by-products and waste. EFSA stated that "the risk with citrus fruit is mainly associated with the waste ...".

Second bullet point. EFSA quoted Agostini *et al.* (2006) as justification for the statement that "Symptomatic citrus fruit/peel can be a source of viable pycnidiospore inoculum for at least 40 days ...". It is inappropriate to extrapolate these laboratory observations to field conditions, especially to say "for at least 40 days" – this is simply without any scientific justification. It is inappropriate to assume that the production of spores from lesions on peel removed from fruit will be as prolonged as with an intact fruit. The Agostini *et al.* (2006) quote was further out of context in that, whereas on page 1423 par. 2, Agostini *et al.* stated that: "*G. citricarpa* could be inoculated from affected fruit or peel at least through 40 days" this referred to isolations made from mycelium, not pycnidiospores. Although it is acknowledged that this cannot be deemed to be an authoritative reference, Korf H.J.G. (unpublished) found that pycnidiospore production on peel exposed to sunlight was limited to a maximum of 4 hours.

Third bullet point. Fruit or peel discarded on the ground under natural field conditions, rapidly decays, it does not slowly mummify and dry up, as was the case in the observations reported by Wager (1949). EFSA's quote of Wager (1949) was therefore out of appropriate context.

Fourth bullet point. The work referred to relates to fruit on the tree (still attached). These quotes by EFSA were out of context with regard to by-products and waste. EFSA further

misquoted McOnie (1967) since he reported on the germination of ascospores, not pycnidiospores (page 743 under Materials and Methods).

P. 79, first paragraph. It is apparent that whereas it is conceivable that waste could be a source of contamination, the likelihood of such waste being placed in sufficiently close contact with susceptible host (under circumstances that are conducive to the possibility of infection) is inconceivable as anything other than an extremely remote possibility.

P. 79, Conclusion on the probability of transfer to a suitable host. EFSA did not present any evidence (that is evenly remotely near to being compelling) to indicate that the likelihood of such an occurrence can be considered to be more likely than an extremely remote possibility.

P. 79, section 3.1.6. Conclusion on the probability of entry. EFSA concluded that *G. citricarpa* may be transferred to suitable hosts, but failed to qualify this statement by indicating that the likelihood of successful dispersal and infection would be extremely remote. The SA WG cannot agree with the way in which EFSA portrayed the likelihood of occurrence of such events. It is simply not true to state that “the organism is associated with the citrus fruit from South Africa”, and “will survive ...”. Considerable quantities of fruit will not be exposed to the organism (production in pest free areas for example) and the overwhelming majority of the fruit simply is not infected. The SA WG considers such deviations from the obvious to seriously compromise EFSA’s subsequent conclusions about risk.

P. 80 to 84, Section 3.2, Probability of establishment.

P. 81, Section 3.2.2, Suitability of environment.

Whereas EFSA criticised the use of the term “Mediterranean climate”, the SA WG notes that it recognises the generally accepted definition for the term “Mediterranean climate” as being a type of climate characterised by hot, dry, sunny summers and a cool, winter rain season. EFSA failed to acknowledge that although the pathogen has established in various climate conditions, summer rainfall is common to all these areas. EFSA quoted Kotzé (1981) to substantiate its assertion that “Past over reliance on a climate barrier for the establishment of CBS is a new area was proven unsatisfactory”. This is quoted out of appropriate context in that the author had referred to the development of epidemic proportions, not to the establishment of the disease. Furthermore, the author himself, both as part of previous SA WG submissions to the EU and in Annexure 2, clearly indicates the importance of climate as a factor limiting the potential distribution of the organism.

The SA WG previously commented on the draft version of Vicent and García-Jiménez (2008), noting that this study demonstrated the similarity of the Western Cape (*i.e.* unsuitable climate for CBS) and European Mediterranean region on a macro-climatic scale. In 2007, the SA WG furthermore provided data from micro-climatic weather stations that clearly demonstrated the similarities between the Spanish and Western Cape climates on a micro-climatic scale. These data were not considered by EFSA. The SA-WG again stresses its conclusion that the observations by Vicent and García-Jiménez (2008) “provide strong support for the SA WG’s position. Alternaria brown spot does indeed occur abundantly in the Western Cape, providing further biological evidence of the similarity between the climatic conditions in Western Cape and Spanish citrus producing areas. However, under these same conditions CBS does not occur in SA and one can therefore reasonably expect the same in Spain”.

Given the various shortcomings of new information presented by EFSA (as indicated in this document and its Annexures), inappropriate context for various quotes of scientists with recognized expertise with the organism under field conditions, and the additional information provided in Annexures 3, 4 & 5, the SA WG does not consider EFSA to have provided any additional information that justifies an amendment to the PRA conclusion that the environment in the PRA area is not suitable for establishment of the organism.

P. 81 to 83, Section 3.2.3, Cultural practices and control measures

P. 82, third bullet point. The relevance of quoting Wager (1949) with regard to cultural practices in the Western Cape Province of South Africa, where CBS does not occur, is unclear and seems irrelevant.

P. 82, fourth bullet point. The practice referred to is not one that is used commercially and Kotzé (1981) is quoted out of context in this regard.

P. 82, fourth last bullet point. EFSA failed to specify how “irrigation methods may affect ascocarp maturation on leaf litter”. In fact, for successful ascocarp maturation and subsequent ascospore dispersal, alternate wetting and drying of leaves is required (Kotzé, 1981). This will not happen in drip-irrigated orchards in the absence of rain (Mediterranean summer). From unpublished results in South Africa, it was clear that ascospore numbers were lower in micro-irrigated orchards, most likely due to accelerated decomposition of leaves.

P. 82, third last paragraph. The removal of late-hanging (out-of-season) fruit is a basic good agricultural practice applied within modern citriculture and is utilized to minimize alternate bearing and suppression of yield.

P. 83, first paragraph. The climatic conditions in Brazil are completely different from any in the EU. The behaviour of CBS under these conditions is therefore irrelevant to considering risk in the PRA area. **P. 83, Section 3.2.3, paragraph 5.** EFSA expressed concern that “cultural practices

and control measures applied in commercial citrus orchards in the PRA area cannot prevent the establishment of the pathogen". This is irrelevant when considering that there is no appreciable risk of the organism establishing in such regions. Furthermore, worldwide, within modern agricultural systems, cultural practices and control measures are put in place for diseases that are currently present in that country. No control measures or cultural practices are put in place for diseases that are not known to occur in a country. Practices will only be implemented if a disease were to become established. The implementation of scientific based import conditions provide for protection against exotic diseases, not preventative cultural and management practices.

P. 83, Section 3.2.4. Other characteristics. Although a lot is known and published about the pathogen (morphology, host range, means of dispersal, economic impact, symptoms and detection) there is comparatively little known about the genotype diversity and the reproductive biology of this pathogen. It is therefore appropriate to conclude that there is insufficient evidence to allow genetic adaptability potential to be incorporated into the risk assessment.

P. 84, Section 3.2.5, Conclusion on the probability of establishment.

EFSA considered that "parts of the PRA area have a climate which can be favourable for CBS establishment", but failed to qualify from their own studies (P.33) that some parts of the EU citrus production area are "marginally suitable for disease development". No areas of EU citrus production are "favourable or highly favourable for disease development" and that "favourable seasons occur infrequently at specific locations". EFSA concluded that "the probability of *G. citricarpa* establishing in the PRA area is greater than that indicated in the South African document", but failed to specify the extent of the deemed "greater" probability.

Considering the various problems with the EFSA opinion, as highlighted in this document and its Annexures, the SA WG concludes that EFSA has not provided scientific evidence that supports changing the conclusion of the PRA with regard to the probability of establishment. The SA WG considers the additional work reported on in the Annexures to this document, as well as some of the information provided by EFSA (when considered in an appropriate context), to provide further support for its contention that there is no risk of *G. citricarpa* in association with the pathway becoming established in the EU.

P. 84, Section 3.3, Uncertainty. The SA WG concurs that uncertainties that may exist in relation to the issues listed by EFSA in this section do not preclude drawing a conclusion to the risk assessment.

P. 85 to 96, Section 4. Evaluation of management options.

P. 86 & 87, Section 4.2, Elements of quarantine regulation of major non-EU citrus producing countries for *G. citricarpa*.

The existence of CBS regulations in other parts of the world does not automatically indicate that they are technically justified.

P. 86, India. *G. citricarpa* is known to occur in India and hence the relevance of the specified regulations is unclear.

P. 87, USA. Details of these regulations are currently under review.

P. 87 to P. 91, Section 4.3, Evaluation of the current EU measures.

P. 89, Option 16.4 (b). The extent of CBS-free areas officially recognized within SA has been expanded beyond that which was listed in EFSA (2008) and the outcome of recent official surveys holds the prospect of further expansion of such areas. Likewise, there has recently been official establishment of an area of low pest prevalence, within which pest free places of production have been established. However, production within such regions constitutes only 20% of the industry. Furthermore, lesser proportions of the total grapefruit, lemon and Valencia orange crops are produced in these areas.

P. 90 & P. 91, Option 16.4 (d). Pre-harvest CBS control practices can be highly effectively in controlling CBS if applied intensively.

P. 91 to 95, Section 4.4, Evaluation of alternative measures.

P. 91, Section 4.4.1.1. The practicality of implementing potentially intensified sampling procedures and the potential for disruption of trade also needs to be taken into consideration. Furthermore, such options are irrelevant in light of the risk.

P. 91, Section 4.4.1.3. Considering the large volumes of citrus fruit that are being traded, the application of such measures would be totally impractical. Furthermore, such options are irrelevant in light of the risk.

P. 93, Section 4.4.1.6. In light of the risk, there is no need to consider such restrictions. Furthermore, considering the current regulations, EFSA's own climate modelling work indicated that the area of risk is restricted to and within some southern Member States. Considering that the bulk of the citrus fruit exported to EU by SA has a destination outside of these areas,

application of the current restrictions to all citrus fruit entering the EU cannot be considered to be appropriate, even in terms of EFSA's own assessment of the areas at risk.

P. 95, section 4.4.6. Such measures would have a devastating effect. The SA citrus industry is a key component of South Africa's agriculture sector. It is a major source of foreign earning, with direct export earnings being in the region of R5 billion annually, excluding the extensive economic value of the associated support services. The industry is a major provider of employment (approximately 100 000 families are directly dependant on citrus industry employment), especially in rural impoverished areas. The citrus industry plays an active role in facilitating successful black economic empowerment in this important component of the agriculture sector. The SA citrus industry is very heavily dependent on exports, with more than 80% of its earnings being derived from fresh fruit export. The SA citrus industry accounts for more than 50% of the country's fresh fruit exports. The SA citrus industry is the second largest exporter of fresh citrus in the world. It accounts for the following approximate proportions of all southern hemisphere fresh citrus exports: 85% of grapefruit, 76% of oranges, 33% of soft citrus and 26% of lemons. The EU market receives more than 50% of its export volume. Closure or severe restrictions of access to this market would result in total collapse of the industry, with far reaching socio-economic implications extending beyond SA in the SADC region.

P. 95 & 96, Section 4.5, Conclusions on pest risk management options.

P. 95, Section 4.5.1, paragraph 1, lines 6 to 9. Monitoring of the CBS free areas in SA is based on quality inspections and official phytosanitary inspections conducted on citrus fruit exported from these areas. No fruit from these areas has ever been rejected for CBS at any of these inspections points in SA. No fruit from these areas has ever been rejected by South African trading partners (including the EU) that list *G. citricarpa* as an organism of quarantine importance. These CBS-free areas are further protected by SA regulations pertaining to the movement of citrus propagating material.

P. 96, first sentence. This conclusion is irrelevant in light of the appropriate assessment of risk.

P. 96, lines 4 to 8. The SA WG strongly disagrees with this conclusion. Scientific information and expert advice provided to the EU over a period of time has demonstrated that CBS associated with fruit exported from SA does not pose a threat to EU citrus producing MS. Relative to an appropriate assessment of risk, the current regulations as they pertain to all EU MS are disproportionately restrictive and without appropriate technical justification. Furthermore, this conclusion is incongruous with EFSA's own assessment of risk, where it is recognized that risk is

restricted to small parts of five MS whereas the regulations restrict trade (including the bulk of the produce) to 27 MS. It is totally inappropriate to suggest that this equates to being “in line with the IPPC principle of minimal impact”. This situation is exacerbated when consideration is additionally given to the severe cost implications associated with the pre-harvest practices that SA has to apply in pursuit of compliance with the current (technically unjustified) regulations.

P. 96, Alternative measures.

Paragraph 1. The stipulation of obligatory post-harvest treatments would be inappropriate in light of the risk.

Paragraph 3. The potential introduction of such additional measures would be inappropriate relative to risk, impractical for implementation in light of the volumes of fruit involved and highly disruptive to trade (being out of proportion to the risk).

Paragraph 4. Whereas market segregation is unnecessary in relation to the risk, it is recognized that it would greatly reduce the extent of disproportionate trade restrictions (relative to even EFSA’s own assessment of areas at risk) resulting from current regulations.

P. 97 to 98, Conclusions and recommendations.

Point 1. The SA WG does not agree with the EFSA conclusion. Given (a) concerns about aspects of EFSA’s comments and additional data, (b) consideration of aspects of the additional data supplied by EFSA (when considered in the relevant context as commented on in this document and its Annexures), and (c) combined with the results of additional investigations reported as Annexures to this document; the SA WG concludes that there is now additional evidence to further support the conclusion that the EU citrus producing areas are not suitable for establishment of CBS.

Point 2. The SA WG does not agree with the EFSA conclusion and does not consider EFSA to have supplied any compelling evidence to support changing the earlier PRA conclusion that there is no appreciable risk of CBS becoming established in the EU through the fruit pathway.

Point 3. The SA WG notes that Option 16.4 (b) is currently utilized and is appropriately maintained, but only applies to a small proportion of the volume produced in SA. In terms of Option 16.4 (d), pre-harvest controls can be highly effective if intensively applied, but are very costly. However, the SA WG does not consider these measures to be justified in terms of the risk. The SA WG strongly disagrees and objects to the EFSA conclusion that the existing measures “are in line with the IPPC principle of minimal impact”. EFSA’s conclusion in this regard is contrary to its own conclusions on the occurrence of risk areas within the EU. The SA

WG contends that the current EU regulations are without technical justification, disproportionately restrictive to trade relative to risk and must be removed to become compliant with IPPC principles.

Point 4. The SA WG does not agree with the recommendations regarding alternative options proposed by EFSA. Such measures would be inappropriate relative to risk, some would be impractical for implementation and would be highly disruptive to trade. Whereas the SA WG considers geographic market segregation to be unnecessary relative to risk, it does recognise that, relative to the current regulations, this would both be better aligned with EFSA's own assessment of risk and reduce the extent of disruption to trade.

3. NEW INFORMATION

J.M. Kotzé, an internationally acclaimed CBS specialist with 52 years of research and field experience with the organism, provided a commentary on the EFSA opinion (Kotzé, 2009; **Annexure 2**). EFSA (2008) criticised aspects of the climate modelling work using CLIMEX that SA had previously supplied to the EU. A new CLIMEX modelling study was undertaken by Yonow & Hattingh (2009) (**Annexure 3**). EFSA (2008) provided new information in the form of illustrative comparisons of aspects of the climate at certain sites in EU and in SA. This topic was investigated further by Fourie *et al.* (2009) (**Annexure 4**). EFSA (2008) also provided additional information in the form of simulations using the "simple generic infection model for foliar fungal plant pathogens" developed by Magarey *et al.* (2005), "recently implemented in the NAPFAST system ... (Magarey *et al.*, 2007)". A revised version of such modelling was undertaken by Magarey *et al.* (2009) (**Annexure 5**).

4. CONCLUSIONS AND RECOMMENDATIONS

SA consolidated relevant scientific information available at the time into a formal Pest Risk Assessment in 2000. This PRA concluded that *G. citricarpa* in association with the pathway of fresh fruit exports from SA to the EU does not pose a risk to the European Union. Subsequent research and exchanges between SA and the EU, added to the original PRA. The SA WG has considered the original PRA, subsequent exchanges between SA and the EU including the recent EFSA (2008) opinion and more recent additional investigations.

The SA WG previously advised the EU WG that it is necessary to consider that a series of improbable events would need to sequentially coincide for there to be any likelihood of an infection event taking place. The EU has consistently argued around the incomplete elimination

of all risk associated with individual steps in the PRA process. However, no evidence has been presented by the EU which supports deviation from the conclusion that the risk of occurrence of an infection event can be no more than an extremely remote possibility.

The SA WG has consistently advised that it is clearly inappropriate to equate risk of an infection event with risk of establishment (requiring the successful recurrent completion of successive generations). However, EU arguments have continuously disregarded this obvious basic principle. There is an abundance of scientific evidence supporting the assertion that the climate in Europe is unsuitable to support establishment of *G. citricarpa*. EFSA (2008) criticised aspects of the studies that the SA WG referred to in support of its position on the unsuitability of the climate in Europe. These criticisms were fully addressed through additional studies, discussed in and appended to this document. In fact the updated studies, taking EFSA comments into consideration, provide further and stronger evidence that the climate in Europe is not suitable for *G. citricarpa* to potentially establish there.

EFSA provided their own data in support of its argument that the climate is less of a barrier to establishment than what the SA WG had contended. However, numerous problems were evident in the information presented by EFSA. These have been further investigated in additional recent studies discussed in and appended to this document. Considering the additional investigations conducted by Fourie *et al.* (2009) and Magarey *et al.* (2009), and taking the data presented by EFSA into consideration in an appropriate context, only served to further support the conclusion that climate in Europe will not support establishment of *G. citricarpa*.

Despite EFSA (2008) concluding (on the basis of its own studies) that the risk is contained to a small part of the EU (being parts of 5 Member States), it incongruously concluded that current EU phytosanitary regulations (that apply equally to 27 EU Member States) are appropriate and are consistent with the IPPC principle of “minimal impact”. EFSA failed to appropriately consider the disruption to trade that would ensue from implementation of various proposed additional or alternative measures. Implementation of most of the proposed measures would only exacerbate the existing disparity between risk and disruption to trade.

The SA WG concludes that there is now considerably more scientific evidence that supports the same conclusion as was made in the original PRA. It further concludes that the current EU phytosanitary regulations pertaining to *G. citricarpa* on fresh citrus fruit imported into the EU are without adequate technical justification and are unnecessarily restrictive and disruptive to trade relevant to risk. In accordance with the IPPC principles of “technical justification” and “minimal

impact”, failure to rescind phytosanitary regulations pertaining to *G. citricarpa* on fresh citrus fruit imports would constitute an unjustified technical barrier to trade.

The SA WG previously indicated to the EU that although the PRA supports removal of phytosanitary regulations pertaining to *G. citricarpa* in association with the fresh citrus fruit pathway, the retention of suitable quality standards would be appropriate. Prior to harmonization of EU phytosanitary regulations, there were no phytosanitary regulations pertaining to fresh citrus fruit imported into northern Europe. This has been the traditional market for SA citrus fruit and remains the destination for the bulk of these exports. In the absence of phytosanitary regulations pertaining to these markets, the maximum permissible level of *G. citricarpa* infection was regulated by quality standards. The SA quality standards are periodically revised to remain aligned with the relevant EU standards as a major SA trading partner. The SA WG previously proposed this to the EU as being an appropriate (technically justified and compliant with minimal impact) mechanism. The EU did not respond to this proposal. It remains the recommendation of the SA WG.

In 2007, the SA WG proposed that if the EU was not prepared to accept the outcome of the PRA, third party intervention be engaged. The EU declined to respond to this proposal and proceeded to sustain bilateral engagement through subjecting the data to review by an internal EU body in the form of EFSA. It remains the opinion of the SA WG that unless the EU is prepared to accept the outcome of the PRA, suitable third party intervention be engaged.

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6. ANNEXURES

Annexure 1. List of prior documentary exchanges between SA and the EU regarding the CBS Pest Risk Assessment.

Annexure 2. Kotzé, J.M. 2009. Comments on: “EFSA (2008) Scientific Opinion of the Panel on Plant Health on a Request from the European Commission on *Guignardia citricarpa* Kiely”. Report, pp. 10.

Annexure 3. Yonow, T. & V. Hattingh. 2009. CLIMEX analysis of the potential distribution of *Guignardia citricarpa* and risk posed to Europe. Report, pp. 37.

Annexure 4. Fourie, P.H., G.C. Schutte & V. Hattingh. 2009. Rainfall and temperature comparison of citrus producing areas with known presence or absence of *Guignardia citricarpa*. Report, pp. 19.

Annexure 5. Magarey, R., S. Chanelli & T. Holtz. 2009. Validation study and risk assessment: *Guignardia citricarpa* (Citrus black spot). NAPPFAST report, pp. 19.

LIST OF PRIOR DOCUMENTARY EXCHANGES BETWEEN SOUTH AFRICA AND EUROPEAN UNION REGARDING THE CBS PEST RISK ASSESSMENT

May 2000 (SA → EU)

Citrus Black Spot: Pest Risk Assessment document for the review of current phytosanitary regulations pertaining to the export of fresh Citrus fruit from the Republic of South Africa to the EU

October 2001 (EU → SA)

Report of the Commission Working Group on evaluation of the Pest Risk Assessment (PRA) prepared by South Africa on Citrus Black Spot (CBS)

August 2002 (SA → EU)

Response from South Africa on the Report (dated 24/10/2001) of the EC Working Group (WG) relating to the WG's evaluation of the Pest Risk Assessment (PRA) by South Africa on Citrus Black Spot (CBS)

December 2003 (SA → EU)

Further Data Relating to the PRA for Citrus Black Spot and the Export of Fresh Citrus Fruit from South Africa to the EU (**Climate Matching Study – Mapping the potential distribution of Citrus Black Spot caused by *Guignardia citricarpa* Kiely – Paul *et al.***)

February 2004 (EU → SA)

Letter: Acknowledgement of receipt of data sent by SA during December 2003 (Climate Matching Study – Mapping the potential distribution of Citrus Black Spot caused by *Guignardia citricarpa* Kiely – Paul *et al.*)

July 2004 (SA → EU)

Further Data Relating to the PRA for Citrus Black Spot and the Export of Fresh Citrus Fruit from South Africa to the EU - **Research Report on Potential Transmission from Fruit to Leaf litter**

July 2004 (EU → SA)

Letter: Acknowledgement of receipt of data sent by SA during July 2004 (Evaluating the colonisation potential of pycnidiospores from *Guignardia citricarpa* infected citrus fruit to leaf litter – Truter *et al.*)

2005: (SA → EU)

Scientific publication: Paul I, Van Jaarsveld AS, Korsten L, Hattingh V (2005) The potential global geographical distribution of Citrus Black Spot caused by *Guignardia citricarpa* (Kiely): likelihood of disease establishment in the European Union. *Crop Protection* **24**, 297-308

November 2006 (EU → SA)

Report of the Commission Working Group on evaluation of the Pest Risk Assessment prepared by South Africa on Citrus Black Spot (caused by *Guignardia citricarpa* Kiely)

August 2007 (SA → EU)

Report of the South African CBS Expert Working Group on evaluation of the Pest Risk Analysis for Citrus Black Spot (*Guignardia citricarpa*) on fresh citrus fruit from South Africa to the European Union. **The two following documents were also attached to the report:**

Microclimatic weather station data and a scientific publication by Truter M, Labuschagne PM, Kotze JM, Meyer L, Korsten L (2007) Failure of *Phyllosticta citricarpa* pycnidiospores to infect Eureka lemon leaf litter. *Australasian Plant Pathology* **36**, 87-93.

January 2009 (EFSA → World wide web)

Pest risk assessment and additional evidence provided by South Africa on *Guignardia citricarpa* Kiely, citrus black spot fungus – CBS1 Scientific Opinion of the Panel on Plant Health (Question No EFSA-Q-2008-299)

March 2009 (SA → EU)

Letter: SA's response about EFSA's opinion on the review of current phytosanitary regulations pertaining to the export of fresh Citrus fruit from the Republic of South Africa to the EU

**Comments on: “EFSA (2008) Scientific Opinion of the Panel
on Plant Health on a request from the European Commission
on *Guignardia citricarpa* Kiely”**

by

J.M. Kotzé

May 2009

INTRODUCTION

According to the Panel on Plant Health CBS does not occur in Europe. How do they explain this fact? We know that citrus is not indigenous in Europe; that there was heavy trade between the East, the Far East and countries like Portugal and Spain in particular. It is logical that the pathogen must have been imported with the host plant. It is also true that Europe has been importing fruit from CBS-positive countries for decades. It has been shown that fruit is not a pathway of introducing the disease to new areas and I cannot find an example where CBS was introduced by fruit. I contend that the climate in Europe does not support the disease life cycle and that the fruit pathway does not offer the possibility for the pathogen to establish. After decades of fruit imports into Europe from CBS-positive countries CBS is absent. The past is a useful basis to predict the future.

INFECTION VERSUS ESTABLISHMENT

Germination, infection and establishment

Several workers tried to identify the most important factors of the CBS epidemic. (McOnie (1964) indicated that temperature and moisture are key factors. In epidemical terms this was a simplified view. If the susceptible host is present (citrus orchards) and the pathogen is close to the host, the key factor is an environment which must be there to enable the pathogen to establish and produce inoculum in large quantities. This brings time (t) into focus because the production of inoculum is dependent on the presence of moisture and temperature within a given time which determines the rate (r) of increase of inoculum. The r values reflect moisture, temperature, time and susceptibility. The r values can be a useful tool to measure the growth of an epidemic. If inoculum is small, like on an infected fruit, the numerical value of inoculum is insignificant (or $I = 0$). If the inoculum is out of contention, the ongoing importance of moisture, temperature and time becomes zero. This is the situation in Europe and the Western Cape Province of South Africa. One can manipulate these factors to suit many arguments, but unless the epidemiological requirements of inoculum are fulfilled, the epidemic fails. Climate conditions are certainly not only important for germination and infection, but for a series of events that follow. These events lead to establishment of infection of the leaf, the conditions for colonisation, maturation and sporulation.

The gateway for CBS into new areas is the leaf. The infected fruit can play a role in the epidemic in very conducive places where the epidemic is established (like in Brazil), but the situation under discussion is predominantly a Mediterranean climate where the disease is **not** established. Pycnidiospores from imported fruit have to get onto a susceptible leaf to establish an infection. If a fruit becomes infected it can never produce ascospores. The fruit is eventually removed from the orchard and plays no further part.

The focus is on the sustainable establishment (not the infection) of *G. citricarpa* to cause a disease or epidemic in certain parts of Europe. The only possible source of inoculum is pycnidiospores from imported fruit. It was already explained why this route is very remotely possible but establishment is virtually impossible because the environment does not favour establishment. The Panel classified

the fruit pathway as well as the frequency of infected fruit/peel which may be discarded under citrus trees, as an “uncertainty”. At this point the risk is not the infection of susceptible leaves, but the real question is whether the infection may lead to a sustainable establishment so that sufficient inoculum can be produced under the prevailing climate over long periods of time (years). The Panel avoids this point and pretends that it is a simple matter of infection. There is consistently confusion between infection and establishment, disease and epidemic, which leads to serious misunderstanding.

In climate analyses conducted by the Panel, the extrapolation is made that the situation in the Eastern Cape Province is relatively similar to some citrus growing areas in the EU. Contrary to the Western Cape, the Eastern Cape has a **summer** rainfall and it is CBS-positive. The Eastern Cape received the CBS pathogen more than 50 years ago when CBS-positive nursery plants were introduced from the Transvaal. The Eastern Cape has a summer rainfall pattern which coincides with the most susceptible stage of the citrus. The infected leaves of the nursery plants came into a conducive climate to complete the life cycle of *G. citricarpa*. **Fruit trade has nothing to do with it.**

One cannot single out contributing factors and thereby elevate their importance. An epidemic model of CBS is an interaction of many complex factors. The message from the EU models is that some areas in the EU are conducive to CBS. The evidence shows only germination and infection models. No data in the establishment of CBS is utilised. This is misleading. If EU is in danger of becoming CBS-positive, why has it not happened long ago? Why has it not happened in the Western Cape Province where exposure to the disease goes over decades and fruit trade moved freely?

In plant pathological terms, the organism must establish. Establishment means that the pathogen must be able to complete the full life cycle in a sustainable way. There is no evidence that these stages of CBS establishment occur in Europe or in the Western Cape Province. It is my considered opinion that the hypothetical arguments about the pycnidiospore threat from imported citrus fruit be dropped.

Polycyclic and Monocyclic Pathogens

In models it will be misleading to utilise data from well known polycyclic pathogens like *Phytophthora infestans*, *Alternaria* blights, *Venturia inaequalis*, etc. etc. These pathogens may start off with very low levels of inoculum, but because of the large number of infection cycles within a season, it can largely influence the final disease outcome. For these diseases, inoculum is so prevalent, that climatic data requirements are easily satisfied.

In general, the epidemic rate (which is designated r and can be calculated from the transformed disease progress curve, if the amount of initial disease is known) for polycyclic diseases is much greater than the epidemic increase (r value) of monocyclic diseases. CBS is a unique monocyclic disease that requires a good understanding to successfully create models.

The significance of infection of leaves

It is only natural that the infected fruit receives much attention. However, the infected leaf is the door of entry and secures that inoculum is provided in a conducive climate. On the ground the pathogen (in an infected fallen leaf) enters a struggle of life and death. Under sub-tropical, summer rainfall conditions at the time of leaf drop (usually autumn to winter) the environment is dry and warm, but seldom cold. The leaf dries out slowly. At the time the leaf is leathery brown but not dry. During this stage, just described, *G. citricarpa* colonises the dead leaf. Colonisation means “the occupation of vacant space”. After this process the pathogen begins to prepare for its survival and reproduction stage. This event which is so vital for a CBS epidemic is ignored by the EU and misunderstood. Months after colonisation mature ascospores are produced in pseudoperithecia on the dead leaf, ready to be released during rain to complete the cycle. The pathogen is not successful under Mediterranean conditions, like for example, in the Western Cape Province. The EU CBS dispute revolves around these biological events. There is a huge gap between infection and the reproduction of spores. That “gap” represents the competition for survival. The pathogen is vitally dependant on the environment factors, like moisture, temperature and time, which differ for the various life stages in the life cycle.

Under conditions of winter rainfall, temperatures are too cool or cold. There may be night frost. The pathogen is slowed down while the saprophytes are active. It rains, the fallen leaves become soaking wet and stay wet for long periods. After 4 days of soaking the leaf becomes colonised by saprophytic bacteria like *Bacillus licheniformis*, *B. subtilis*, etc. Such a leaf is soft and pliable. Even if it dries, the internal structure is broken down and fungal saprophytes like *Trichoderma*, *Aspergillus sydowi*, *A. caespitosus*, etc. occupy the organic space. The process has turned to compost and *G. citricarpa* is eliminated.

The processes which have been described very briefly, are the determining factors in the life cycle of *G. citricarpa*. If this is overlooked irrelevant questions will keep coming up. Spore germination does not equate to infection and infection does not equate to completion of the life cycle. The environment plays a vital role in each facet of the completion of the life cycle. Therefore, if an infection of a leaf takes place in Europe, with all the odds against such an event, the further development as described is still very dependent on the environment. In this description of the pathogenic relationship and interaction of host, pathogen and environment, lies part of the answer to why CBS is not a problem in Europe.

Lesser known stages in the CBS cycle

For CBS, the monocyclic disease causing organism, *Guignardia citricarpa*, kindly consider the following route of the disease:

Spore release: Ascospores which are the primary source of inoculum are released with rising temperatures in spring or summer, from myriads of pseudothecia on leaves on the ground when it rains. Wetting by irrigation or heavy dew can also sometimes cause limited releases but if the plant does not remain wet long enough no infection will take place. The spores in the asci in the

fruiting body must be mature and release will only happen when the “rotten” leaves on the ground become sufficiently wet. The spores are ejected vertically by force and may reach a height of approximately 3 cm. From that point onwards the ascospore is completely dependent on atmospheric conditions to reach its target. Wind, air currents, convection streams, etc. play a major role in the spread of spores. The majority of spores do not succeed in getting an airlift and many are returned to the ground by rain.

A significant observation was made repeatedly for years by three independent operators in completely different areas in South Africa: Ascospores are not observed in spore traps when the air temperature is below 17.5°C. This implies that spore germination studies below 17.5°C are of academic value only. This point must be noted in CLIMEX studies.

The germination and penetration process takes place in the presence of moisture. The plant surface must remain wet during the process. The temperature can enhance the growth of the germ tube and when too warm, the infection process is stopped. Low temperatures inhibit germ tube development and appressorium development.

Latent period: “Normal” environmental conditions do not appear to affect the “infected” status of the leaf, but systemic fungicides do affect it.

The fallen leaf: During studies to develop reliable DNA techniques to detect CBS accurately under all the different climatic conditions in South Africa, it was concluded that the establishment of CBS depends largely on what happens in the fallen leaf.

When the infected leaf (mostly symptomless) lands on the orchard floor the pathogen needs a substrate to grow (colonise) in preparation for meiosis and completion of the sexual process to produce ascospores. The “ideal” conditions were found to be as follows: the leaf must dry slowly over a period of 5-10 days with alternating wetting and drying, and brief exposure to sunlight (or filtered light under a tree) at temperatures above 25 °C. The leaf changes colour from dark green to paper brown. When exposed to direct sunlight when the ground temperature may exceed 60°C, it turns creamy-white and the pathogen dies. When the leaf remains wet for long spells other organisms will take over and *G. citricarpa* is eliminated. The alternate wetting and drying under sub-tropical conditions are absolutely necessary for the colonisation of the leaf and the reproduction process.

In the Western Cape where repeated surveys confirmed that CBS is absent, the autumn, winter and spring temperatures are too low to stimulate the production of ascospores or the completion of the cycle. Ascospores have never been found.

The importance of pycnidiospores

The role of pycnidiospores in the EU context was dealt with in detail in previous communications. The EU Panel contended that pycnidiospores may be as important as ascospores in the initial stages

of disease establishment. I respectfully must point out that this is out of context in the EU situation. The fruit may carry water-borne pycnidiospores, not airborne ascospores.

CBS does not occur in EU Member States. Why? The classic model for disease (epidemic) dictates that three components must be present for the establishment of an epidemic. The first is that susceptible host plants must be present in large numbers. This is the first component. The second is the presence of the abundance of inoculum of the pathogen. No disease can exist on the presence of two components. This is a basic principle and is accepted as a law of nature. This is certainly true for Citrus Black Spot. History shows that EU countries were trading citrus fruit from CBS positive areas for many decades. It is not new. Indeed lemons were extensively used to control scurvy on ships, long before South Africa started to export citrus to Europe more than a hundred years back.

How it is possible that EU importers of citrus material and fruit from CBS positive countries, are CBS free? If there is one area which was extensively exposed to all kinds of citrus imports for 3.5 centuries, it is the Western Cape Province of South Africa. The Cape Town area has produced citrus since the 17th century. The Western Cape has a Mediterranean climate. EU has a Mediterranean climate. There is one conclusion and that is: a Mediterranean climate does not support the CBS disease.

The fact that CBS does not occur under Mediterranean climatic conditions outweighs all hypothetical speculations about possible splash infections during rain and whatever is supposed to follow.

MODELLING IN PERSPECTIVE

Parameter values for *G. citricarpa*

Magarey and Borchert (2003) and Magarey *et. al.* (2005) are quoted for many foliar fungal pathogen studies for modelling. These workers **estimated** parameter values for *G. citricarpa* by using infection data by pycnidiospores of *Guignardia bidwellii*, a pathogen of grapes (*Vitis vinifera*). No wonder that they recorded high infection efficiencies at temperatures between 10 to 15°C. Fortunately, *G. bidwellii* was found to be inappropriate for *G. citricarpa* modelling. Infection models which are based on germination data can only be done when assumptions are made, because the available data from literature differ greatly. Certainly one reason for the variation is the confusion that existed between *G. mangiferae* and *G. citricarpa* until 10 years ago. Another reason is that infection studies are difficult to perform, because of the time it takes to see the final result. A further problem is that in the case of *G. citricarpa* infection can not be equated to establishment. The Panel apparently do not appreciate the difference. Generic infection models are therefore of academic interest.

The real problem is the most improbable route of infection by pycnidiospores from imported fruit. It was already shown that this risk, in practice is negligible. Furthermore, *G. citricarpa* is a sub-tropical disease which requires a summer rainfall pattern to establish. Parameter values must be based on conditions required for the full life cycle. The importance of generic models can easily be

overrated because they appeal to the less informed decision makers; create wrong perceptions and further disputes.

Modelling climate suitability for *G. citricarpa* infections

Guided by polycyclic diseases, infection and infection periods receive most attention in the models. At this stage pycnidiospores as the only possible source of inoculum should receive the most attention. But, it must not be forgotten that the chances of pycnidiospores to start an epidemic are remote as has been pointed out many times. Published data on germination and infection are in a state of confusion. Germination studies in literature unfortunately also contain *G. mangiferae*. Germination and infection are different events.

Generic infection models

It was conceded that the studies of Magarey *et. al.* (2005) were exploratory and that considerable uncertainty concerning the parameter values existed. I reject the statement that **'pycnidiospores are critical to *G. citricarpa* establishment only for the first infection event following entry and that the climatic conditions at any location therefore only need to remain suitable for the time required to complete one infection'**. This is out of line with the basic principles of an epidemic. CBS in Europe will not establish in this manner. Any epidemic is inoculum driven and if the climate cannot guarantee numerous re-infections to generate high inoculum values the epidemic fails anywhere in the world. Generic models, like this can only confuse and bedevil progress.

The generic infection models based on the work of Magarey *et. al.* (2005) were studied with great interest, because the shortcomings and misunderstandings between the EU and South Africa are clearly exposed.

1. Forecasting of future events has a built-in uncertainty. The more estimates and assumptions are made, the greater the uncertainty of the outcome. Therefore, when such models are used as a tool to demarcate possible future outbreaks in a region where the disease does not even exist, the scientific value can be likened to predicting the outcome of horse racing.
2. I am aware of the fact that forecasts of Late Blight of potatoes were useful since late forties when Van Everdingen proposed the "Dutch Rules" (which was very climate based). The "Mills chart", integrated leaf wetness, temperature and infection data since 1944. These "models" were exclusively used to forecast possible infection periods and then to apply control measures.
3. Modelling with the aid of computers became fashionable since 1969 (EPIDEM) and are now used extensively for polycyclic diseases. CBS is monocyclic with a very complex cycle.

4. Generic modelling of Magarey *et al.* (2005) is based on polycyclic diseases and is only marginally suitable as a template for CBS.
5. Germination and infection information of the pre-PCR era are not reliable because there was no way to differentiate between *G. citricarpa* and *G. mangiferae* which caused great confusion. Again, assumptions are made.
6. If generic potential successful infection models are created for the sake of debate or academic studies there is no objection but if such models are used to justify quarantine of a country's much needed fruit export it is rejected. Fairness commands firmer evidence and much more accuracy.
7. Germination and infection data are insufficient to predict epidemics of CBS in the situation under discussion.
8. It is argued that pycnidiospores are "critical to *G. citricarpa* establishment". There is indeed no other inoculum available. In earlier communications a lot was already done to explain the remoteness of a pycnidiospore infection. It was also stated that "climatic conditions at any location therefore only need to remain suitable for the time required to complete infection". If the conditions are not suitable for the events that follow **after** infection, the disease will not (can not) establish. The discussion is about **establishing** not infection only.
9. It is agreed that the "importance of winter temperatures for the establishment of *G. citricarpa* in EU needs to be considered". Add to this, the climatic conditions needed for meiosis, the sexual process and the completion of the life cycle to increase inoculum as ascospores. If inoculum cannot increase, the disease is in a dead end.
10. It is true that pseudothecia of *G. citricarpa* can be produced in the "short" time of ± 40 days under very conducive sub-tropical conditions. In the cooler climates this event took ± 150 days in wire baskets, but under wet, cold ground conditions the leaf has deteriorated to such a state that it is no longer a substrate for *G. citricarpa*, but a long list of saprophytes.

Probability of entry (3.1)

The statement was made that the greatest number of diseased fruit observed in the lower part of a citrus canopy (Sposito *et al* 2008) is indirect evidence that splash-dispersed pycnidiospores have an important role in increasing the disease severity. Sposito works in Brazil under unique conditions and cultural methods where the rainfall is ± 1500 mm per year and up to six fruit-sets occur on the same tree where massive loads of pycnidiospores are produced continuously.

This is a misplaced assumption. Ascospores are windborne but they are also released from the ground level. Is it not logical that more ascospores will reach their target the closer they are to the

target? One would expect that more ascospores would land on the lower half of the tree than the top.

Nature often works differently. In the studies of Kotze (1963) it was repeatedly found that the severity of CBS on fruit in the top half of the tree was up to three times **higher** than the lower half. It was established that due to poor control of Red Scale, the top half of the tree was more exposed to direct sunlight. Light strongly stimulates symptom development (Broderick *et al* 1970), which explains the symptom distribution. Pycnidiospores move downwards.

The reference to Whiteside (1967) is unfortunate. In 1967 technology to detect ascospores was not available in Rhodesia and Whiteside made an assumption, which turned out to be incorrect. Ascospores were found later in abundance in Zimbabwe (Kotze 1981).

The Panel again notes that splash dispersal and wind dispersal in small droplets are possible. Fitt *et al* (1989) considered the dispersal by rain drops, using different Ascomycetes, but not *Guignardia citricarpa*. All these quotations are irrelevant. At this stage Europe is concerned about the possible establishment of CBS in citrus orchards by citrus peel from symptomised fruit imported from South Africa. We have already shown in previous communications that this pathway, although theoretically remotely possible, is most unlikely. It is underlined by the fact that it has not happened over the many years of fruit trade. There seems to be a consistent tendency by the EU to argue along theoretical lines. The fruit carries no ascospores. The leaf, if infected will probably not show any symptoms. If the Mediterranean climate does not support the process further (EU climate does not support it) nothing will come of it. This is the situation in EU Member States. There is no CBS, a fact that is confirmed on each occasion.

The Panel presented their views on the availability of susceptible citrus trees (there is no other host known) and the possible suitability of climate especially in certain areas in Italy and Greece. Most of the presentation does not address the real issue and that is:

1. There is no CBS disease anywhere in EU Member States. The EU is firm on this point.
2. EU does not import infected citrus leaves but fruit only. No evidence exists where infected fruit started an epidemic.
3. The biological entry point of CBS to new areas is the leaf. The leaf becomes infected while it is still young. This infection usually happens via ascospores once the epidemic is established. This is not the situation in Europe. The disease can not establish in Europe because the climate can not sustain inoculum.
4. Europe does not have a sub-tropical climate with summer rains to secure inoculum increase which can only happen on dead leaves under conditions which can be described as sub-tropical.

The paradox is that infected fruits do enter the EU. This process has gone on for many decades. History confirms that the infected fruits do not present a threat to Europe. The absence of CBS in Europe and the Western Cape as well as citrus growing areas with a winter rainfall climate, can not be ignored by the Panel.

CONCLUSION

CBS does not appear in the Member Countries of the EU and that the reason for this phenomenon is dictated by the climatic conditions that do not support the sustainable establishment of the disease. Furthermore, it has been debated for long that CBS does not establish in a new area via fruit which means that there is no source of inoculum in Europe and no chance to generate further inoculum. If fruit is not a pathway, there is really no substance in the present dispute.

**CLIMEX Analysis of the Potential Distribution of *Guignardia
citricarpa* and the Risk Posed to Europe**

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1 Executive Summary

EFSA (2008) assessed a CLIMEX model of *Guignardia citricarpa* Kiely produced by Paul *et al.* (2005). EFSA (2008) asserted that a number of problems with this CLIMEX analysis invalidated Paul *et al.*'s claim that *G. citricarpa* posed no threat to the European citrus industry. A new CLIMEX analysis of *G. citricarpa* was therefore undertaken to build a more robust model with substantiated and defensible parameter values.

The new CLIMEX model of *G. citricarpa* is presented, with documentation to support the choice of the new parameter values. In describing the new CLIMEX model, any specific criticisms that EFSA (2008) made of the Paul *et al.* (2005) model are also addressed. Most of Paul *et al.*'s (2005) original parameter values have been changed; the Cold Stress Index has been removed; a Cold-Wet Stress Index has been implemented; and the PDD parameter (annual number of degree-days above the lower temperature threshold for development) has been added. The current model is thus significantly different to that of Paul *et al.* (2005). With justification given for the choice of parameter values, this model is believed to be more robust, and to better predict the potential distribution of *G. citricarpa*.

Using the new parameters, an analysis is made as to the suitability of Europe for *G. citricarpa*. For Europe in general, the Growth Index is relatively low due to a temporal disparity in the Temperature and Moisture Indices; alpine areas are too cold; and Cold-Wet Stress is a major limiting factor. Most of Europe (including most of the citrus producing area) is predicted to be completely unsuitable for the establishment and persistence of *G. citricarpa*, with an Ecoclimatic Index of 0. The remainder of the citrus growing regions are predicted to be, at best, only marginally suitable for *G. citricarpa*. Most of these areas have an Ecoclimatic Index of 1, a few grid cells in Spain have an Ecoclimatic Index of 2, and a single grid cell in Spain has a marginally suitable Ecoclimatic Index of 3. These values are all lower than EI values for sites in South Africa and Australia where CBS occurs (mostly in excess of 9 and many in excess of 29). It is therefore concluded that based on climate alone, the potential threat of *G. citricarpa* to the European citrus industry is exceedingly small indeed, and that if it were accidentally introduced to Europe, the likelihood of establishment and persistence is remote.

The report additionally addresses other issues raised by EFSA (2008), regarding (a) the suitability of CLIMEX to define the distribution of a pathogen, (b) the use of the CLIMEX Match Climates technique, and (c) some aspects of the CLIMEX Compare Locations technique.

2 CLIMEX Model

The model was parameterised for South Africa and Australasia, using a map of known *G. citricarpa* occurrence locations (Paul *et al.*, 2005), revised in accordance with: (a) recent surveys (E. Carstens, Citrus Research International, South Africa, pers. comm., Figure 1); (b) the Australian National Pest Database (A.K. Miles, Department of Primary Industries, Australia, pers. comm.; Australian Citrus Growers Association, www.auscitrus.org.au; Figure 2); and (c) references to occurrence in Taiwan (CPC, 2007; CABI/EPPO, no date; and Liu, no date). This ensured that all

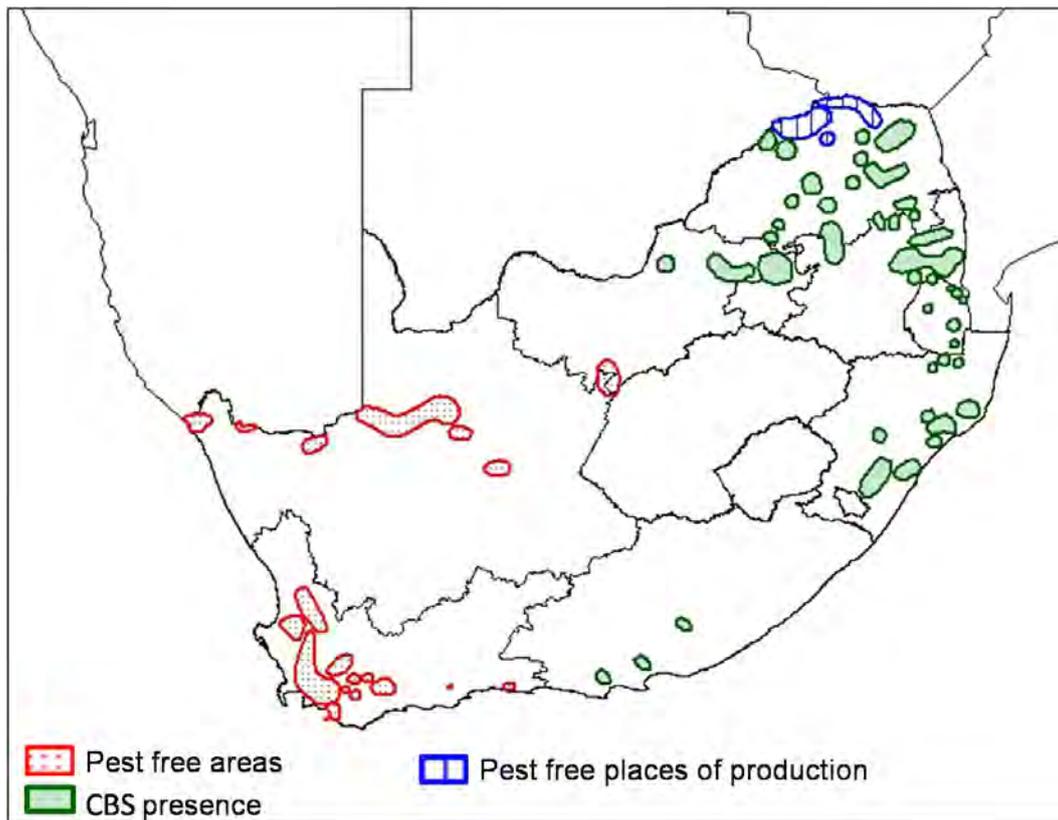


Figure 1. Map of commercial citrus growing areas in South Africa indicating the status of *G. citricarpa*, using International Plant Protection Convention terminology (FAO, 2009).

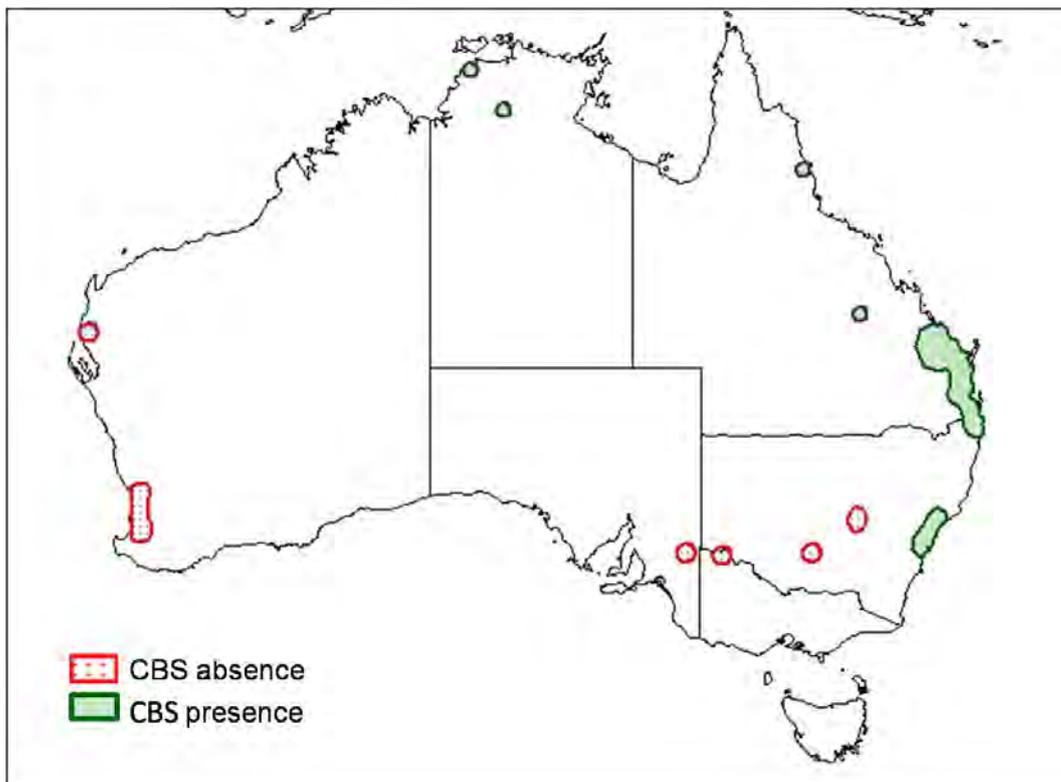


Figure 2. Map of commercial citrus growing areas in Australia indicating the status of *G. citricarpa*.

locations from which this species was recorded in these regions were defined as suitable. *G. citricarpa* has been present in South Africa and Australia over long periods (Cobb, 1897; Doidge, 1929), enabling the organism to spread to the limits of its potential distribution in these regions. Once constructed, the model was verified for the rest of Africa and South America, again using maps of known distribution in these areas (Baayen, *et al.*, 2002; EPPO, 2006; CPC, 2007; Reeder *et al.*, 2008; Dewdney & Timmer, 2009 and G.C. Schutte, Citrus Research International, South Africa, pers. comm.) to assess the model fit. Only at this point, when no discrepancies were found to exist between the model and the known distribution of *G. citricarpa*, was the model run for Europe.

As one of the criticisms of EFSA (2008) was the use of outdated meteorological data in the original CLIMEX analysis, this analysis utilised CRU CL1.0 (New *et al.* 2002) gridded climate data, which is based on 1960 to 1990 climate data. Whilst it may not be “current” to 2009, it is the best available long term data at this point in time.

Reference to available literature is provided where available to support the selection of parameter values. This was done to address the EFSA (2008) criticism of Paul *et al.* (2005) that “the procedure used to produce the parameters is not described in detail”.

EFSA (2008) also criticised Paul *et al.* (2005) for using an EI value of 4 as the “cut-off” value, such that any locations with an $EI \leq 4$ were considered to be unsuitable. The current model uses an EI value of 0 to designate an area as being climatically unsuitable. EI values of 1 would generally be considered as exceedingly marginal: it is highly unlikely that any species will establish and persist in a location where $EI = 1$. Similarly, EI values up to 4 are considered to be marginal, although obviously increasing in suitability. A location with an EI of 5 or higher is suitable for the establishment and persistence of a species, with climatic suitability increasing with increasing EI values. Although defining levels of suitability is arbitrary, climatic suitability can be broadly categorised according to Table 1.

Ecoclimatic Index	Suitability
0	Unsuitable
1 – 4	Marginal
5 – 9	Suitable
10 – 29	Highly suitable
30 – 100	Optimal

Table 1. Indication of climatic suitability relative to the CLIMEX Ecoclimatic Index.

2.1 Soil Moisture Parameters

The original soil moisture parameters of Paul *et al.* (2005) prevented the disease from being able to persist in Taiwan. In the version of CLIMEX that Paul *et al.* (2005) used, both locations for which there are meteorological data in Taiwan have EI values of 0. Both sites, T’aipei and Hwa-Lien, are predicted to be unsuitable because the Moisture Index (MI) is 0 for the entire year: despite ample rainfall, at no time is the MI suitable for *G. citricarpa*. EFSA (2008) suggested that the predicted unsuitability of Taiwan “may be due to the unrepresentative location of weather

stations...”. However, *G. citricarpa* is known to occur in T’aipei, as well as in other areas (Liu, KC, Studies on citrus black spot. 1. Infection of fruit by the causal fungus. pp 20 – 25: www.tari.gov.tw/taric/uploads/journal_arc_15-2-4.pdf).

Paul *et al.*'s (2005) parameters also provided some inconsistencies in South Africa. Areas in the west of the Western Cape Province which do not have *G. citricarpa* despite numerous introductions had EI values of 4; Addo and Hermitage in the Eastern Cape Province, where *G. citricarpa* is present, had EI values of 1 and 0 respectively; and Messina in the northern Limpopo Province had an EI of 6, despite being an exceedingly marginal and probably unsuitable location (South Africa, 1983). The discrepancy between EI values and suitability for *G. citricarpa* was related to differences in the MI values, indicating that the moisture parameters used by Paul *et al.* (2005) needed to be revised.

2.1.1 SM0: low limit for any growth

Paul *et al.* (2005) used a value for SM0 of 0.18. This would indicate that *G. citricarpa* can persist in relatively dry conditions – probably drier than is realistic. This was increased to 0.22, to limit growth in very dry areas, but to provide sufficient moisture for spring and autumn growth of *G. citricarpa* in Addo, South Africa, where this species persists.

2.1.2 SM1: low limit for optimal growth

Paul *et al.* (2005) set SM1 to 0.45, indicating that the lower optimal soil moisture is reasonably high. This was decreased to 0.37 to increase the suitability (EI) for *G. citricarpa* at Addo. The current value of 0.37 for SM1 results in Addo having an EI of 4. Whilst *G. citricarpa* persists at Addo, it does not flourish, indicating that this area is just marginal. Changes in SM1 alter the EI of Addo as per Table 2.

	SM1 Value	EI Value
Addo	0.33	5
	0.37	4
	0.43	3
	0.45	2

Table 2. Changes in EI values at Addo, South Africa, as a result of changing SM1.

The value of 0.37 was selected in order to place Addo at the boundary between marginal and suitable, whilst balancing the changes in EI values in the rest of South Africa. Values lower than 0.37 for SM1 increase the suitability of the Western Cape Province, making a larger area suitable, as well as increasing the EIs of those areas in the Western Cape already being indicated as marginal. As *G. citricarpa* does not occur in the Western Cape despite numerous introductions there, a value of 0.37 for SM1 was considered to be appropriate.

2.1.3 SM2: high limit for optimal growth

The value of 0.85 for SM2 used by Paul *et al.* (2005) prohibited *G. citricarpa* from occurring in Taiwan. SM2 was therefore increased to 1.3, which results in greatly enhanced suitability of Taiwan’s climate in the model.

2.1.4 SM3: high limit for any growth

The SM3 value of 1.0 used by Paul *et al.* (2005) does not allow predominantly wet climates to be modelled as suitable. Although a value of 1 represents soil saturation,

many wet tropical areas have rainfall patterns that effectively result in run-off. As with the low original value of SM2, this excluded *G. citricarpa* from Taiwan in the model. SM3 was increased to 1.8 to model Taiwan's climate as suitable from a soil moisture perspective.

2.2 Temperature Parameters

EFSA (2008) was of the opinion that "the lower limiting temperature and the cold stress temperature threshold parameters for CLIMEX are set very high by Paul *et al.* (2005) and are not related to published information on cold temperature survival for *G. citricarpa*". After conducting a literature search, EFSA (2008) produced a table indicating temperature values thought to be appropriate for the climatic modelling of *G. citricarpa*. Based on the available information, the temperature parameters were reassessed and new values were set.

2.2.1 DV0: low limit for any growth

Paul *et al.* (2005) set DV0 to 17°C. EFSA (2008) queried this value, suggesting that perhaps DV0 should be reduced to 15°C or even 10°C. Despite the fact that there are no reports of successful infection or spore release at low temperatures, the available literature did seem to indicate that a lower DV0 value may be warranted:

- Agostini *et al.* (2006): *G. citricarpa* was readily isolated from fruit maintained at 8°C.
- EFSA (2008): minimum temperatures used in modelling were 10°C or 15°C.
- Kotzé (1963): ascospores germinated at 15°C.
- Lee & Huang (1973): no pseudothecia were produced at temperatures below 7°C; 14°C was the lowest temperature at which formation of pseudothecia was observed.
- Magarey & Borchert (2003): a minimum temperature of 7°C was used for prediction modelling.
- Noronha (2002): appressoria were formed from pycnidiospores incubated at 10°C.
- Wager (1952): no fruit lesions developed when held at 15.5°C.

Based on the above information and the EFSA (2008) suggestion that DV0 be reduced, various attempts were made to fit a model with DV0 < 17°C. However, when DV0 was reduced to either 10°C, 12°C, or 15°C (and all other parameters based on DV0 were also adjusted accordingly), it was impossible to produce an acceptable distribution of *G. citricarpa* in South Africa. Any reduction in DV0 below 17°C resulted in winter growth of *G. citricarpa* throughout most of the Western Cape Province, as well as in other parts of S. Africa. *G. citricarpa* does not occur in the Western Cape Province, despite repeated introductions there, and the population cannot increase in winter. It proved impossible to use any combination of all of the other parameter values and stress indices to restrict the distribution of *G. citricarpa* to its known range. Consequently, these low values of DV0 had to be abandoned.

CLIMEX is a robust modelling tool, and the results with DV0 set to less than 17°C were surprising. However, examination of growth charts for a number of locations throughout South Africa indicated that when DV0 was reduced to 10°C, 12°C, or 15°C, the Temperature Index was suitable for almost the entire year. Such results suggest that in the absence of any other limiting factors in winter, such as moisture (either insufficient or excessive) or cold stress, *G. citricarpa* would thrive (increase)

throughout most of the winter season in South Africa. This is clearly not the case. Whilst *G. citricarpa* can certainly persist in many locations over winter, there is no active population growth at this time (Kotzé, 1981). Growth (i.e. spore release and new infections) only occurs in spring and summer, when temperatures are higher (Kotzé, 1981). Field pathologists in South Africa report that although low levels of spore release may take place on rare occasions at 15°C, they do not see subsequent infection from such spore release events (S.H. Swart & G.C. Schutte, unpublished). This implies that 15°C is below the infection temperature threshold. Fourie *et al.* (2009) studied the combination of field spore monitoring and weather station data and established that 98% of spore release events were observed at temperatures above 16.7°C and that 95% of spore release events were observed at temperatures above 18°C.

Even with DV0 at 17°C, as per the original value of Paul *et al.* (2005), CLIMEX indicates that parts of the Western Cape Province as well as areas further eastwards (Patensie, Addo and Hermitage) are suitable for winter growth of *G. citricarpa*. Extensive attempts to prevent this winter growth through the use of other parameter values and stress indices proved completely unsuccessful.

Because of (a) the failure to fit an acceptable CLIMEX model using DV0 at values of 10°C, 12°C, and 15°C; (b) observations in South Africa indicating that field infections do not occur until temperatures exceed 17°C, and (c) the erroneous CLIMEX prediction of winter growth of *G. citricarpa* in South Africa when DV0 is set at 17°C, DV0 was incrementally increased above 17°C (and all other parameters associated with DV0 were adjusted accordingly) until winter growth in South Africa was prevented. Ultimately, the only way to suitably restrict the predicted distribution of *G. citricarpa* in South Africa and prevent winter growth was to set DV0 to 20°C.

2.2.2 DV1 and DV2: optimal range for growth

Paul *et al.* (2005) originally set DV1 to 24.5°C and DV2 to 32°C. Most of the available literature indicates that these values are reasonable.

- Chiu (1955): the optimal temperature for hyphal growth was 25°C to 28°C.
- EFSA (2008): used 25°C or 27°C as optimal temperature range.
- Korf (1998): in the laboratory, conidia germinated optimally at 22°C.
- Kotzé (1963): the highest germination rate was obtained at 29.5°C, which was also the highest temperature tested.
- Kotzé (1981): the optimal temperature for the growth of *G. citricarpa* on liquid basal synthetic medium was 27°C.
- Lee & Huang (1973): optimal temperature for pseudothecia formation was 21°C to 28°C.
- Magarey & Borchert (2003): an optimum temperature of 27°C was used in prediction modelling.
- Magarey *et al.* (2005): the highest value used for the optimal temperature was 28°C.
- Noronha (2002): for pycnidiospores, the highest rates of appressoria formation occurred between 25 and 30°C.
- Wager (1952): optimal growth on agar occurred at 24°C to 27°C.

Apart from the lower optima of 21°C (Lee & Huang, 1973) and 22°C (Korf, 1998), the remainder of the available literature indicates that the optimal temperature range for

G. citricarpa may be 24°C to 30°C. Therefore, DV1 was reduced from the original value of 24.5°C to 24°C and DV2 was reduced from the original value of 32°C to 30°C.

2.2.3 DV3: high limit for any growth

Paul *et al.* (2005) set DV3 to 40°C. Based on the available literature, and as suggested by EFSA (2008), this seemed too high:

- EFSA (2008): used a maximum temperature of 35°C.
- Korf (1998): the highest temperature at which conidial germination and appressorium formation was observed was 35°C.
- Lee & Huang (1973): no development of existing pseudothecia occurred at 35°C.
- Magarey & Borchert (2003): used a maximum temperature of 35°C.
- Magarey *et al.* (2005): maximum temperature may be set at 35°C when, as in the case of *G.citricarpa*, there is no information on the upper temperature limit for infection.
- Noronha (2002): germination of pycnidiospores occurred between 10°C and 40°C.

As a result, DV3 was reduced from 40°C to 35°C.

2.3 Growth Index

The Growth Index (GI) in CLIMEX is the combination of the Temperature Index (TI) as defined by the temperature parameters, DV0 to DV3, and the Moisture Index (MI) as defined by the soil moisture parameters, SM0 to SM3. To get a positive GI, both the TI and MI must be positive at the same time. Thus, the GI will be zero if the TI is positive but the MI is zero, or if the TI is zero whilst the MI is positive. In South Africa (Figure 3), much of the Northern Cape Province and the northern part of the Western Cape Province have a GI of zero. In Australia, the bulk of the central and southern regions have a GI of zero (Figure 4). In Taiwan, only one high altitude area in the north central part of the island has a GI of zero (Figure 5).

In both Australia and South Africa, the areas of zero GI are due to insufficient rain in summer. Thus, although the TI is positive, the MI is not. The relatively small zone in the far northern part of the Limpopo Province in South Africa, where Pest Free Places of Production occur (FAO, 2009; South Africa, 1983), also has a zero GI value. This reflects the high level of sensitivity of the model to small differences in climatic conditions that are biologically meaningful for the distribution of *G. citricarpa*. In Taiwan, the single grid cell with a GI of zero is a high altitude location (altitude of 2054m) which never becomes sufficiently warm – the TI is never positive, as the maximum temperature never reaches 20°C.

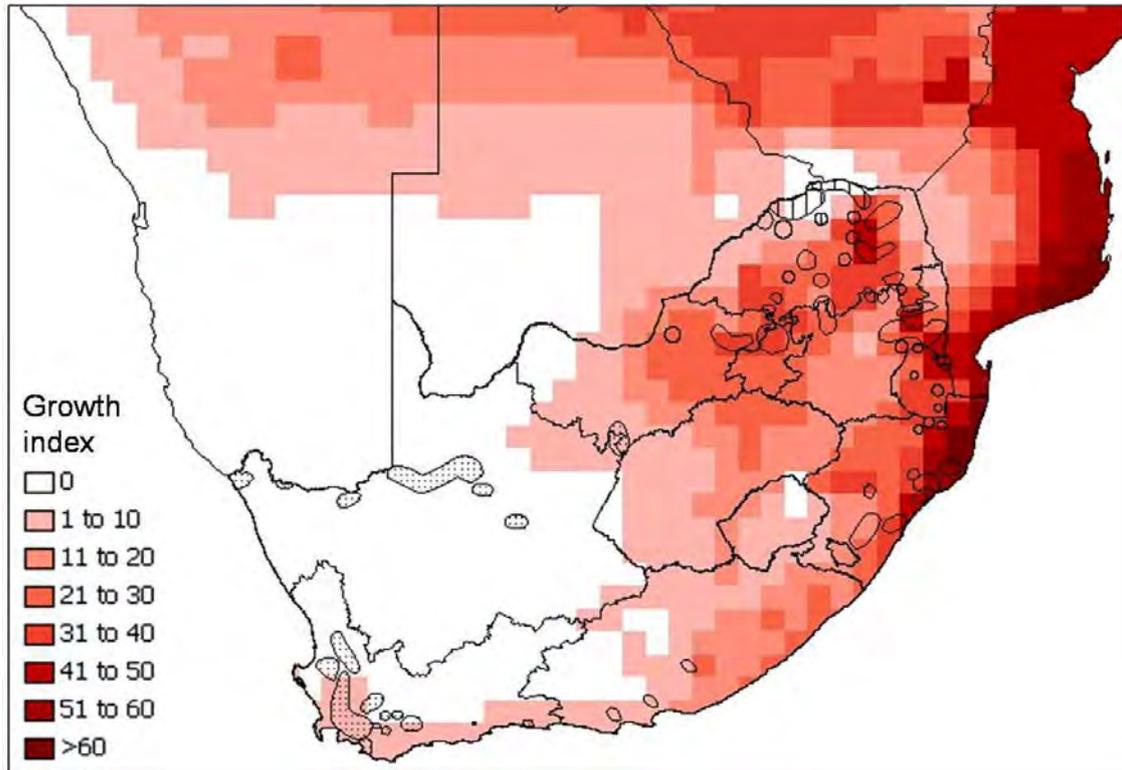


Figure 3. Map of the Growth Index (GI) for South Africa. Areas indicated as unsuitable (GI=0) are due to the absence of a temporal overlap of a positive Temperature Index (TI) and a positive Moisture Index (MI). The highest GI value is 66.

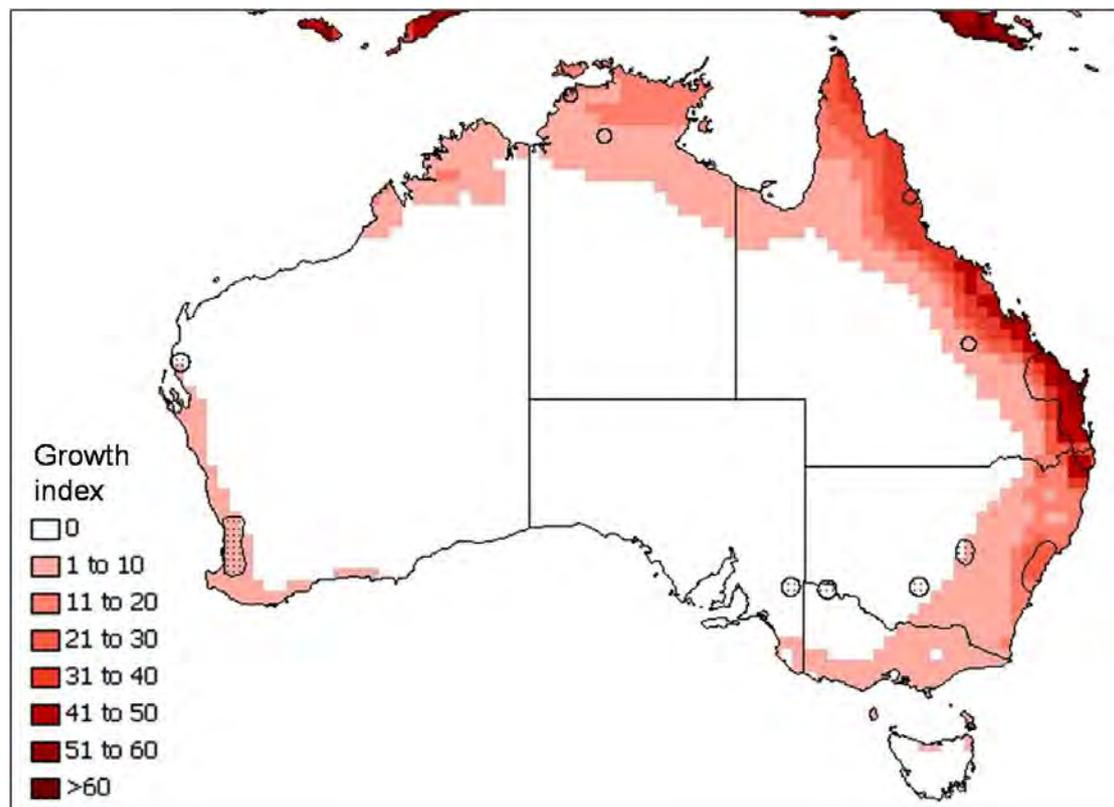


Figure 4. Map of the Growth Index for Australia. Areas indicated as unsuitable (GI=0) are due to the absence of a temporal overlap of a positive Temperature Index (TI) and a positive Moisture Index (MI). The highest GI value is 67.

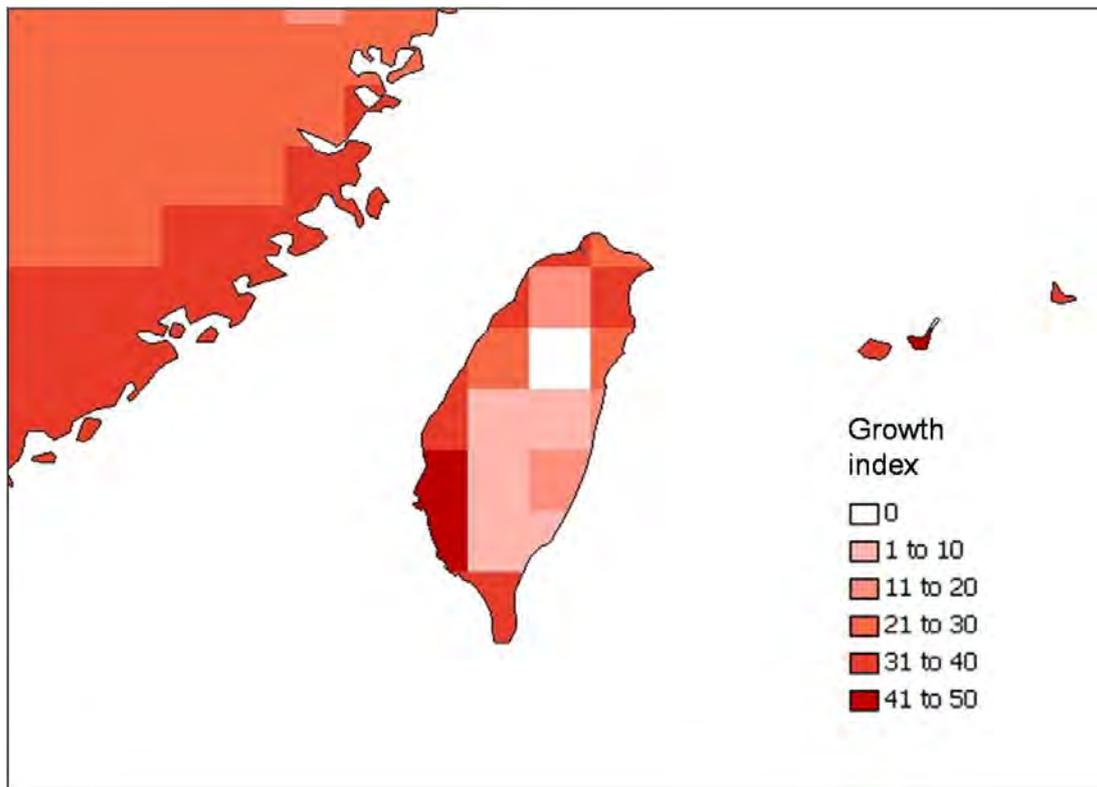


Figure 5. Map of the Growth Index for Taiwan. Areas indicated as unsuitable (GI=0) are due to the absence of a temporal overlap of a positive Temperature Index (TI) and a positive Moisture Index (MI). The highest GI value is 43.

2.4 Light Index

This index was not used.

2.5 Diapause Index

This index was not used.

2.6 Cold Stress

In Paul *et al.*'s (2005) model, Cold Stress accumulates in two ways. With the Temperature Threshold Model, CS accumulates at the rate of -0.0001 when the temperature drops below 11°C, and with the Degree-Day Threshold Model, CS accumulates at a rate of -0.00025 if there are fewer than 6 degree-days above DV0 (17°C).

EFSA (2008) considered that “the lower limiting temperature and the cold stress temperature threshold parameters for CLIMEX are set very high by Paul *et al.* (2005) and are not related to published information on cold temperature survival for *G. citricarpa*”. It is true that with the model of Paul *et al.* (2005), most places that accumulate any amount of degree-day cold stress are made unsuitable by the temperature threshold cold stress. Paul *et al.*'s (2005) temperature threshold model of CS is extremely severe.

After initial infection, *G. citricarpa* can remain latent in the plant material for many months (Kotzé, 1981), making elimination of the pathogen directly through exposure to cold unlikely, unless the cold is sufficient to kill the host plant material. Thus, the

CS mechanisms in Paul *et al.*'s (2005) model were disabled and CS was not used in the current model to limit the distribution of *G. citricarpa*.

2.7 Heat Stress

As with Cold Stress, Paul *et al.*'s (2005) model utilises two mechanisms of Heat Stress. With the Temperature Threshold Model, HS accumulates at a rate of 0.001 when the temperature exceeds 40°C, and with the Degree-Day Threshold Model, HS accumulates at a rate of 0.001 when there are more than 25 degree-days above 40°C.

Since the temperature parameters were adjusted, the HS parameters also needed to be adjusted. As seen earlier under the temperature parameters, it would appear that 35°C is a reasonable assumption of the upper limit for this species. However, it was not clear if both mechanisms of HS were necessary. Thus, after adjusting the parameter values for both models of HS, the temperature threshold model of HS was compared with the degree-day model of HS.

2.7.1 Temperature threshold model of heat stress

For the Temperature Threshold model of HS, parameters were set as follows. TTHS, the temperature threshold above which HS accumulates, was set to 35°C, which is also the upper optimum temperature for development, DV3. THHS, the rate at which HS accumulates, was set to 0.0007.

With this model of HS, lethal HS occurs in parts of Saharan Africa (Mauritania, Mali, Algeria, Niger, Chad and Sudan), parts of the Arabian Peninsula into Iraq, Iran and Afghanistan, and small areas of Pakistan. A very marginal amount of HS is experienced in the north of the Northern Cape Province in South Africa.

2.7.2 Degree-day threshold model of heat stress

DTHS, the number of degree-days above DV3 (35°C) needed for HS to accumulate, was set at 1, the rate of HS accumulation, DHHS, was set at 0.0007.

2.7.3 Comparison of the heat stress models

In Australia, Africa and the Middle East, the area experiencing HS is somewhat larger with the temperature threshold model than with the degree-day model. However, excessive HS only occurs in Western Australia with the degree-day model of HS and similarly, in northern Africa (Sahara) and the Middle East, a slightly larger area overall experiences high levels (> 90) of heat stress with the degree-day model. In other words, by comparison with the temperature threshold model of HS, the degree-day model of HS produces an overall smaller area experiencing HS, but the "hot spots" are much hotter, more excessive levels of HS are obtained, and the area experiencing high levels of HS are also larger.

Because there is no basis for using a degree-day model of HS, i.e., there is no biological information to indicate that *G. citricarpa* cannot persist because the daily heat load above 35°C is too high, the degree-day model of HS was deleted and only the temperature threshold model of HS was retained. In terms of fitting the model to known distribution points in South Africa, Asia and Australia, HS has no impact. However, the temperature threshold model of HS was retained because (a) it is unlikely that *G. citricarpa* would be able to survive the extreme temperatures in the

places where HS occurs, (b) extensive citrus cultivation does not occur in such areas and (c) a HS model makes biological sense.

In South Africa (Figure 6), HS only occurs in a small area that is already predicted to be unsuitable by the GI (see Figure 3 above). In Australia (Figure 7), HS accumulates in the inland and northern regions, and there is an overlap in the areas where HS and GI (see Figure 4) are both positive. As there is no HS in Taiwan, a HS map was not provided for this region.

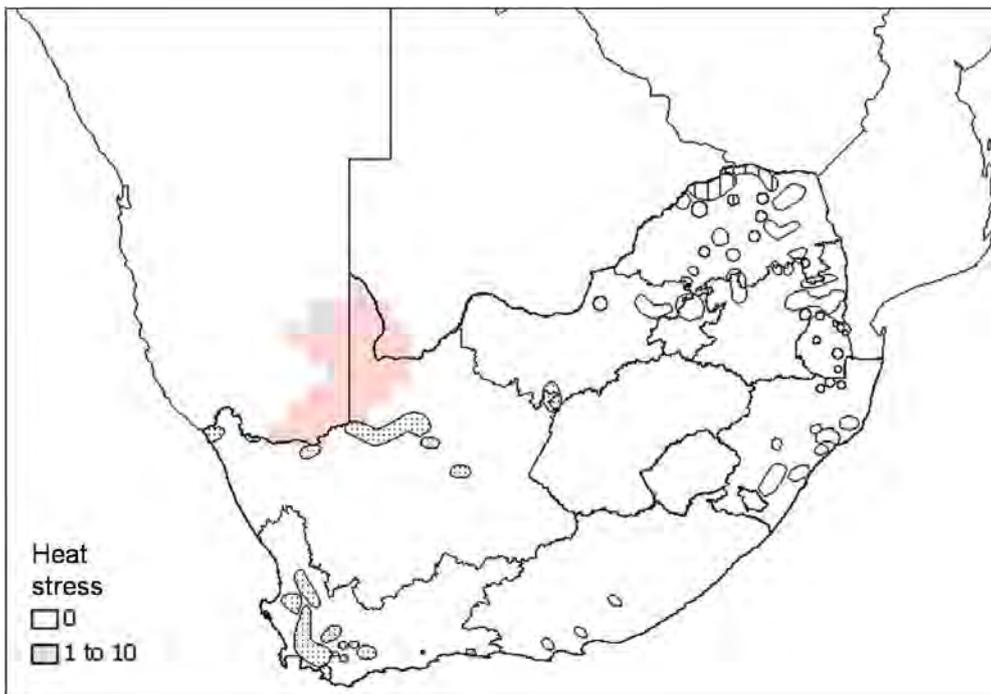


Figure 6. Map of Heat Stress for South Africa. The highest heat stress value is 4.

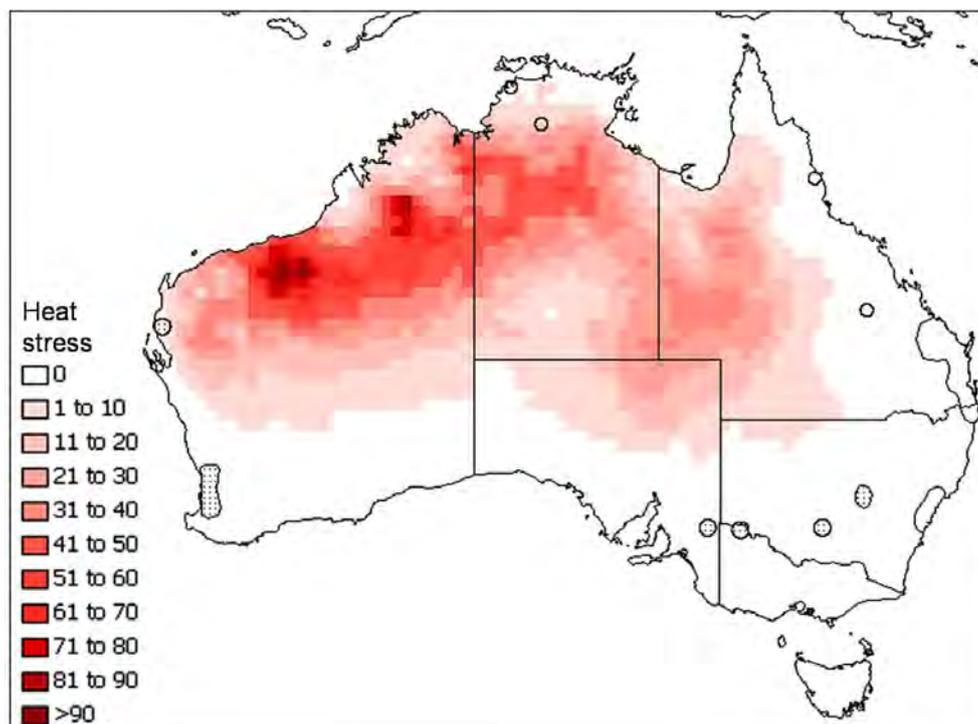


Figure 7. Map of Heat Stress for Australia.

2.8 Dry Stress

In CLIMEX, Dry Stress accumulates at a set rate when soil moisture drops below SM0. Paul *et al.*'s (2005) model had no Dry Stress included in the parameter set. EFSA (2008) pointed out that this was “a surprising omission considering the importance of moisture in the disease cycle.” To address this, the soil moisture threshold (SMDS) below which DS accumulates was set to 0.22 (SM0), and the rate of DS accumulation (HDS) was set to -0.001.

This allows Dry Stress to accumulate in many parts of the world. In fitting the model, it was deemed appropriate to allow lethal DS to accumulate in the west and central areas of the Northern Cape Province in South Africa (Figure 7) and in parts of central Australia (Figure 8). These regions receive minimal rainfall, and they fall outside the distribution range of *G. citricarpa*.

No DS accumulates in Taiwan. In both South Africa (Figure 8) and Australia (Figure 9), lethal amounts of DS occur in areas that already have unsuitable GIs (Figures 3 and 4). However, in both of these countries, some DS does occur in areas with a positive GI (Figures 3 and 4).

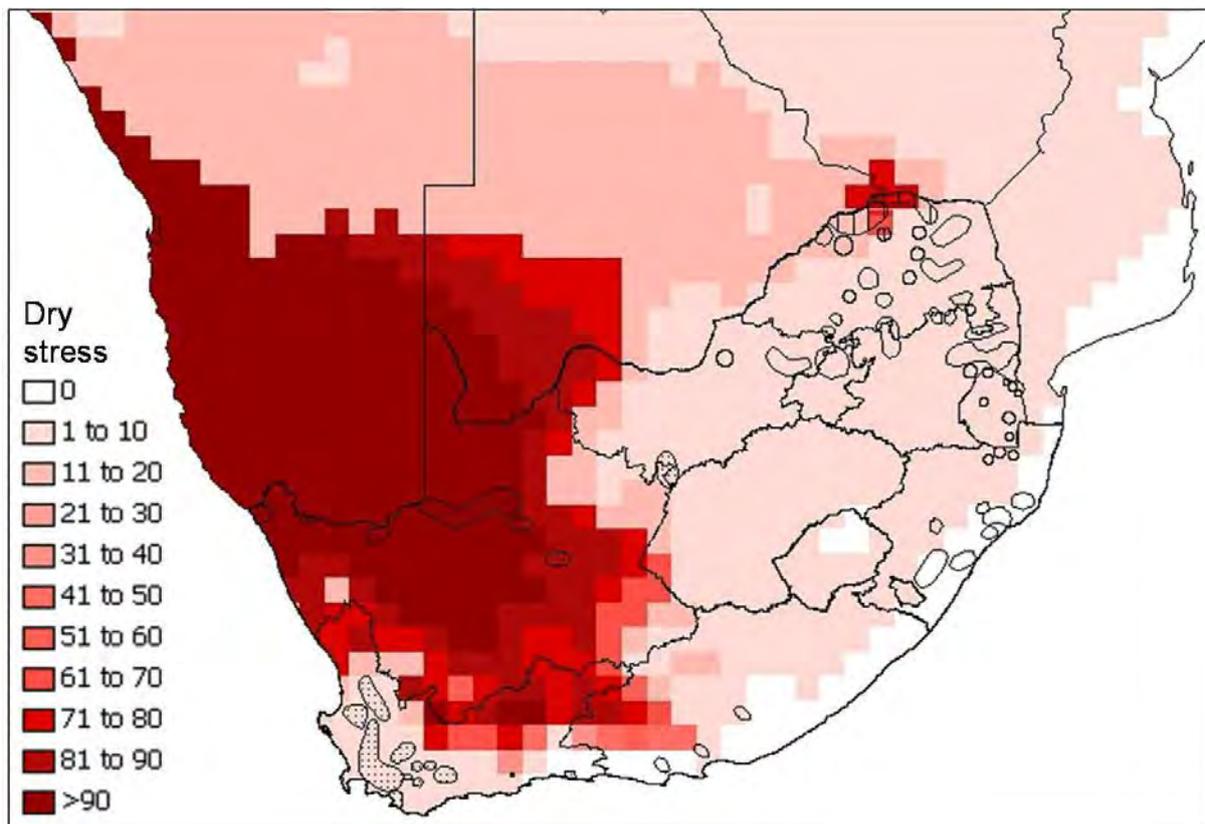


Figure 8. Map of Dry Stress for South Africa.

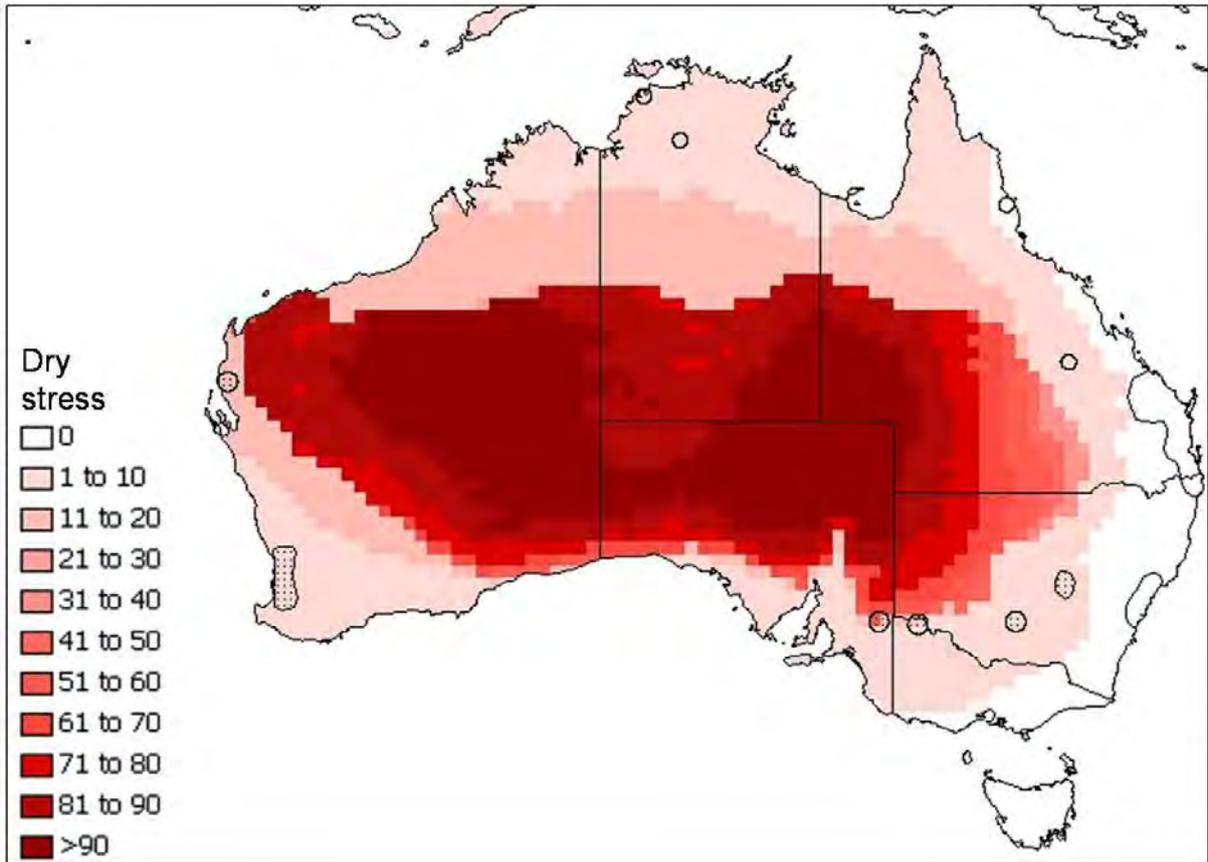


Figure 9. Map of Dry Stress for Australia.

2.9 Wet Stress

Paul *et al.*'s (2005) model includes a Wet Stress Index, with a soil moisture threshold of 1 and an accumulation rate of 0.0001. With these parameter values, WS has minimal impact worldwide, and will not restrict the distribution of *G. citricarpa*. However, Lee & Huang (1973) reported that excessive moisture was detrimental to *G. citricarpa*.

Because the upper soil moisture threshold (SM3) was increased to 1.8, the wet stress threshold (SMWS) had to be increased accordingly: wet stress cannot accumulate at a soil moisture level lower than the upper soil moisture limit for growth (SM3). SMWS was therefore also set at 1.8. The accumulation rate was increased to 0.005 in order to get some wet stress occurring in various parts of the world.

Small amounts of WS accumulate along the north-eastern tip and coast of the Cape York Peninsula in Australia ($WS \leq 6$) and in the Hong Kong & Taiwan regions ($WS \leq 4$).

Wet Stress made the following areas unsuitable for *G. citricarpa*: the western coast of Columbia in South America, some small areas in India and Myanmar; and parts of New Guinea and Papua New Guinea. In all of these areas, rainfall is well above 4500mm annually. *G. citricarpa* is not reported to occur in these areas and would not be expected to thrive under such conditions.

2.10 Cold-Dry Stress

This index was not used since there is no indication this is a biological constraint, apart from potentially making a site unsuitable for the host plant.

2.11 Cold-Wet Stress

By contrast to Cold-Dry Stress, Cold-Wet Stress was considered to be an appropriate mechanism by which to potentially limit the distribution of *G. citricarpa*. This species thrives in warm wet climates, but around the world has failed to establish in Mediterranean climates (Baayen, *et al.*, 2002) which are characterised by relatively warm dry summers and cool wet winters. Lee & Huang (1973) described how the occurrence of excessive moisture leads to accelerated degradation of the inoculum substrate. Sufficient warmth is also recorded as a requirement for maturation preceding spore release (Kotze, 1981). The combined effect of cold and wet can therefore be expected to be a relevant potential biological constraint.

Parameter values were set as follows: the threshold number of degree-days (DTCW) below which CWS accumulates was set to 10; the soil moisture threshold (MTCW) above which CWS accumulates was set to 0.18; the rate (PCW) at which CWS accumulates was set to 0.00017; and the temperature threshold (DVCS) used to calculate degree-days was set to 20. Thus, CWS accumulates at the rate of 0.00017 when there are fewer than 10 degree-days below 20°C and the soil moisture exceeds 0.18.

With no evidence to suggest otherwise, DVCS was left at the default value, equivalent to DV0. For numerous locations in the Western Cape Province, an examination was made of soil moisture levels and the number of degree-days above 20°C, to guide the choices for the DTCW and MTCW parameters. The use of CWS in the model precluded *G. citricarpa* from persisting in the Western Cape Province of South Africa. The rate of 0.00017 makes suitable a known occurrence point on the east coast of Taiwan and ensures that all known occurrence locations in Australia are predicted to be suitable.

CWS accumulates to sufficiently high levels to have an impact upon the distribution of *G. citricarpa* in South Africa (Figure 10), Australia (Figure 11) and Taiwan (Figure 12).

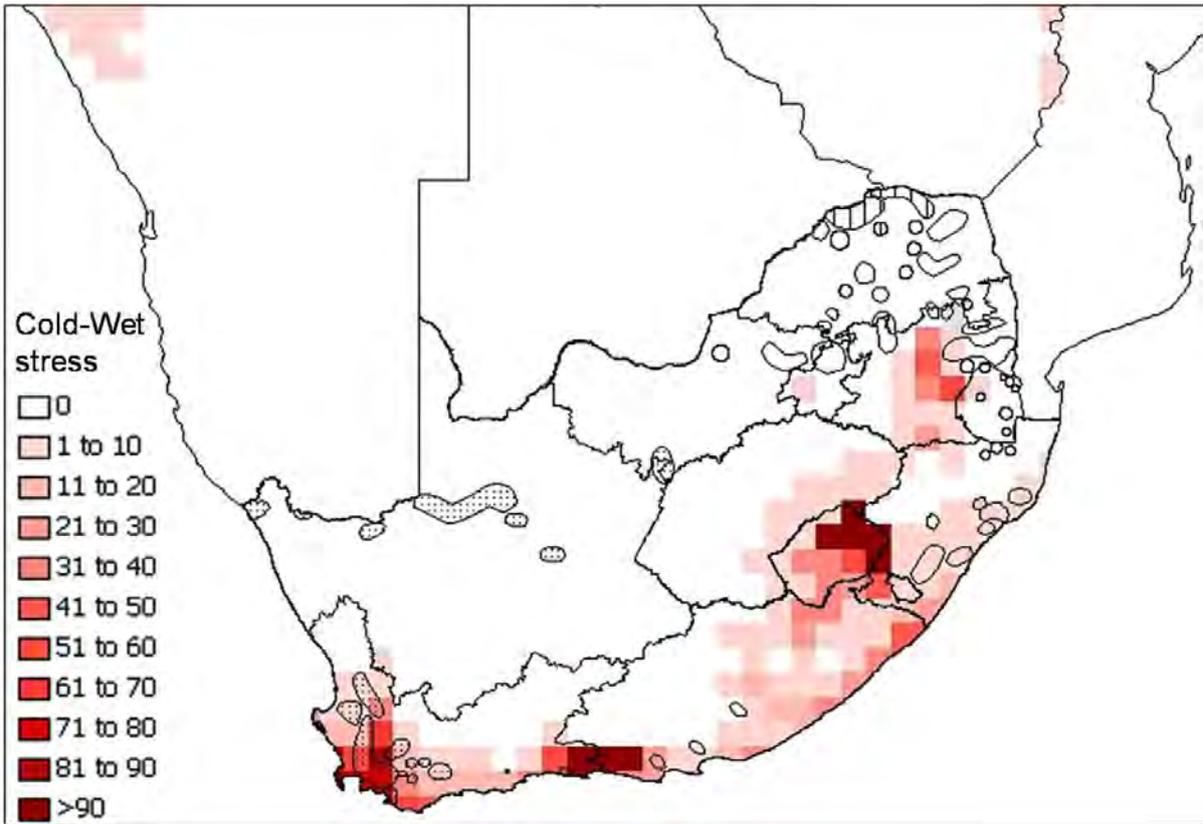


Figure 10. Map of Cold-Wet Stress for South Africa.

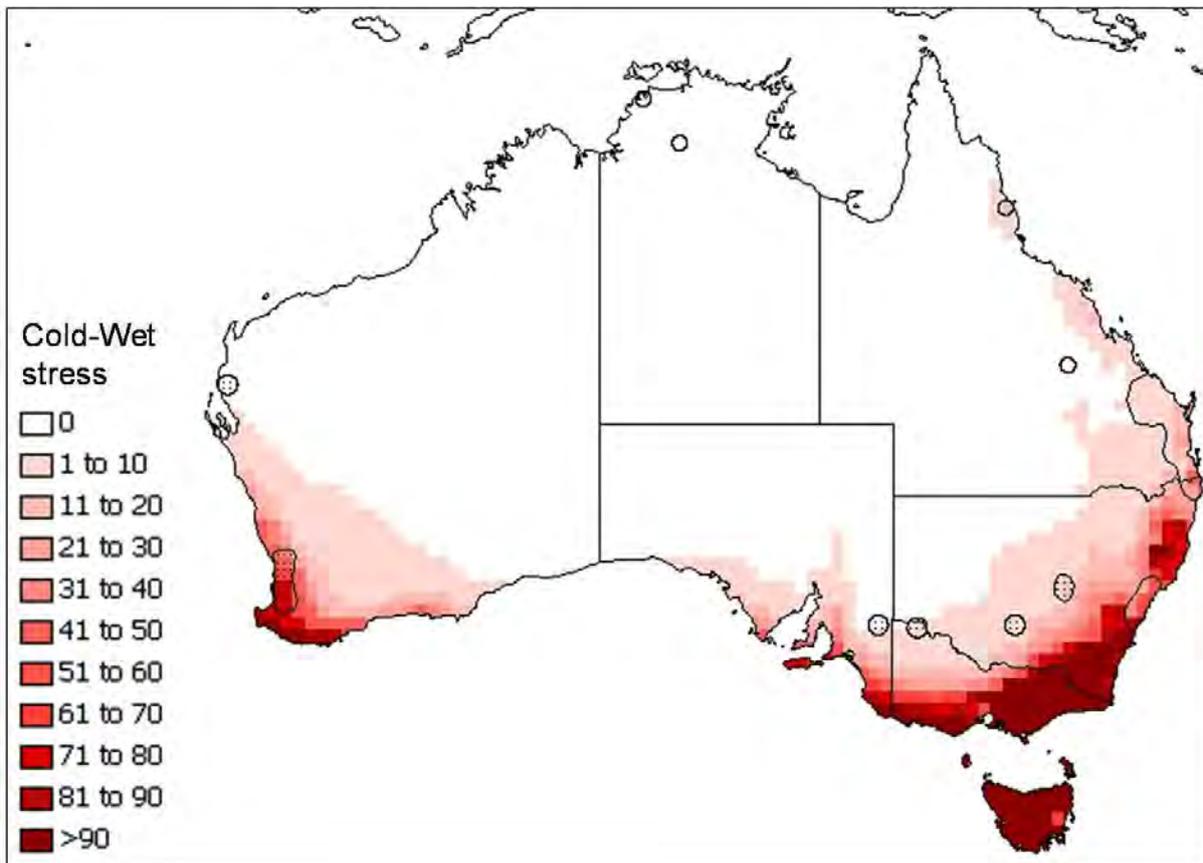


Figure 11. Map of Cold-Wet Stress for Australia.

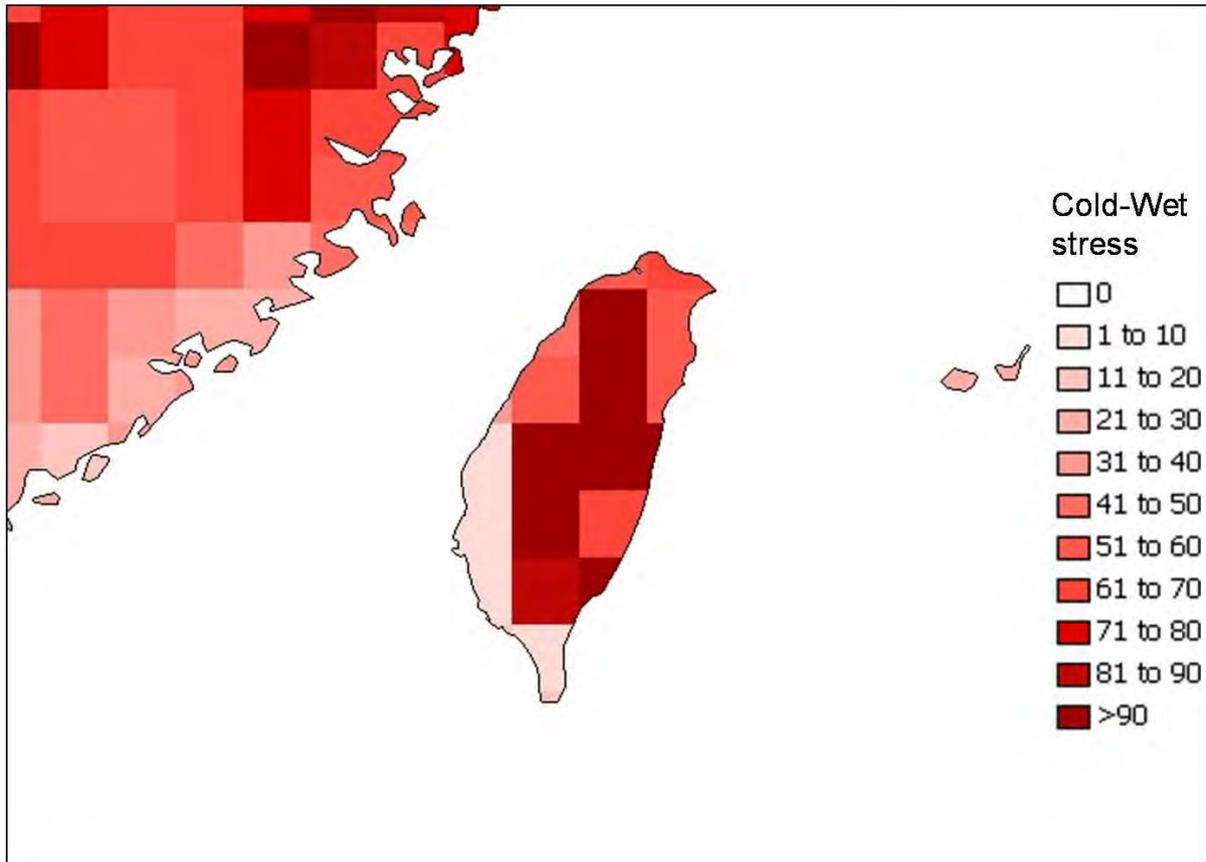


Figure 12. Map of Cold-Wet Stress for Taiwan.

2.12 Hot-Dry Stress

This index was not used. A number of combinations of HDS parameters were examined, but HDS did not assist in modelling the distribution of *G. citricarpa*: any areas where HDS accumulated were already accumulating Dry Stress.

2.13 Hot-Wet Stress

This index was not used, since *G. citricarpa* persists in warm wet locations such as parts of the KwaZulu Natal Province in South Africa, parts of Australia and parts of Asia (CPC, 2007).

2.14 PDD

PDD is an annual heat sum threshold, measured as the number of degree-days above DV0 (20°C). This threshold must be reached (or surpassed) for a species to persist in a location. If the annual number of degree-days above DV0 is below the PDD value, then the EI value is set to zero. For *G. citricarpa*, PDD relates to the need for the rate of pathogen maturation to be sufficiently rapid in the post spring leaf drop period, so that sufficient pseudothecium maturation and spore release can take place before the leaves decay to the point that they become unsuitable to support an inoculum. Retarded maturation (due to insufficient heat accumulation) may also delay spore release into a period that is climatically unsuitable both for spore release and for infection.

PDD was set to 175. This is the maximum value enabling the Patensie area in the Eastern Cape Province of South Africa to be predicted suitable for *G. citricarpa*.

Figures 13 to 15 indicate areas made unsuitable for *G. citricarpa* due to insufficient heat accumulation (PDD < 175). In Taiwan, the small area made unsuitable by the PDD parameter (Figure 15) is already unsuitable due to CWS (Figure 12), and so including PDD does not change the projected distribution of *G. citricarpa* in this country. Although PDD has an impact on the distribution of *G. citricarpa* in both South Africa (Figure 13) and in Australia (Figure 14), the impact of PDD in addition to the impact of CWS (Figures 10 and 11) is not obvious, as there is much overlap in the areas designated unsuitable by both mechanisms. Thus, for these two countries, predictions with and without PDD were compared. Figures 16 and 17 show those areas additionally made unsuitable for *G. citricarpa* by the PDD parameter, and for both countries, including PDD results in a slightly more restricted distribution and provides a marginally better fit to the known distribution of *G. citricarpa*, indicating that it should be retained in the model.

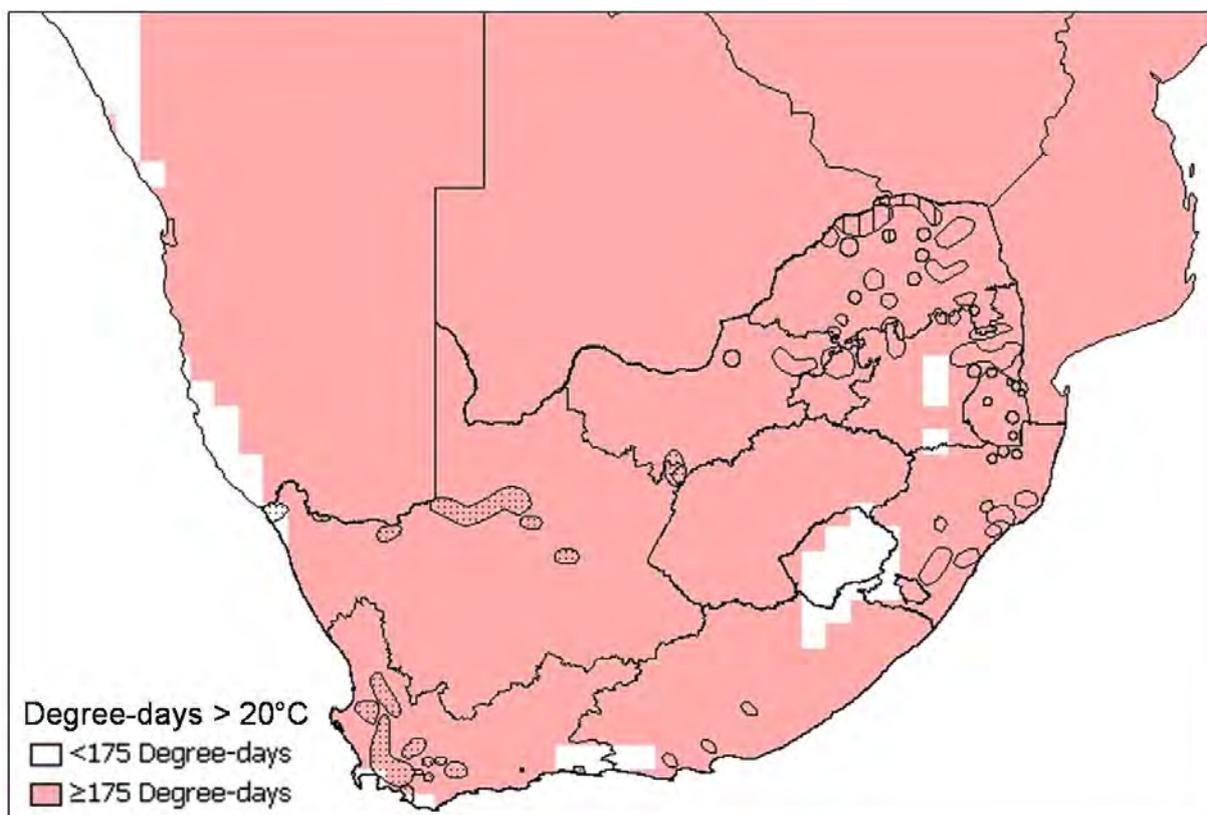


Figure 13. Map showing where heat accumulation is adequate for *G. citricarpa* to persist in South Africa. Areas in white have fewer than 175 degree-days >20°C and are unsuitable for *G. citricarpa*.

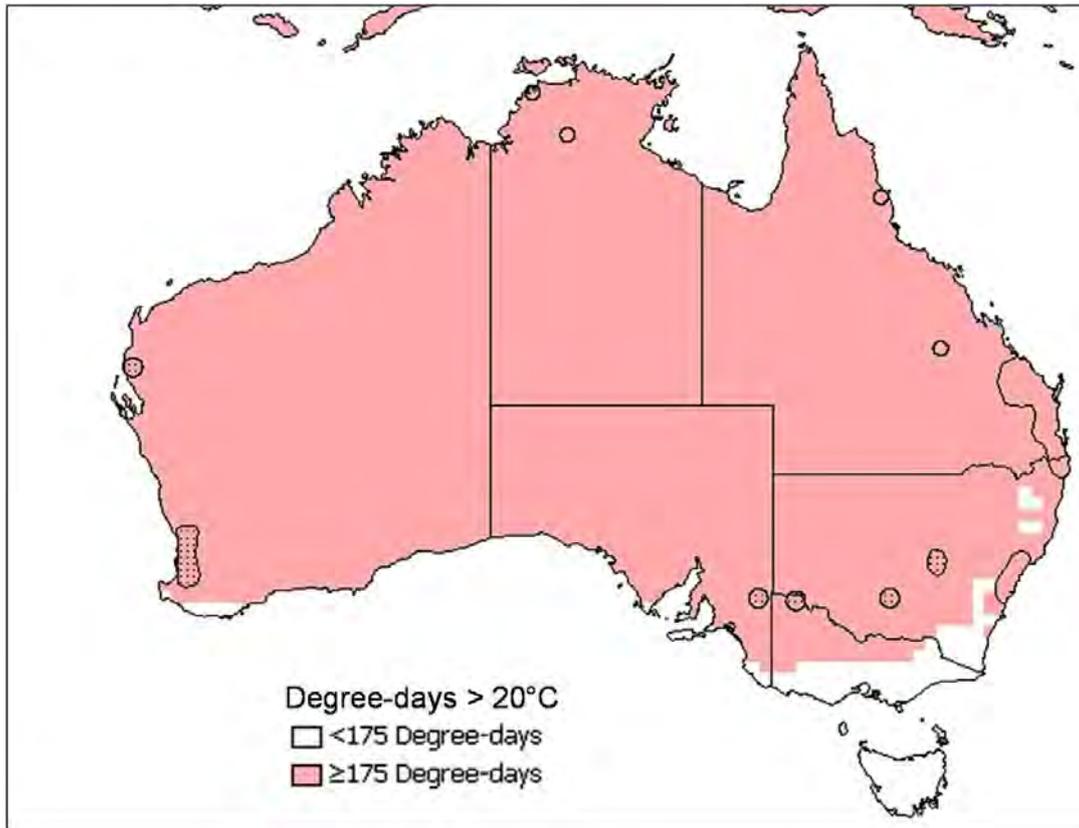


Figure 14. Map showing where heat accumulation is adequate for *G. citricarpa* to persist in Australia. Areas in white have fewer than 175 degree-days $>20^{\circ}\text{C}$ and are unsuitable for *G. citricarpa*.

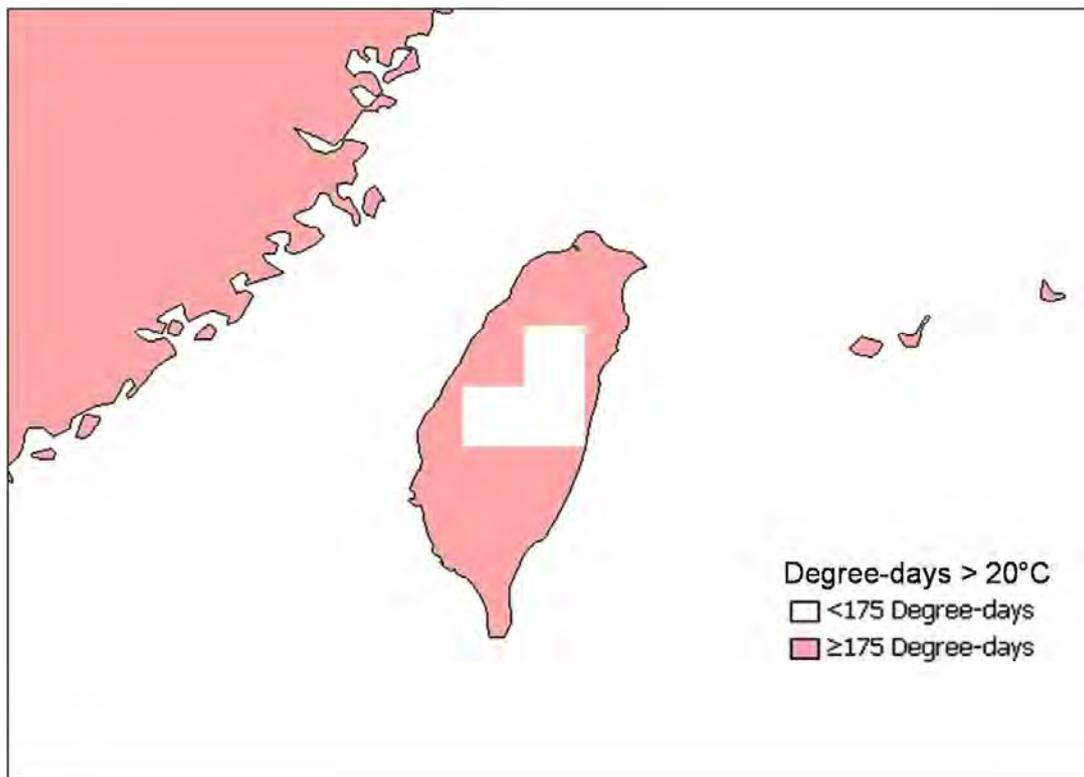


Figure 15. Map showing where the heat accumulation is adequate for *G. citricarpa* to persist in Taiwan. Areas in white have fewer than 175 degree-days $>20^{\circ}\text{C}$ and are unsuitable for *G. citricarpa*.

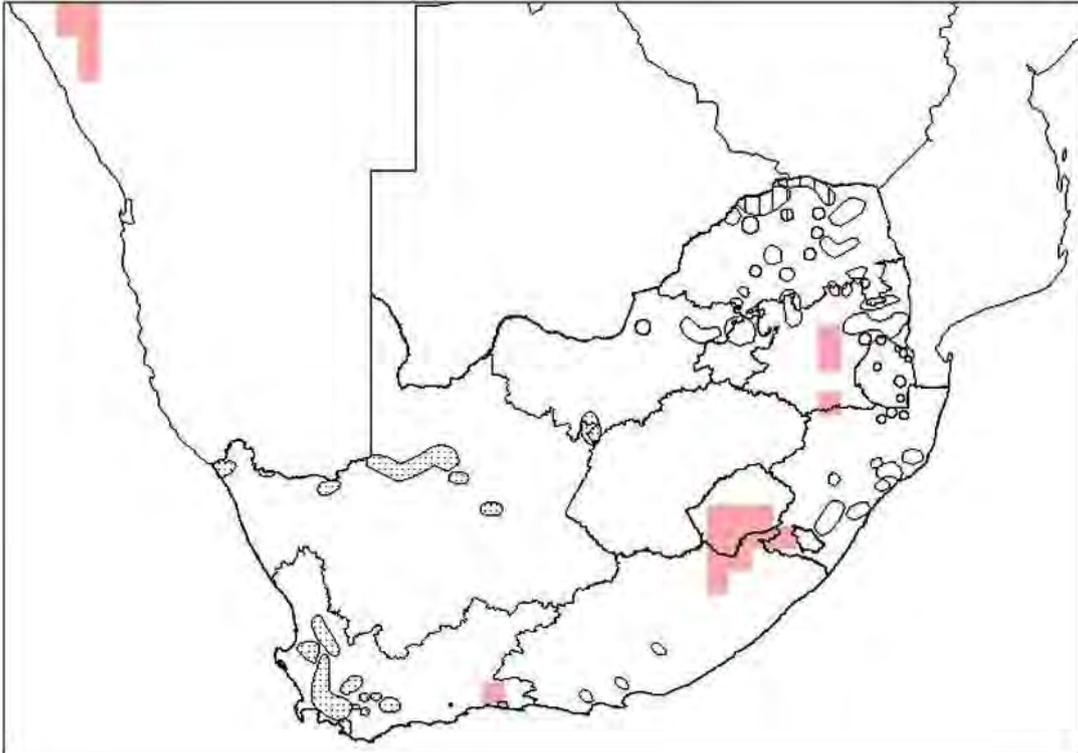


Figure 16. Additional areas in South Africa made unsuitable for *G. citricarpa* by including PDD in the CLIMEX model (shaded areas become unsuitable).

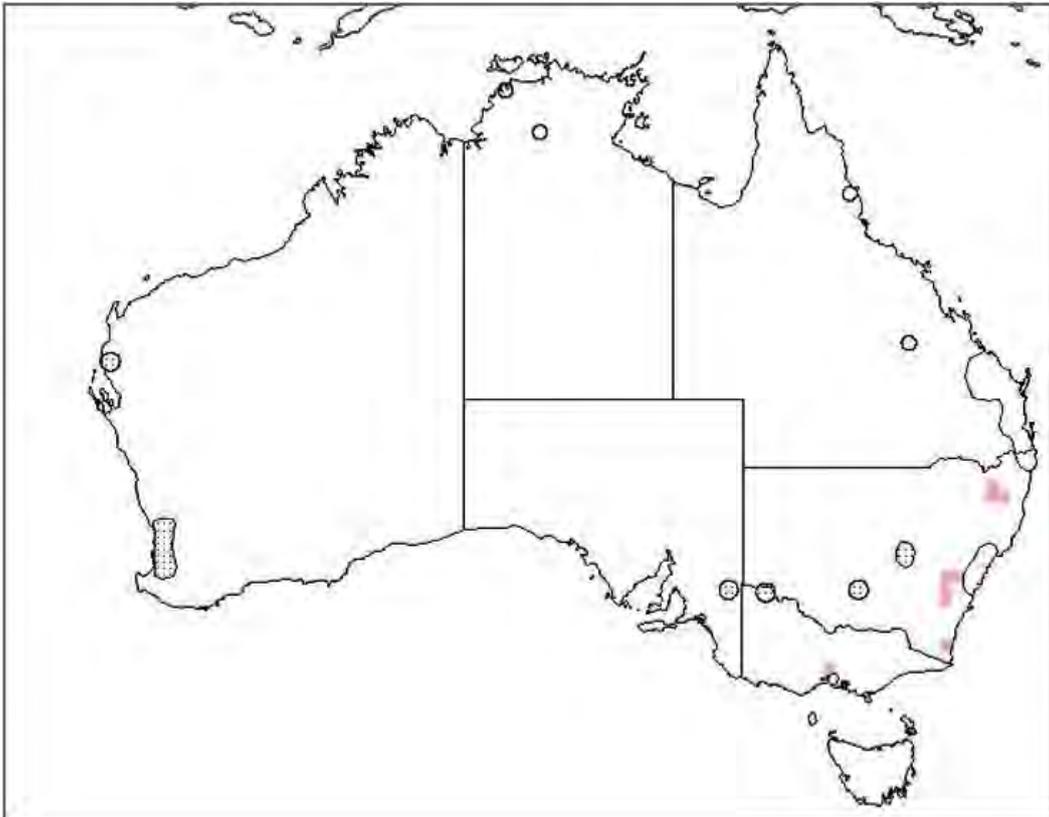


Figure 17. Additional areas in Australia made unsuitable for *G. citricarpa* by including PDD in the CLIMEX model (shaded areas become unsuitable).

2.15 CLIMEX Parameters and EI Values

Table 3 summarises the CLIMEX parameter values for *G. citricarpa*.

Table 3. CLIMEX parameter values for *G. citricarpa*.

CLIMEX Parameter	Description	Value
Moisture		
SM0	Lower soil moisture threshold	0.22
SM1	Lower optimal soil moisture	0.37
SM2	Upper optimal soil moisture	1.3
SM3	Upper soil moisture threshold	1.8
Temperature		
DV0	Lower temperature threshold	20°C
DV1	Lower optimal temperature	24°C
DV2	Upper optimal temperature	30°C
DV3	Upper temperature threshold	35°C
Heat Stress		
TTHS	Temperature threshold	35°C
THHS	Heat stress accumulation rate	0.0007
Dry Stress		
SMSD	Soil moisture dry stress threshold	0.22
HDS	Dry stress accumulation rate	-0.001
Wet Stress		
SMWS	Soil moisture wet stress threshold	1.8
HWS	Wet stress accumulation rate	0.005
Cold-Wet Stress		
DTCW	Degree-day threshold below which CWS will accumulate	10
MTCW	Soil moisture threshold above which CWS will accumulate	0.18
PCW	Cold-wet stress accumulation rate	0.00017
PDD		
PDD	Annual heat sum threshold	175
DVCS	Temperature threshold used to calculate degree-days for cold stress functions; default = DV0	20°C
DVHS	Temperature threshold used to calculate degree-days for heat stress functions; default = DV3	35°C

With these parameter values, CLIMEX accurately predicts the areas suitable for *G. citricarpa* in the three regions (Figures 18 to 22) used to fit the parameter values. There is limited extension of the predicted marginally suitable area into regions where the organism does not occur (southern central Western Cape Province and eastern Northern Cape Province). This could be due to a slight over-prediction by the CLIMEX model or it could be due to an absence of exposure to sufficient

inoculum pressure in the past. Given the limited extent of this discrepancy and the fact that it is restricted to isolated areas predicted to be only marginally suitable, the parameter values were deemed to be appropriate.

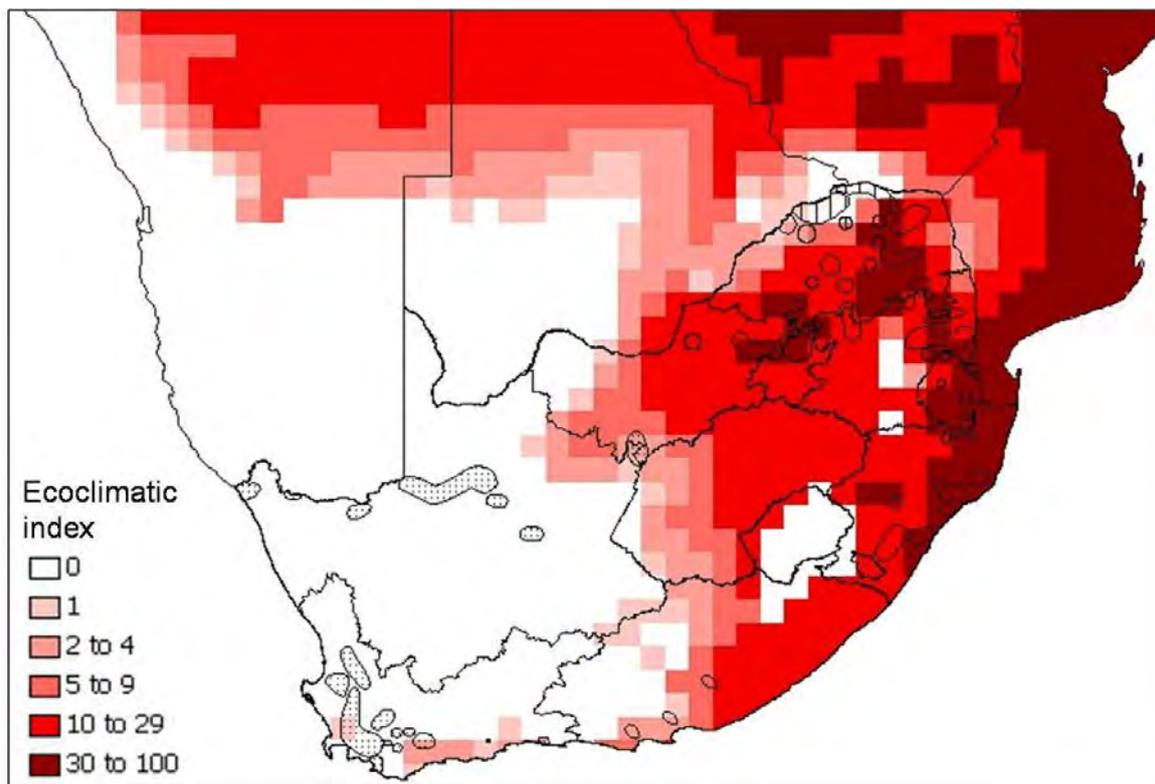


Figure 18. Predicted distribution of *G. citricarpa* in South Africa.

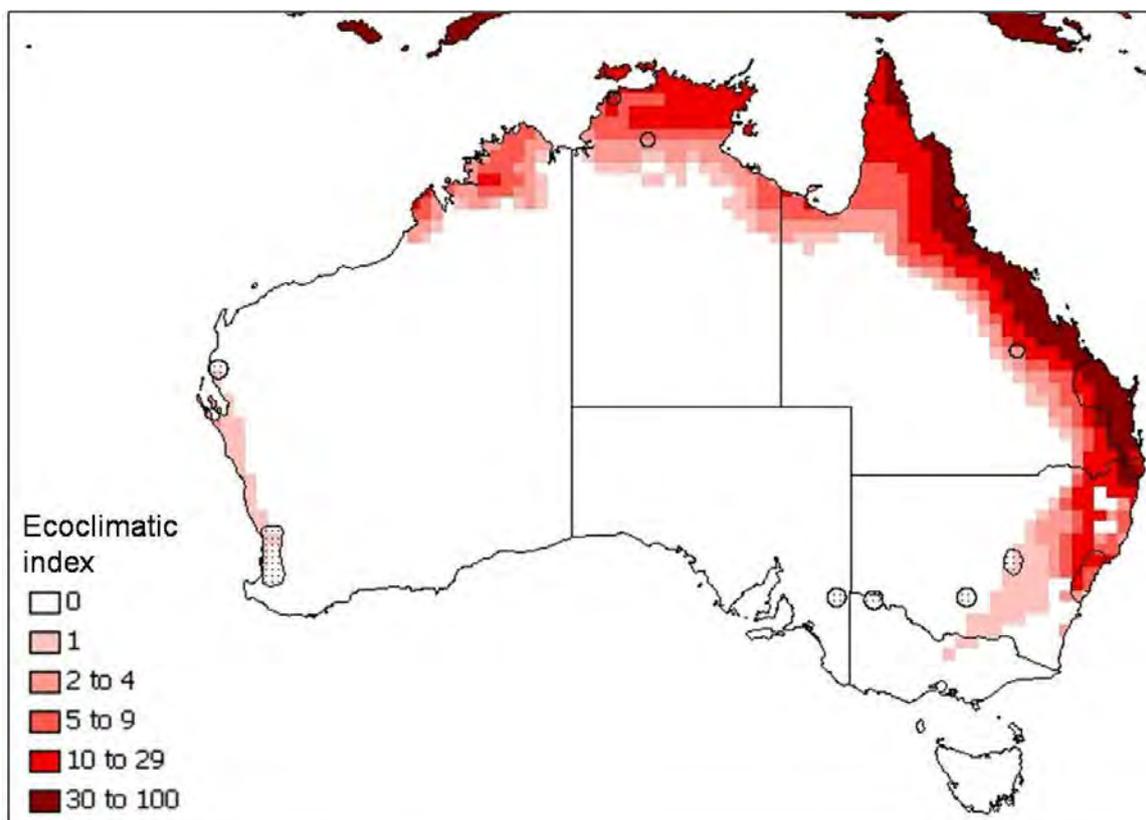


Figure 19. Predicted distribution of *G. citricarpa* in Australia.

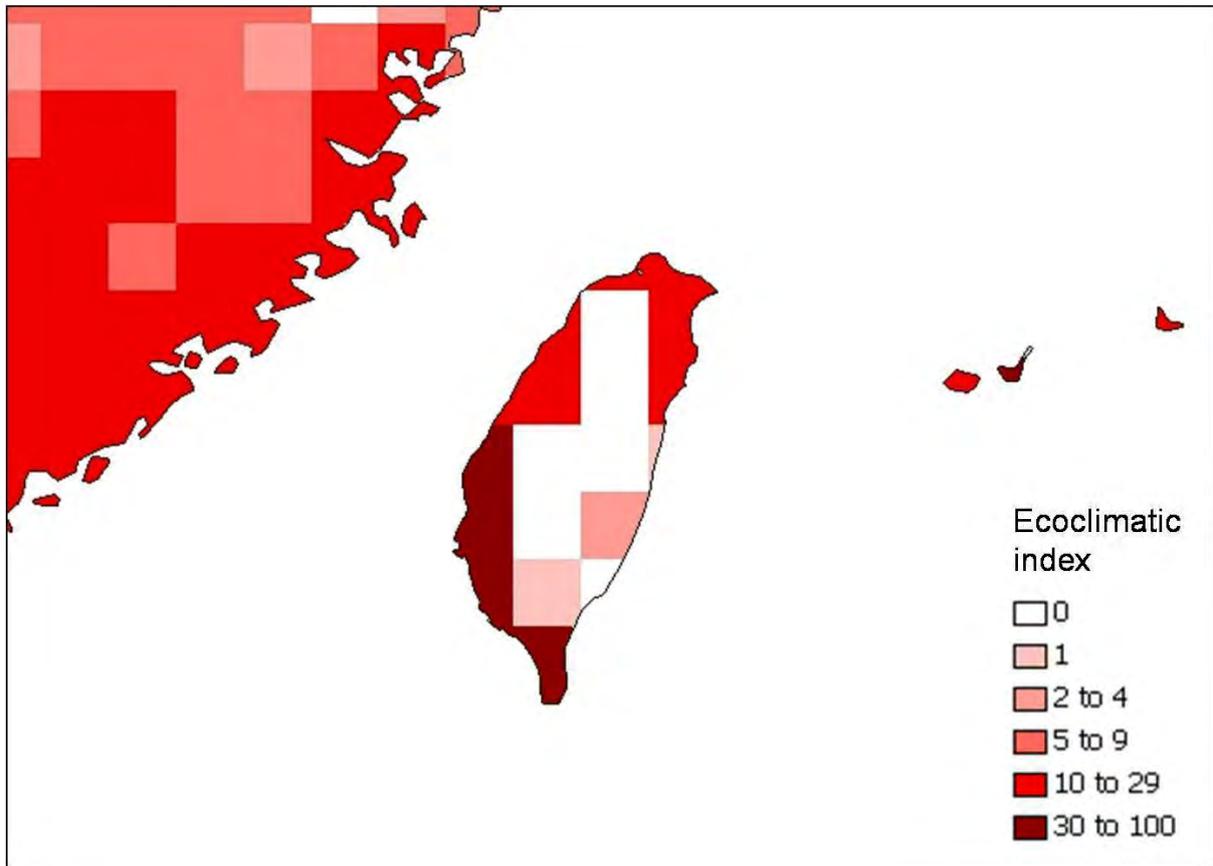


Figure 20. Predicted distribution of *G. citricarpa* in Taiwan.

2.16 Validation of Model

To validate the model, CLIMEX was run with the new parameter values for the continents of Africa and South America, to confirm that all known distribution points for *G. citricarpa* are predicted to be suitable. Results of these runs (Figures 21 and 22) confirm that this is the case.

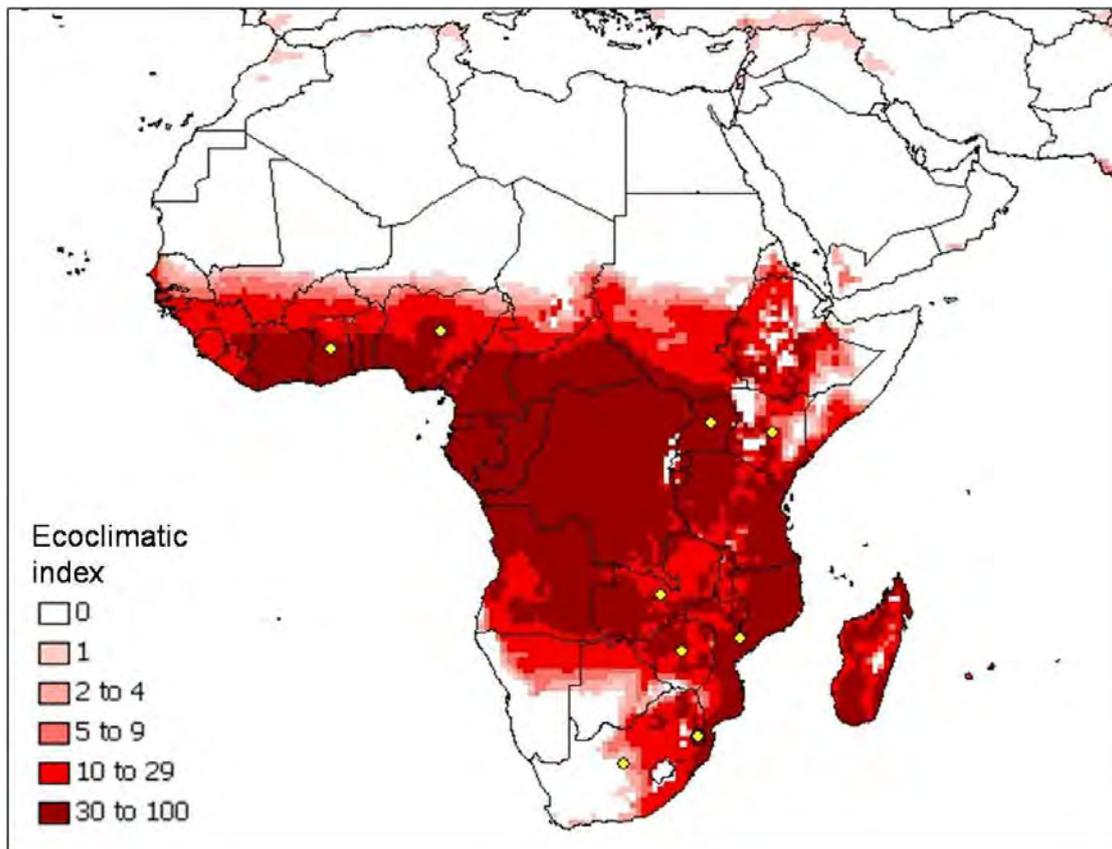


Figure 21. Predicted distribution of *G. citricarpa* in Africa. Symbols indicate countries where *G. citricarpa* is known to occur.

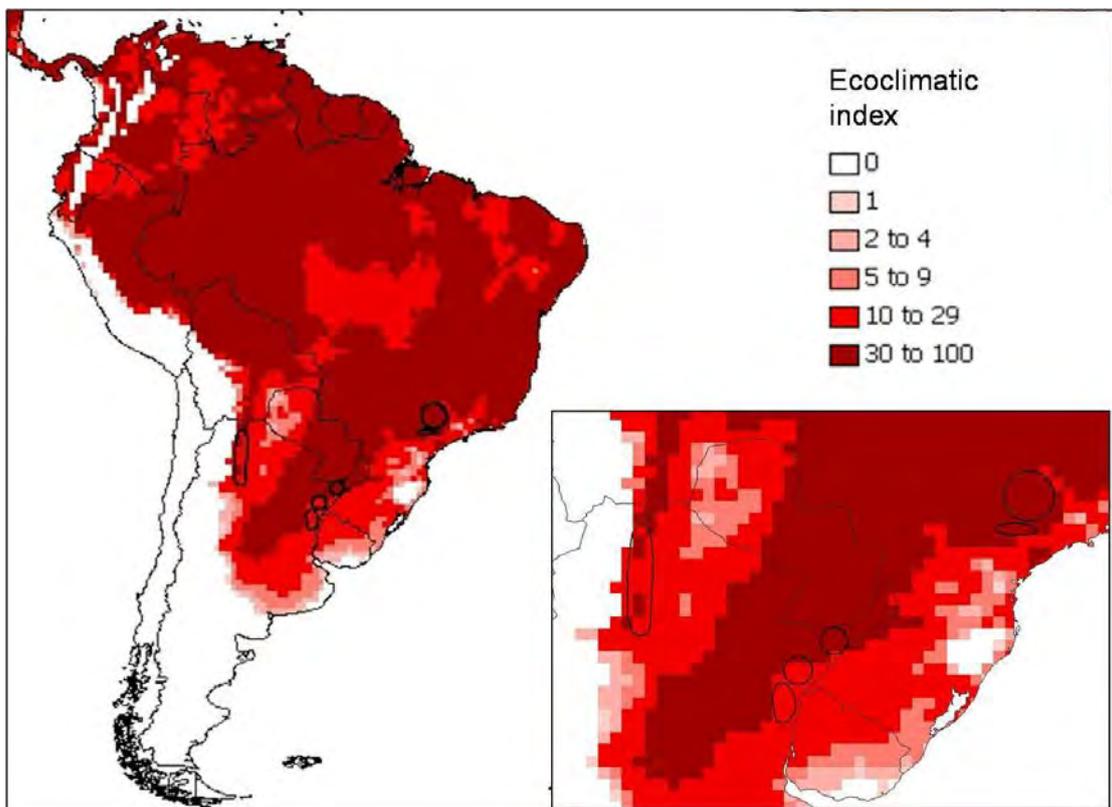


Figure 22. Predicted distribution of *G. citricarpa* in South America. Overlaid polygons indicate *G. citricarpa* occurrence.

2.17 Summary of CLIMEX Model, Including Reference to EFSA (2008) Criticisms

The new CLIMEX model for *G. citricarpa* is comprised of entirely new parameter values. In addition, Cold Stress has been replaced by Cold-Wet Stress, and both Dry Stress and PDD have been introduced into the new model.

Changes in the moisture parameters have resolved an issue with the predicted distribution of *G. citricarpa* in Taiwan. This particular issue was addressed by increasing both the SM2 and SM3 parameters, thereby predicting *G. citricarpa* to persist in much wetter locations than previously. Changes to both SM0 and SM1 restrict this species from slightly drier conditions than previously, whilst still ensuring that spring and autumn growth can occur in some of the drier areas of South Africa where *G. citricarpa* persists.

The temperature parameters were all re-examined and adjusted. Extensive attempts were made to reduce DV0 as suggested by EFSA (2008) and since there are indications in the literature to suggest that various aspects of the life cycle can be completed at temperatures lower than 17°C. However, it proved impossible to produce an acceptable distribution of *G. citricarpa* in South Africa with lower values of DV0. In fact, a closer examination of growth charts in the Western Cape Province (and other provinces) using DV0 at 17°C indicated that this temperature was enabling *G. citricarpa* to have a positive Growth Index in winter, which is contrary to well studied aspects of the disease cycle. DV0 had to be increased to 20°C to prevent winter growth. The other temperature parameters were also all adjusted, and are now all in line with published data. The EFSA (2008) criticisms of the Paul *et al.* (2005) temperature parameters were thoroughly investigated in the process of selecting the new parameter values.

Examination of the Paul *et al.* (2005) Cold Stress parameters supported the EFSA (2008) contention that these were very severe. Cold Stress *per se* was considered to be an inappropriate mechanism limiting the distribution of this species, and so was removed.

Changes were made to the Heat Stress parameters, partly as a result of re-fitting the temperature parameters, and partly as a result of a closer examination of the different Heat Stress mechanisms. The current Heat Stress parameters are consistent with the temperature parameters, and, despite the lack of any real data from which to set the values, are also biologically realistic.

EFSA (2008) commented on the lack of a Dry Stress mechanism in the Paul *et al.* (2005) CLIMEX model. Dry Stress has been included in the new model. The Dry Stress threshold was set equivalent to the lower soil moisture threshold for growth (SM0), and the rate of stress accumulation was set to preclude prediction of *G. citricarpa* occurrence from exceptionally dry areas of the world.

Because changes were made to the soil moisture parameters, the Wet Stress parameter values needed to be reconsidered. Where the original model of Paul *et al.* (2005) did not allow for any Wet Stress to occur at lethal levels anywhere in the world, the new parameter values preclude prediction of *G. citricarpa* occurrence from regions experiencing over 4500mm of rain annually (> 100mm per week).

G. citricarpa is not known to occur in Mediterranean climates, typified by hot dry summers and cold wet winters. Cold-Wet Stress was considered more appropriate as a limiting mechanism than Cold Stress. Parameter values were selected after detailed examination of the climates in numerous locations in South Africa, and the rate of stress accumulation was set so as to predict persistence of the species in known localities of occurrence in South Africa, Australia and Taiwan.

The final modification to the CLIMEX model was the introduction of PDD, to preclude *G. citricarpa* from occurring in areas where there is insufficient annual thermal accumulation. The PDD value was set to predict the persistence of *G. citricarpa* in the Patensie region (Eastern Cape Province) of South Africa, and enables all other known localities of this species to be predicted as suitable.

EFSA (2008) criticised Paul *et al.*'s (2005) use of an EI value of 4 to denote unsuitability, as this led to inconsistencies in Paul *et al.*'s (2005) analysis. The new parameter values provided above have been selected so that EI = 0 represents climatic unsuitability. Any value in excess of 1 denotes some level of suitability, with EI values of 1 to 4 being reflective of marginal climates, and higher EI values indicating increased levels of suitability.

3 Suitability of Europe for the Establishment and Persistence of *G. citricarpa*

As the current CLIMEX model provides a good fit to the known distribution of *G. citricarpa* where it occurs, it was run for Europe, using the same CRU CL1.0 gridded climate data (New *et al.* 2002).

Figure 23 maps the Growth Index of *G. citricarpa* for Europe. As indicated above, the GI is a composite of the Temperature Index (TI), defined by the four temperature parameters, and the Moisture Index (MI), defined by the four soil moisture parameters. In the absence of all other stresses or indices, this map indicates all of the areas where the TI and MI are both positive at the same time, resulting in conditions suitable for the growth of *G. citricarpa*.

As can be seen from Figure 23, much of central Spain, extending down to the south-eastern coast, is unsuitable, with a GI of 0. This is because when the TI is positive and temperatures are sufficiently warm (the period of approximately May through to mid-October), the MI is unsuitable. The alpine regions of Europe are unsuitable for *G. citricarpa*, since the TI is zero (temperatures never reach 20°C).

It is important to note that the maximum GI in Europe is 21. This is thus the maximum EI value possible, if no other restrictions are imposed by any of the stress factors. In reality, the overall distribution will be much smaller than that indicated by Figure 23. It is also noteworthy that areas with the higher GI values are not within the commercial citrus growing areas.

Factors which may further restrict the distribution of *G. citricarpa* in Europe are Heat Stress, Dry Stress, Wet Stress, Cold-Wet Stress, and PDD (annual sum of degree day heat accumulation). No Heat Stress accumulates anywhere; Dry Stress is irrelevant, with a maximum value of 7 in a tiny area on the eastern coast of Spain that already has a GI of 0; and Wet Stress is irrelevant, with a maximum value of 1 in

the south western corner of Scotland. However, both Cold-Wet Stress (Figure 24), and PDD (Figure 25) are limiting.

It is clear from Figures 24 and 25 that whilst there are areas of overlap, there are also areas where only one factor might preclude *G. citricarpa* from occurring. Thus, as was done for South Africa (Figure 16) and Australia (Figure 17), a map was produced for the areas in Europe that would be predicted as being suitable if PDD were not used (Figure 26).

Apart from two grid cells in north eastern Spain, the areas made unsuitable by including PDD are not within the citrus growing regions of Europe. Both grid cells in north eastern Spain would have an EI value of 1 if PDD were not included in the model, so they are at best only marginal.

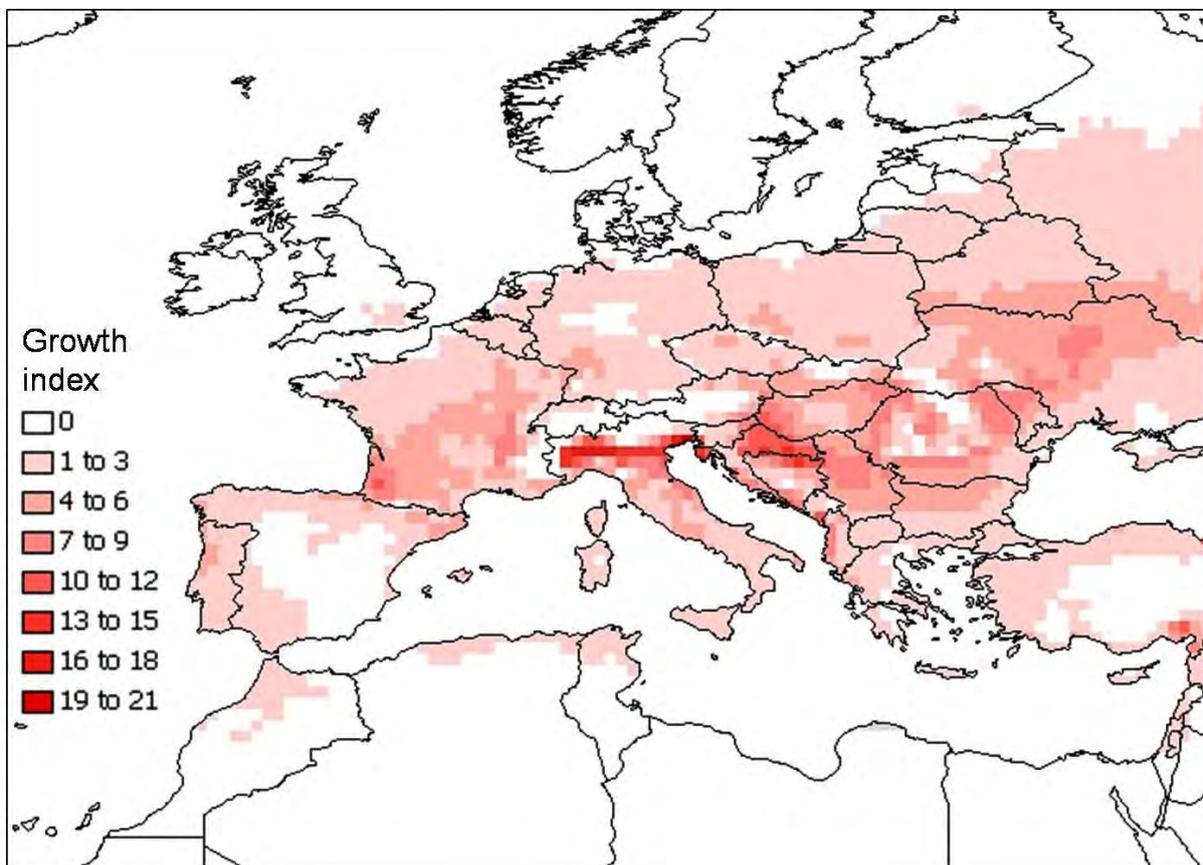


Figure 23. CLIMEX Growth Index for *G. citricarpa* in Europe. The highest GI value is 21.

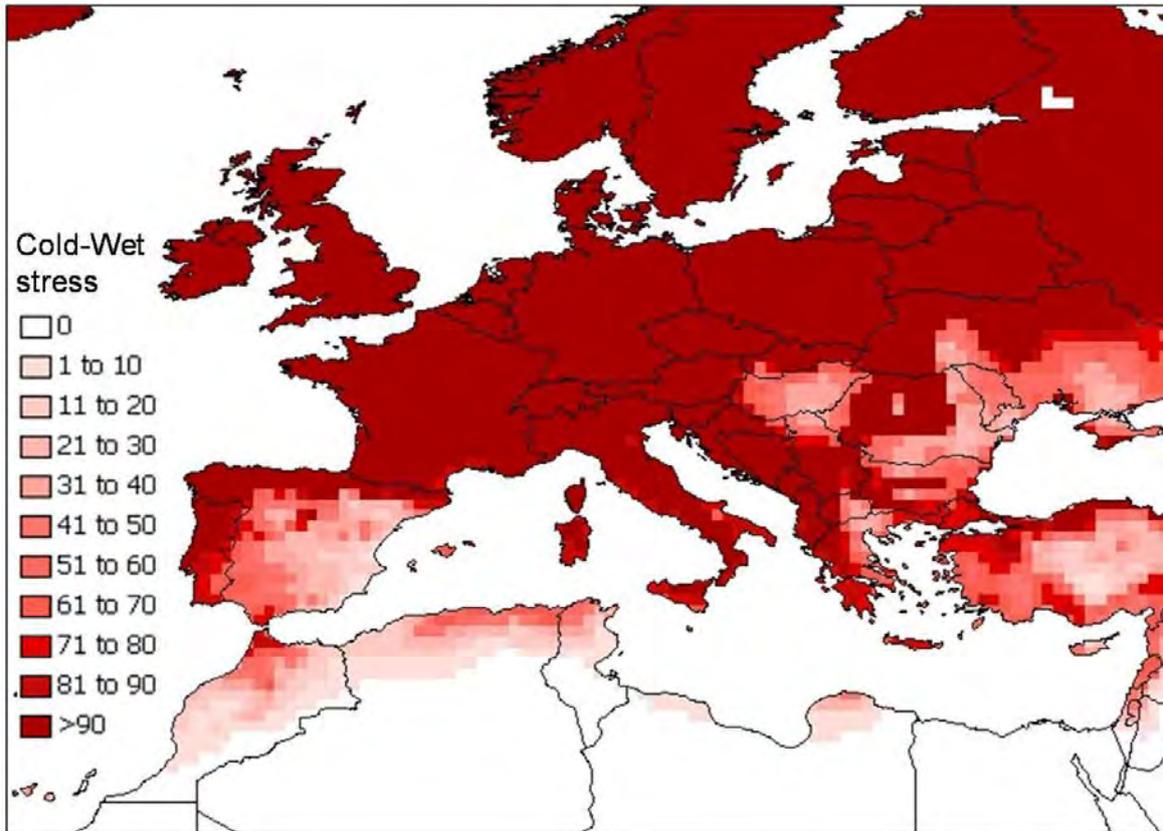


Figure 24. Cold Wet Stress Index for *G. citricarpa* in Europe.

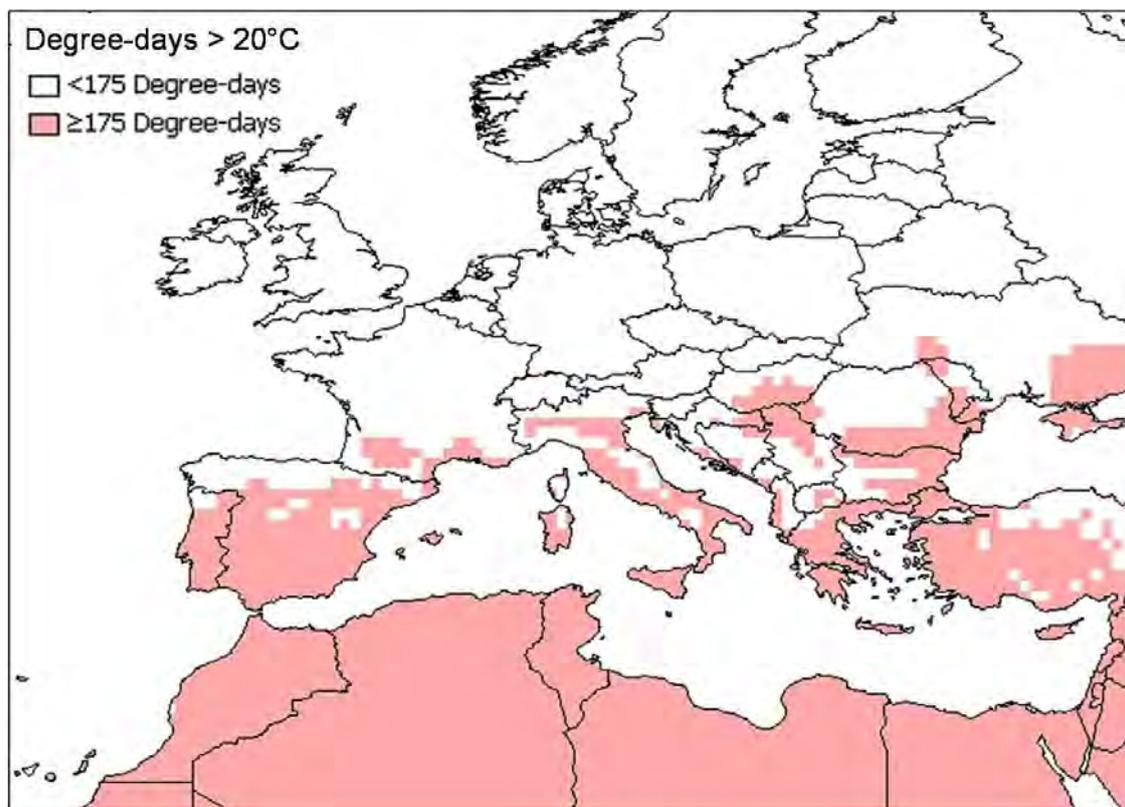


Figure 25. Map showing whether or not *G. citricarpa* can complete a generation in Europe. Areas in white have fewer than 175 degree-days >20°C and *G. citricarpa* will be precluded from these areas.

Using all of the parameter values given in Table 3, the potential distribution of *G. citricarpa* in Europe is shown in Figure 27. Spain is predicted to be unsuitable (EI = 0) to marginal (maximum EI = 3 in one grid cell); and Portugal, France, Italy and Greece are even more marginal, each with a maximum EI of 1 in restricted areas. Given the results presented in Figure 27, it is clear that the factor having the greatest impact on the distribution of *G. citricarpa* is CWS (Figure 24). Whereas Paul *et al.* (2005) showed Cold Stress to be the primary constraint to *G. citricarpa* in Europe, the use of Cold-Wet Stress in this model is considered to have greater biological relevance and hence be an improvement.

These results show that the maximum potential threat of *G. citricarpa* to the European citrus industry is very small indeed, and that if it were accidentally introduced to Europe, the chances of this pathogen establishing and persisting are minimal.

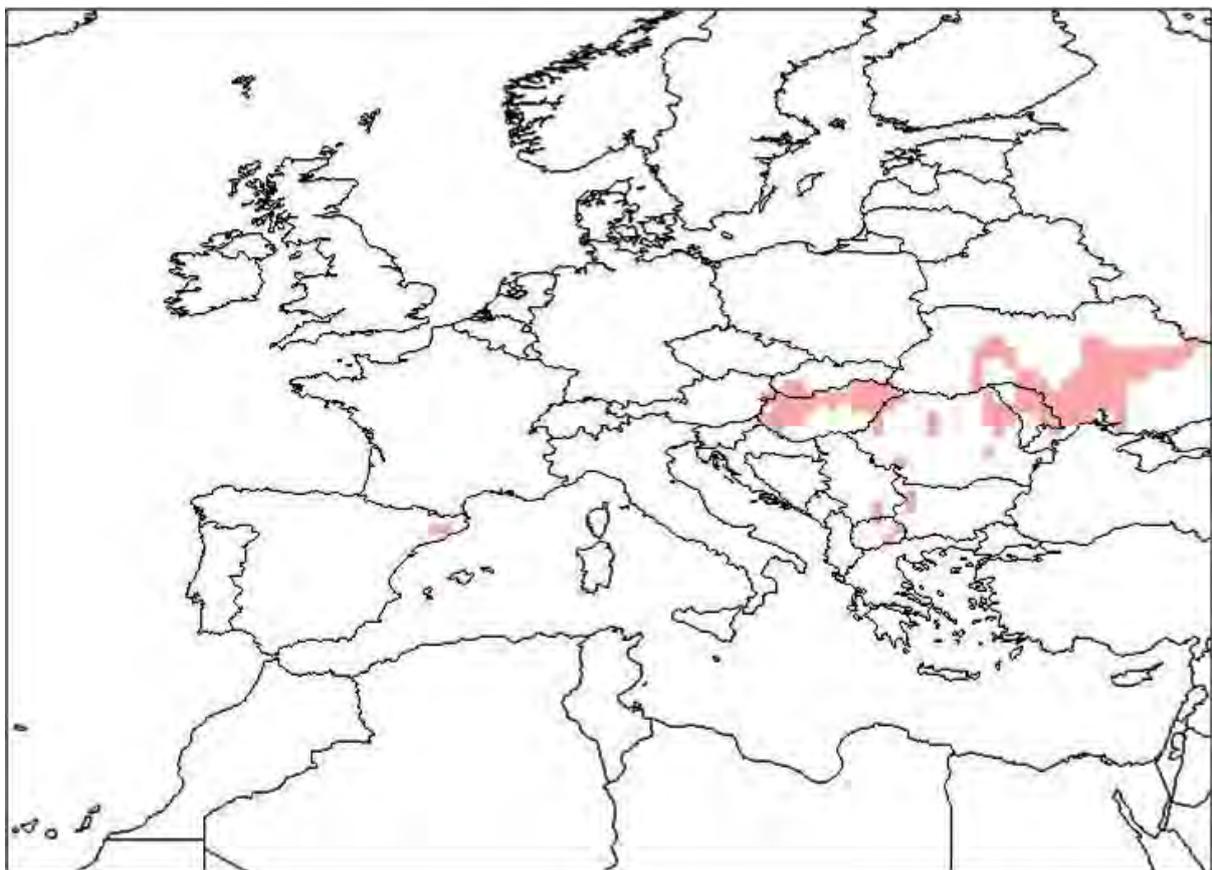


Figure 26. Map showing locations that would be predicted to be potentially suitable for *G. citricarpa* in Europe if PDD were not included in the CLIMEX model (shaded areas become unsuitable).

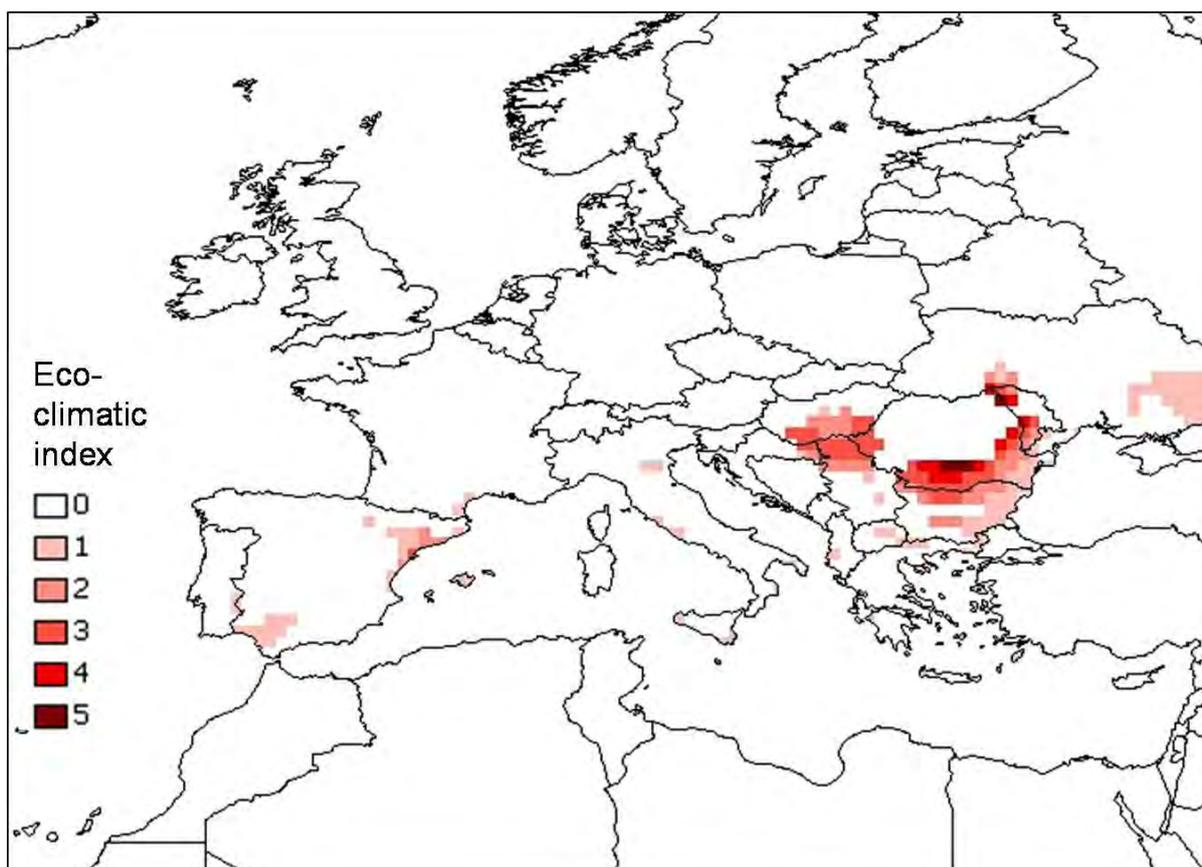


Figure 27. Predicted potential distribution of *G. citricarpa* in Europe. The highest EI value (in Romania, not a commercial citrus producing area) is only 5.

4 Responses to EFSA (2008) Regarding the Use of CLIMEX

4.1 Limitation of CLIMEX for use with pathogens

EFSA (2008) states that as a result of three factors there are “limitations of CLIMEX in predicting the potential distribution of pathogens such as *G. citricarpa*...”:

1. the relationship between pathogen infection and host phenology,
2. discrepancies between the pathogen and host’s climatic response, and
3. the importance of complex variables, such as leaf wetness, that are not taken into account by CLIMEX and may act at a much shorter time scale.

For the first factor, EFSA (2008) argue that the climate during the period of host susceptibility alone should be considered, rather than the climate over the entire year. Whilst it is true that climatic conditions must be suitable at the appropriate time of host susceptibility for the presence of *G. citricarpa* spores to potentially result in an infection, it is not true that a window of opportunity for host infection will necessarily lead to the permanent establishment of a population of *G. citricarpa*. An infection incident will not result in the establishment of a pathogen population unless the climate is suitable for the persistence of that population until the next infection incident can occur and a full life cycle can be completed. Furthermore this needs to recur persistently for there to be an opportunity for permanent establishment.

For the second factor, it is of course true that a pathogen and its host may have differing climatic responses. Thus, it may be that the host’s distribution is a limiting

factor to the pathogen's distribution, but it is equally possible that the pathogen has a more limited distribution than its host. In the case of *G. citricarpa* and citrus, there is evidence in South Africa that despite numerous introductions of this pathogen to citrus growing regions of the Western Cape Province, it has never established. In this case, the area is evidently climatically suitable for citrus, but apparently unsuitable for *G. citricarpa*. Because the CLIMEX model was accordingly parameterised so as to make this Western Cape region unsuitable, it also indicates that parts of Australia (South Australia and Victoria) are unsuitable for *G. citricarpa*, although Figure 7.8 in Paul's thesis (Paul, 2006) suggests that citrus is grown in this region. In other words, there are several citrus regions that the model predicts to be unsuitable for the long-term persistence of *G. citricarpa*. Thus, the CLIMEX model does in fact address the concerns of EFSA (2008) and appropriately indicates that the pathogen's distribution is more restricted than that of its host.

For the third factor, it is true that CLIMEX does not consider the effects of a whole range of complex variables (which may or may not be driven by climate), such as leaf wetness, and it is true that the time scale at which a factor such as leaf wetness occurs is very short by comparison to the time scale at which CLIMEX operates. However, such issues are related to the first factor, where EFSA (2008) argue that only short periods of climate should be considered, and the counter-argument remains the same: short periods of suitability that may result in an infection incident will not necessarily result in the establishment and persistence of the pathogen.

If the CLIMEX parameters are set so that CLIMEX correctly reflects the known records of presence and absence of *G. citricarpa*, and there has been ample opportunity for the distribution of the organism to expand to its full potential extent, especially if this spans a wide range of climates (as is the case with *G. citricarpa* in South Africa and Australia), then confidence in the model is justified. With appropriate parameter values, the interactions between the various temperature, moisture and stress indices in CLIMEX should reflect the climatic conditions that are suitable or otherwise for this species. Although soil moisture is used instead of leaf wetness, the soil moisture parameters at locations where *G. citricarpa* occurs (and does not occur) must nonetheless reflect suitable (or unsuitable) conditions. *G. citricarpa* requires leaf wetness, which in turn requires free water, i.e., rainfall. The soil moisture parameters should reflect the level of rainfall sufficient to allow *G. citricarpa* to persist, even in those areas where rainfall may be very low but still sufficient to enable this pathogen to grow and persist.

In summary, there is no reason that CLIMEX cannot successfully and adequately describe the potential distribution of a pathogen at a scale suitable for biosecurity concerns. The predicted distribution will be that defined by climate, and does not consider other factors; however, this should be seen as the maximum potential distribution. Other factors, such as host availability, host phenology, periods of suitable leaf wetness, soil type, etc., can only further limit the pathogen distribution within this range. Thus, the "true" distribution of the pathogen is likely to be smaller, not larger, than that predicted by CLIMEX.

4.2 EFSA (2008) criticism of the CLIMEX Match Climates technique

In the first instance, EFSA (2008) makes several criticisms of Paul's (2006) analysis using the CLIMEX Match Climates technique on the basis that this analysis is based

on the annual climate data rather than shorter time periods, when specific factors may allow for an infection event to take place. This is an erroneous criticism. CLIMEX defines the suitability of a location for the growth and persistence of a species – i.e., its establishment. If it is necessary to identify the possibility of an infection event at a specific time of year, rather than the establishment of the species, then the growth charts (available for any location) may be used to do so. What the Match Climates technique provides is a way of identifying the degree of similarity in the climates of different locations, so as to provide a very basic estimation of the suitability of a target location for the establishment of a species. Whilst the new version of CLIMEX allows somewhat more flexibility in the use of the Match Climates technique, in that more specific time frames may be considered in the analysis, the only advantage is to potentially better identify target locations susceptible to an infection occurrence. Such an analysis, using a specified time frame as opposed to the annual climate data, would provide no additional support for or against the potential establishment of the species at these target locations. In this regard, Paul's (2006) analysis was appropriately conducted to ascertain the potential suitability of European locations for the establishment of *G. citricarpa*.

Secondly, EFSA (2008) suggest that "...the 60% threshold limit applied is unlikely to provide an accurate indicator of climatic suitability for *G. citricarpa*". This is a valid criticism of Paul's (2006) analysis. When doing a Match Climates analysis, the Match Index value should be at least 85% to indicate a reasonable similarity in the climates and therefore indicate potential suitability of the target location. The lower the Match Index value, the less similar the climates being compared are, and thus the lower the degree of confidence that can be placed in making any predictions as to the suitability of the target location for the species. However, using a lower threshold would indicate a wider potential suitability than may be appropriate and hence may result in over-predicting potentially suitable target locations.

Thirdly, EFSA (2008) criticises the choice of the 16 stations employed by Paul (2006) to represent the climate where *G. citricarpa* occurs in South Africa, on the basis that the stations all have similar climatic conditions, and are not representative of the entire range of *G. citricarpa* in South Africa. Whilst this is a valid criticism, the use of the Match Climates technique is largely irrelevant to the discussion on whether or not *G. citricarpa* could establish and persist in Europe if it were introduced there. As stated by EFSA (2008), "The CLIMEX "match climates" technique does not take into account any biological information on the pest and therefore just provides a general index of climatic similarity". At best, this technique therefore only provides a very basic first estimation of the suitability of a target location for the potential establishment and persistence of a species. This type of analysis is only to be used as a first basis approximation of the likelihood of a species establishing somewhere. If the Match Index value between the climate of origin and that of the target location are very close, then it is probable that the species of interest could potentially establish at the target location. But that is as much as can be said. This sort of analysis is clearly far less meaningful than an analysis based on some level of biological information, as is the Compare Locations function in CLIMEX. If there is a robust model available for use with the Compare Locations function, then the Match Index analysis is irrelevant and unnecessary. It will not provide any additional or useful information.

In response to their criticisms of Paul's (2006) Match Climates analysis, EFSA (2008) conducted their own analyses of climates (temperature and rainfall patterns) in Europe and South Africa. As indicated above, such an analysis comparing climates can only indicate the likelihood of an infection event, not of the establishment of *G. citricarpa*.

4.3 EFSA (2008) criticism of the CLIMEX Compare Locations technique

Apart from the EFSA (2008) concerns addressed above, two other specific concerns were raised regarding the application of the CLIMEX "Compare Locations" technique used by Paul *et al.* (2005) and Paul (2006):

1. thirty year averages mask the annual variability and
2. irrigation in many areas of the world is essential for viable commercial citrus production. Some methods of irrigation, e.g. by flooding the soil, may greatly enhance the suitability of locations for CBS that have low EIs.

The first point is not a valid criticism when predicting population establishment, rather than isolated infection events. It is true that some years will be more favourable than others, and that if a species is introduced somewhere at the right time in a favourable year, infection may take place. However, in the absence of the continued recurrence of conditions that are suitable for repeated completion of the life cycle, the organism will not persist at the site because the climate is unsuitable for permanent establishment.

The second criticism by EFSA (2008) is not in fact a direct criticism of the Compare Locations technique in CLIMEX; however, it can be addressed here. Because flooding irrigation is not a common practice anymore, it can be discounted. Citrus is also not generally irrigated with overhead irrigation systems. Irrigation systems are either drip or micro jet. With drip systems, there is no deposition of water onto leaf litter surface and hence *G. citricarpa* is not influenced or affected (i.e., potential for growth is not enhanced) as this irrigation system does not provide the necessary leaf wetting. Micro jets are mini sprinklers positioned under the tree. These generally do not result in leaf wetting, but do deposit surface water onto leaf litter on the orchard floor beneath the trees. This may stimulate spore release from infected leaves with suitably developed fungal fruiting bodies. However, since there is a requirement for surface wetting of susceptible leaves and fruit for an infection event to take place, even micro jet irrigation on its own will not make an unsuitable climate more suitable.

Irrigation is not considered to directly enhance the suitability of a location for *G. citricarpa*, although it is recognised that irrigation is essential in maintaining citrus trees in some production areas. The current CLIMEX model was therefore constructed without the use of irrigation, and areas predicted to be suitable for *G. citricarpa* have sufficiently high soil moisture levels to enable this pathogen to persist in the absence of irrigation. The inclusion of the irrigation scenario available in the CLIMEX Compare Locations technique would increase the soil moisture and make drier areas more suitable. However, based on the current CLIMEX model and the goodness of fit between the predicted and observed distribution of *G. citricarpa*, the use of irrigation would not be a valid modification to the model.

5 Conclusions

A new CLIMEX model for *G. citricarpa* was built, resulting in a different parameter set to that developed by Paul in her thesis (2006) and used by Paul *et al.* (2005) and EFSA (2008). In building the new model, the best currently available climate data was used, and care was taken to document each of the new parameters and justify the final choice of parameter values.

The only areas considered to be totally unsuitable for this pathogen are those which have an Ecoclimatic Index (EI) of zero. A positive EI value indicates some level of suitability, ranging from marginal (EI ranges from 1 to 4) to optimal (EI \geq 30).

Whilst virtually all of the original parameter values have been examined and changed, much attention was given to re-fitting the temperature parameters. Attempts were made to reduce DV0 below its original value of 17°C. Ultimately, this proved to be impossible, and in the final model, DV0 is in fact increased to 20°C. This may seem anomalous in light of the temperature data in the literature. However, none of the published temperature data refer directly to infection. The increased temperature value accords with field information and well established knowledge relating to the disease cycle. The higher temperature value is furthermore defensible in a model that describes the entire life cycle of a species and the likelihood of its establishment and persistence, rather than just a particular life stage and/or the likelihood of an infection event.

The new parameters permit the new CLIMEX model to appropriately describe the current distribution of *G. citricarpa*, as well as its potential distribution worldwide. The new model indicates that whilst there is the potential for infection events to occur, it is highly unlikely that this pathogen could establish in Europe.

Finally, all of the criticisms made by EFSA (2008) regarding the original CLIMEX model described by Paul *et al.* (2005) have been addressed in some detail. Those criticisms thought to be valid have been acknowledged as such, and arguments have been presented to refute those criticisms considered to be groundless.

6 Acknowledgements

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Report:

**Rainfall and temperature comparison of citrus producing areas
with known presence or absence of *Guignardia citricarpa***

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Introduction

In a recent study, EFSA used a “match climates” approach to demonstrate climatic similarities between selected citrus producing localities in the European Union and localities in South Africa where Citrus Black spot occurs (EFSA, 2008a). This portion of the EFSA study can by no means be seen as conclusive as it did not include any citrus producing localities where CBS failed to establish (*i.e.* true-negative controls) despite repeated exposure to the pathogen, *Guignardia citricarpa*. The aim of the present study was therefore to expand on the “match climates” approach as employed by EFSA, but to include various true-negative localities as well as localities with varying levels of CBS prevalence. Additionally, monthly climate data were compared through statistical analysis.

Materials and Methods

Mean monthly rainfall (mm), minimum, average and maximum daily temperatures (°C) were collected for citrus producing locations in South Africa, Greece, Italy, Portugal and Spain (Table 1). The specific localities in Europe were chosen based on their ‘marginal suitability’ (EFSA, 2008a), specifically with regard to higher CLIMEX ecoclimatic index values (Paul *et al.*, 2005; EFSA, 2008b). The weather data from these localities were compared with those from localities in climatically variable areas in South Africa where relative CBS presence / absence can be verified (*i.e.* true positive and negative controls; Kotze, 1981; Paul *et al.*, 2005; Magarey *et al.*, 2009; E Carstens, V Hattingh, M Holtzhausen, CR Kellerman, JM Kotze, L Korsten, HF le Roux, I Paul, GC Schutte, SH Swart, M Truter, pers. comm.).

The monthly weather data from these citrus producing localities were grouped according to origin into Greece, Italy, Portugal and Spain for the European localities, and for the South African localities also according to the relative CBS prevalence or absence into Kwazulu-Natal, Limpopo+Mpumalanga for the moderate/high CBS prevalence sites, Low-CBS (Limpopo and Eastern Cape) and CBS absence (Western and Northern Cape provinces). Data were subjected to analyses of variance ($P = 0.05$) using the afore-mentioned groupings as qualitative variables. Fisher’s LSD tests for least significant difference at 95% confidence level were conducted to compare monthly means.

Implications of reduced winter and spring temperatures. In order to investigate the potential implications of reduced winter and spring temperatures on CBS, preliminary findings from a work in progress (Appendix A) were used, which involved analysis of 635 *Guignardia* ascospore dispersal events and the associated temperature-

related data (8 data sets from 2006/7 through 2008/9 at three localities). For comparative purposes, degree-day accumulation for selected Moderate-CBS, Low-CBS, CBS-absent sites in South Africa and selected European sites were calculated using 1 July as biofix and 10°C as base temperature (Lovell *et al.*, 2003) from monthly T_{\min} and T_{\max} data (Table 1).

Results and Discussion

For illustrative purposes, weather data from selected sites were plotted on a monthly basis in Figs 1-4. In order to compare northern and southern hemisphere climatic data, the data for the European localities were shifted by 6 months.

Rainfall. The CBS-prevalent sites, particularly those with a high relative abundance (moderate to high prevalence; Table 2 and Figure 1) such as Nelspruit, Hoedspruit and Letsitele (Limpopo+Mpumalanga), Eshowe and Pongola (Kwazulu-Natal), showed a clear summer rainfall pattern with *circa* 80% of total 450-800 mm annual rainfall occurring during the months of October to March. For localities with lower CBS abundance, grouped as Low-CBS Limpopo or E-Cape, the respective summer rainfall proportions were 87% of 352 mm, 64% of 453 mm, respectively. In contrast, the European localities in Portugal, Spain, Italy and Greece depicted by EFSA as 'moderately suitable' had clear winter rainfall patterns with generally less than 30% of annual rainfall in the corresponding period, *i.e.* April to September in the northern hemisphere. Likewise, the CBS-absent (W-Cape) sites receives only 21% of their mean 570 mm annual rainfall during October through March, and are also classed as a winter rainfall area. The CBS-absent sites in the Northern Cape province receive less than 155 mm rainfall per annum, of which 75% falls from October through March, *i.e.* a low-summer rainfall area.

Monthly rainfall in the localities with higher CBS prevalence, *i.e.* Limpopo+Mpumalanga and Kwazulu-Natal, were significantly higher than the CBS-absent and European sites during November through March. The European localities generally had significantly higher rainfall during April to September (mid-autumn, through winter to early-spring) compared with the Low-CBS localities in South Africa (Table 2). In October, rainfall at the European localities was still markedly higher than the Low-CBS (Limpopo) locality, but similar to that observed at the Eastern Cape Low-CBS localities. The Western Cape rainfall pattern was generally similar to those described for the European sites, especially Portugal, Spain and Italy. Similar to what can be observed for the CBS-absent sites in the Western Cape, the European sites had 21-51%, 40-72%, 59-95% and 48-94% less rainfall during November, December, January and February, respectively, than recorded for the Eastern Cape province's Low-CBS sites. It can therefore be concluded that the European sites had a rainfall pattern similar to that of the CBS-absent sites in the Western Cape, and quite dissimilar to that of the Low-CBS sites in the Eastern Cape province.

Temperature. Clear divergence in the monthly average temperatures (T_{avg}) during the autumn, winter and spring can be observed between CBS-prevalent and -absent sites (Fig. 2). These differences are largely attributed to lower maximum daily temperatures (T_{max} ; Fig. 3), as the minimum daily temperatures (T_{min} ; Fig. 4) did not differ as much. In fact, T_{min} values of the Low-CBS sites in the Eastern Cape did not differ significantly from the European sites or the CBS-absent sites in the Western Cape from late-winter (August) through spring (September to November), although these sites differed significantly from the moderate-CBS sites in Kwazulu-Natal and Limpopo+Mpumalanga, as well as the Low-CBS site in Limpopo (Table 2). T_{max} means of the Spain and

Italy sites from May through to November (late-autumn, winter and spring) were significantly lower than the CBS-prevalent sites, specifically also the Low-CBS sites in the Eastern Cape, where T_{\max} values were in turn statistically lower than those recorded at the Kwazulu-Natal, Limpopo and Mpumalanga sites from April through November. T_{avg} means of the Low-CBS sites in the Eastern Cape were also significantly lower than the more sub-tropical sites in Kwazulu-Natal, Limpopo and Mpumalanga throughout the year. However, the Low-CBS sites in the Eastern Cape were generally significantly warmer (T_{avg}) than the European sites from June through November.

Interestingly, the CBS-absent sites in the Western Cape province were significantly cooler (T_{\max}) than the Low-CBS sites in the Eastern Cape, but significantly warmer than the European sites from May through November. In terms of T_{avg} , the differences between the CBS-absent sites in the Western Cape province and the Low-CBS sites in the Eastern Cape were also apparent, although not significant from May through July. Nonetheless, the Western Cape's CBS-absent sites were significantly warmer than the Greece sites from August through October, than the Italy sites from July through November, than the Portugal sites from October through December and significantly warmer than the Spain sites during October

Implications of reduced winter and spring temperatures. Preliminary results from the work summarised in Appendix A demonstrated that the 5th percentile for T_{\min} varied from 13.9 to 16.3°C with a mean of 15.3°C, and the mean for the 25th percentile for T_{\min} was 18.2°C (16.7 to 19.2°C). The 5th percentile of mean daily T_{\min} for the 7 days leading up to a spore event was 15.9°C (15.3 to 16.6°C), and the 25th percentile was 18.4°C (17.3 to 19.2°C). These observations support previous reports for ascospore dispersal and germination at temperatures above 15°C (Kotzé, 1963; Lee and Huang, 1973; McOnie, 1964). Mean daily T_{\min} during the spring months were below 15°C for the Low-CBS sites in the Eastern Cape and the European sites and ascospore dispersal, and large numbers thereof, might therefore not be expected during these months. T_{\min} values higher than 15°C were only recorded during December through March in Low-CBS sites in the Eastern Cape, CBS-absent sites in the Western Cape and European sites. In contrast, T_{\min} means for the Kwazulu-Natal, Limpopo and Mpumalanga sites exceeded 15°C in October. Moreover, T_{avg} values in the Mediterranean sites during spring months (<19°C) were sub-optimal for ascospore germination and infection (*i.e.* 25°C) and would most likely prolong the wetness duration required for successful infection (*i.e.* 15-38 hours of wetness at 15-29.5°C; Kotze, 1963).

Lower late-winter and spring temperatures will also delay ascospore maturation, therewith potentially causing asynchrony between the pathogen and the host (Kiely, 1948; Kotzé, 1981; Lee and Huang, 1973). Empirical evidence of the importance of higher late-winter and spring temperatures was obtained in the descriptive statistics of degree-day data of 635 *Guignardia* ascospore dispersal events (Appendix A). The mean 5th and 25th percentiles for degree-days accumulated from 1 July until days on which ascospores were trapped were 932.5°C and 1329.9°C, respectively. Degree-days accumulated from 1 July were plotted over time for selected moderate/high CBS sites in Limpopo and Mpumalanga, Low-CBS sites in the Eastern Cape, CBS-absent sites in the Western Cape and European sites (Fig. 5) in order to predict ascospore dispersal. Onset (5th percentile) of ascospore dispersal was predicted during the first two weeks in October for the Limpopo and Mpumalanga sites, Letsitele and Nelspruit, and the 25th percentile approximately one month later. For the Low-CBS sites in the Eastern Cape province, onset of ascospore dispersal was predicted for mid-November, and first quartile of spore events during mid-December only. This is in accordance with actual observations in South Africa (Kotze, 1981;

unpublished report of one season's trapping data in Addo, South Africa; Dr GC Schutte and Dr SH Swart, pers. comm.).

Of the European sites, Valencia and Messina accumulated degree-days faster than Pontecagnano, but markedly slower than the Western Cape CBS-absent sites, Stellenbosch and Citrusdal. Valencia and Messina had similar degree-day accumulation curves, with onset of ascospore dispersal predicted for beginning of December (one month later than the Low-CBS sites in the Eastern Cape province) and 25th percentile one month later (summer). Pontecagnano with its cooler temperatures accumulated degree-days slowly and pseudothecium maturation and first ascospore release was predicted for beginning of January only (25th percentile during mid-February).

Conclusion

Comparison of long term rainfall and temperature data from selected European localities [suggested by the EFSA study as the most 'suitable' sites in the EU for CBS establishment (EFSA, 2008a)] and localities in South Africa with proven presence or absence of CBS (*i.e.* true positive and negative controls) clearly demonstrated that climates of these European localities varies considerably from the CBS-prevalent sites. Although these localities showed similarities in rainfall with some low-prevalence sites during early-spring months, November and summer rainfall was 21-51% and 40-95% less, respectively. Hence, this climate comparison study supports the EFSA-study that the Mediterranean summer is unsuitable for any form of *G. citricarpa* infection, most likely due to dry-stress as was suggested by EFSA. Thus, when comparing rainfall alone, only spring and autumn months might offer sufficient wetness to sustain infection.

For pycnidiospore infection, lower Mediterranean spring temperatures (daily averages ranging from 11.75 to 18.9°C) will also limit the probability of successful pycnidiospore infections as the optimum temperature for such infections are reported to be 25°C (Kotze, 1981). For ascospore infection during spring this argument will also apply. Moreover, it is highly unlikely that ascospore inoculum would be available during spring: the significantly lower average and maximum daily temperatures during winter and spring at these EU localities would most likely delay ascospore dispersal into the very dry Mediterranean summer months when infection would be highly improbable (EFSA, 2008a). During the autumn months, the EU localities had similar temperatures and similar or higher rainfall compared with the low-prevalence sites in the Eastern Cape, and leaves this period as the only potentially suitable period for ascospore infection.

Thus, for successful ascospore infection, a prerequisite for CBS establishment (as was acknowledged by EFSA), ascospore dispersal needs to be delayed until autumn, which is asynchronous with fruit susceptibility. As most leaves fall during spring (Erickson and Brannaman, 1960), this delay is also approaching or exceeding the maximum limit (180 days) reported for pseudothecium maturation (Kotze, 1981). Through use of sprinkler- or micro-irrigation during the hot summer months and following sufficient degree-day accumulation, it should be considered that ascospore dispersal in these EU climates might nonetheless occur during summer. Consequently, the ascospore inoculum source will be substantially depleted by the time weather conditions are marginally suitable for infection during autumn. An additional limiting factor that should be considered is the unfavourable summer conditions through which fallen leaves should be preserved, in order to sustain pseudothecium maturation and ascospore dispersal in autumn.

In conclusion, this study clearly demonstrates that the European localities shown by EFSA to be the most 'suitable' sites in the EU for CBS establishment had climates similar to low CBS-prevalence localities in autumn only. Most notably, the limiting factors for CBS establishment in these European localities are colder winter and spring temperatures and dry summers. Thus, for *G. citricarpa* to establish in these climatic conditions, it would have to behave as it never has before in any part of the world, *i.e.* primary infection period in autumn as opposed to late-spring and summer.

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Table 1. Citrus producing locations for which climate data was sourced

Location	CBS prevalence	Data sourced*	Weather station information			
			Coordinates	Altitude	Years	Source
South Africa						
Nelspruit (Mpumalanga)	Moderate	Rain, Tmin-avg-max	25.45°S 30.97°E	660	65	ARC Agromet**
Letsitele (Limpopo)	Moderate	Rain, Tmin-avg-max	23.87°S 30.32°E	623	34	ARC Agromet
Messina (Limpopo)	Low	Rain, Tmin-avg-max	22.27°S 29.90°E	522	68	ARC Agromet
Hoedspruit (Limpopo)	Moderate	Rain, Tmin-avg-max	24.32°S 30.90°E	457	6	ARC Agromet
Pongola (Kz-Natal)	Moderate	Rain, Tmin-avg-max	27.41°S 31.59°E	321	8	ARC Agromet
Eshowe (Kz-Natal)	Moderate	Rain, Tmin-avg-max	28.72°S 31.53°E	154	34	ARC Agromet
Kirkwood (E-Cape)	Low	Rain, Tmin-avg-max	33.40°S 25.35°E	96	16	ARC Agromet
Addo (E-Cape)	Low	Rain, Tmin-avg-max	33.57°S 25.70°E	85	21	ARC Agromet
Coerney (E-Cape)	Low	Rain, Tmin-avg-max	33.45°S 25.75°E	152	21	ARC Agromet
Adelaide (E-Cape)	Low	Rain, Tmin-avg-max	32.67°S 26.28°E	600	28	ARC Agromet
Fort Beaufort (E-Cape)	Low	Rain, Tmin-avg-max	32.78°S 26.63°E	456	32	ARC Agromet
Somerset-East (E-Cape)	Low	Rain, Tmin-avg-max	32.73°S 25.58°E	717	17	ARC Agromet
Addo (E-Cape)	Low	Rain, Tmin-avg-max	33.57°S 25.70°E	85	21	ARC Agromet
Patensie (E-Cape)	Low	Rain, Tmin-avg-max	33.77°S 24.82°E	93	29	ARC Agromet
Citrusdal (W-Cape)	Absent	Rain, Tmin-avg-max	32.57°S 18.98°E	198	30	ARC Agromet
Stellenbosch (W-Cape)	Absent	Rain, Tmin-avg-max	33.90°S 18.87°E	146	36	ARC Agromet
Upington (N-Cape)	Absent	Rain, Tmin-avg-max	28.45°S 21.25°E	793	56	ARC Agromet
Kakamas (N-Cape)	Absent	Rain, Tmin-avg-max	28.78°S 20.61°E	720	31	ARC Agromet
Greece						
Kerkyra	Absent	Rain, Tavg	39.61°N 19.89°E	2	10	www.worldclimate.com
Andravidia	Absent	Tavg	37.92°N 21.20°E	17	44	www.worldclimate.com
Naxos	Absent	Tavg	37.10°N 25.30°E	9	10	www.worldclimate.com
Italy						
Messina	Absent	Rain, Tmin-avg-max	38.20°N 15.50°E	59	30	www.worldclimate.com
Pontecagnano	Absent	Tmin-avg-max	40.60°N 14.90°E	30	27	www.worldclimate.com
Salerno-Pontecagnano	Absent	Rain	40.61°N 14.91°E	19	30	www.euroweather.net
Cozzo Spadaro	Absent	Tmin-avg-max	36.70°N 15.10°E	51	30	www.worldclimate.com
Cozzo Spadaro	Absent	Rain	36.68°N 15.13°E	51	30	www.euroweather.net
Napoli/Capodichino	Absent	Rain, Tmin-avg-max	40.90°N 14.30°E	72	30	www.worldclimate.com
Portugal						
Faro	Absent	Rain, Tavg	37.02°N 7.90°W	7	24	www.worldclimate.com
Lisbon	Absent	Rain, Tavg	38.70°N 9.10°W	95	125	www.worldclimate.com
Sagres	Absent	Rain, Tavg	37.00°N 8.90°W	25	18	www.worldclimate.com
Spain						
Rota Nas	Absent	Tmin-avg-max	36.65°N 6.35°W	27	7	www.worldclimate.com
Sevilla	Absent	Rain, Tavg	37.36°N 6.00°W	8	68	www.worldclimate.com
Moron de la Frontera	Absent	Tmin-max	37.18°N 5.60°W	91	10	www.worldclimate.com
Valencia*	Absent	Rain, Tmin-avg-max	39.50°N 0.46°W	62	30	www.euroweather.net

*Longterm monthly averages for rainfall and daily temperature (minimum, average and maximum).

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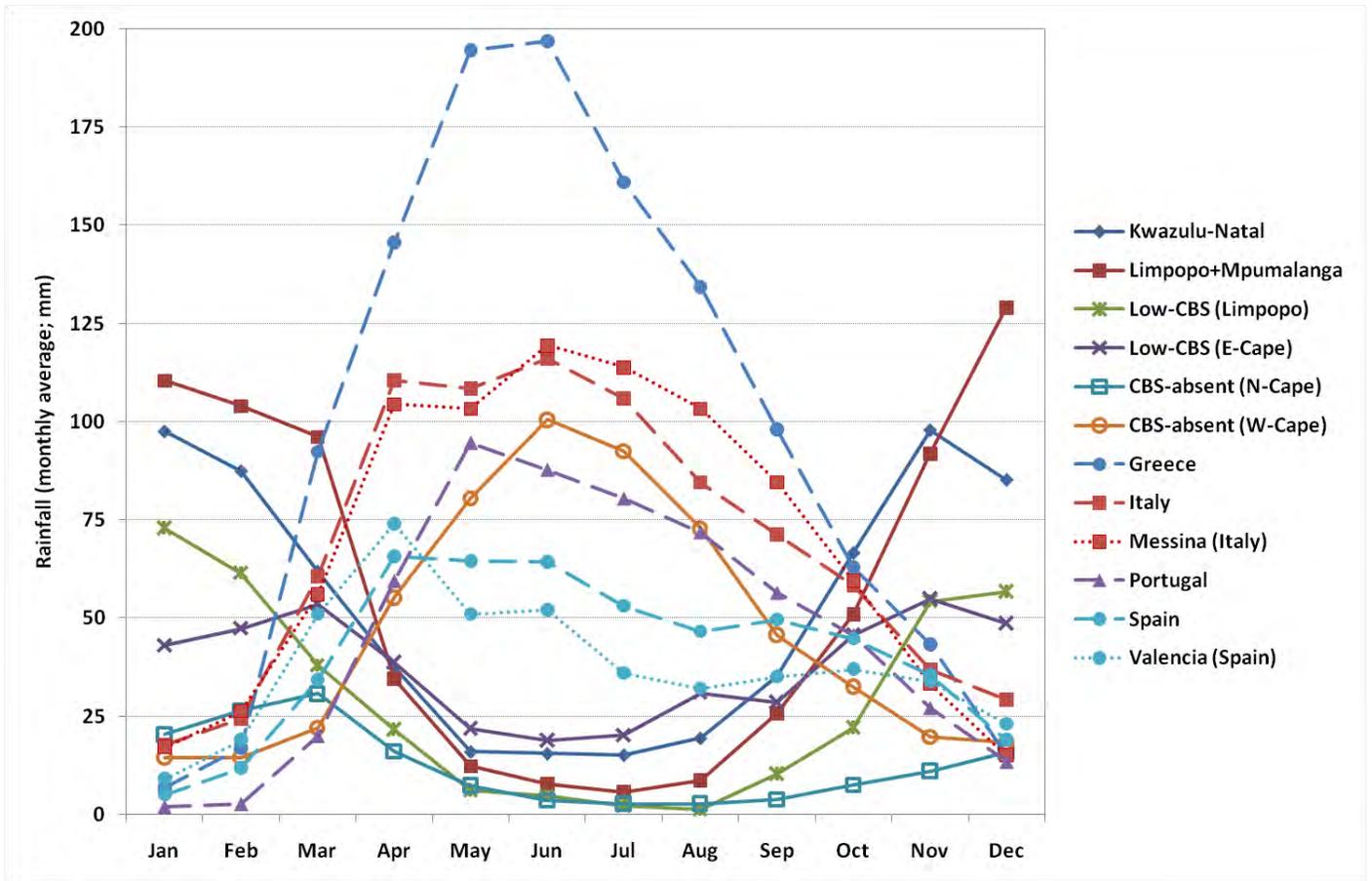


Figure 1. Means of long term averages for monthly rainfall for selected citrus producing localities in the European Union (Greece, Italy, Portugal and Spain) compared with localities in South Africa where *Guignardia citricarpa* has established causing moderate to high levels (Kwazulu-Natal, Limpopo and Mpumalanga provinces), or low to moderate levels (Low-CBS in Limpopo and Eastern Cape provinces) of Citrus Black Spot, and localities where it has failed to become established (CBS-absent in Northern and Western Cape province). Data for the European localities were shifted by 6 months.

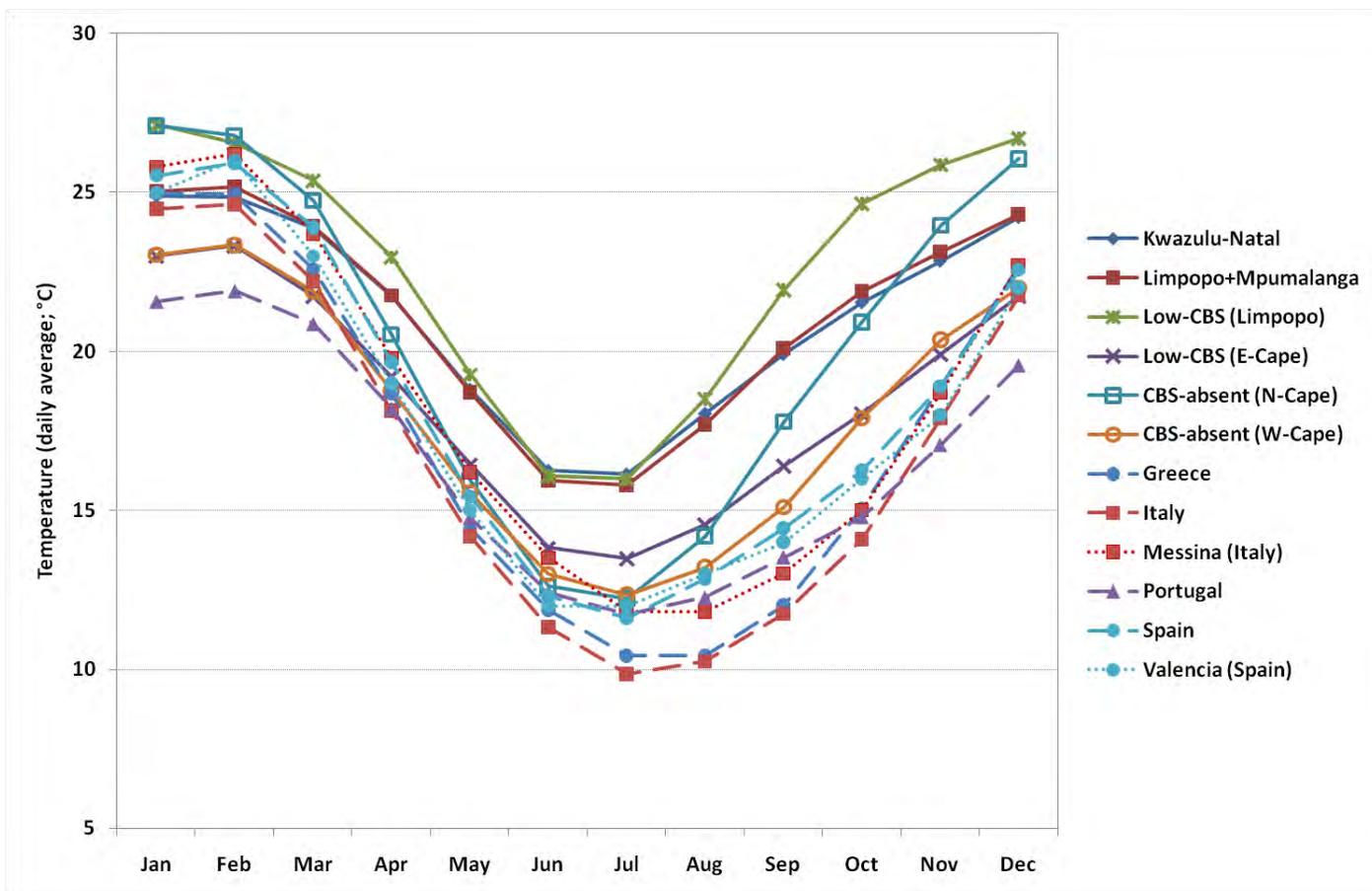


Figure 2. Means of long term monthly averages for mean daily temperatures for selected citrus producing localities in the European Union (Greece, Italy, Portugal and Spain) compared with localities in South Africa where *Guignardia citricarpa* has established causing moderate to high levels (Kwazulu-Natal, Limpopo and Mpumalanga provinces), or low to moderate levels (Low-CBS in Limpopo and Eastern Cape provinces) of Citrus Black Spot, and localities where it has failed to become established (CBS-absent in Northern and Western Cape province). Data for the European localities were shifted by 6 months.

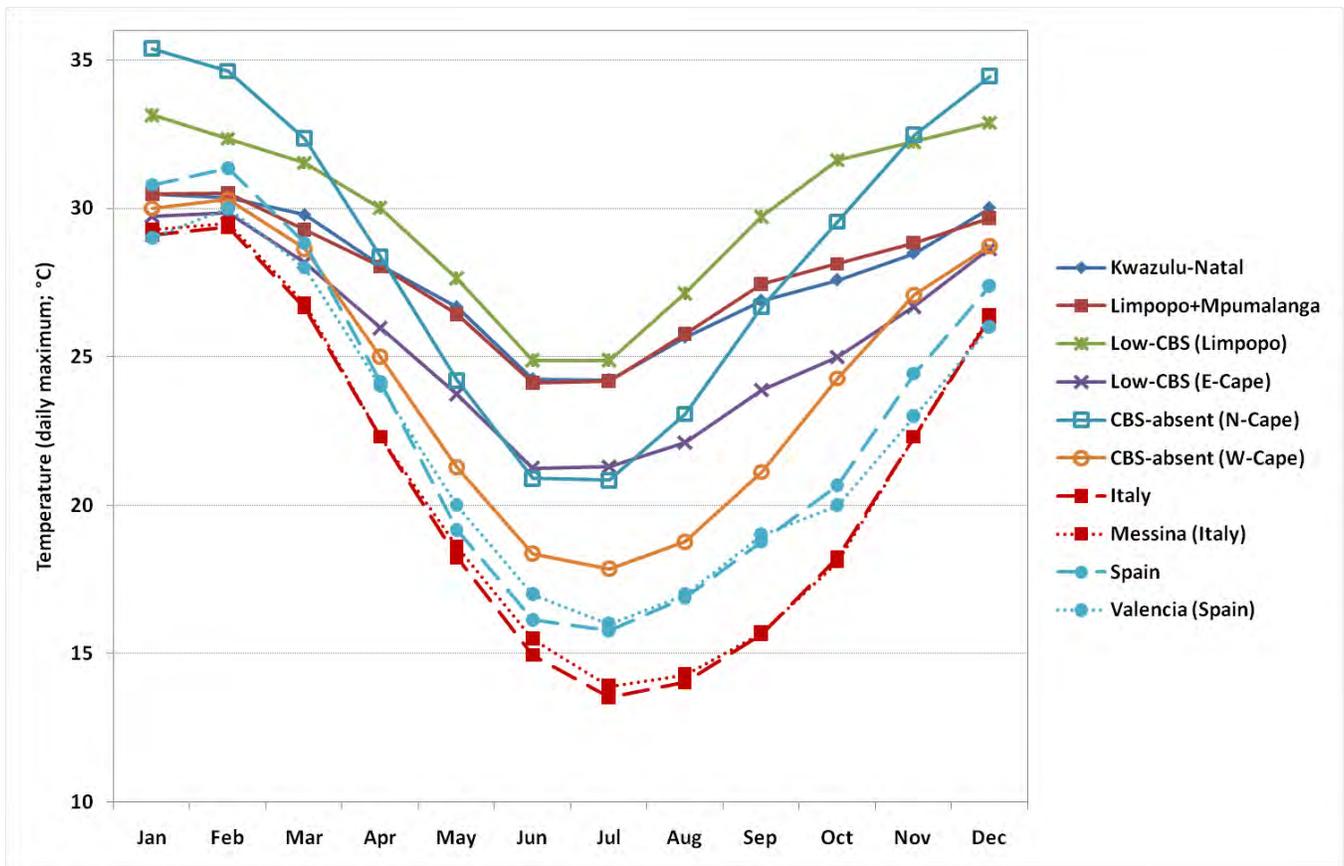


Figure 3. Means of long term monthly averages for maximum daily temperatures for selected citrus producing localities in the European Union (Greece, Italy, Portugal and Spain) compared with localities in South Africa where *Guignardia citricarpa* has established causing moderate to high levels (Kwazulu-Natal, Limpopo and Mpumalanga provinces), or low to moderate levels (Low-CBS in Limpopo and Eastern Cape provinces) of Citrus Black Spot, and localities where it has failed to become established (CBS-absent in Northern and Western Cape province). Data for the European localities were shifted by 6 months.

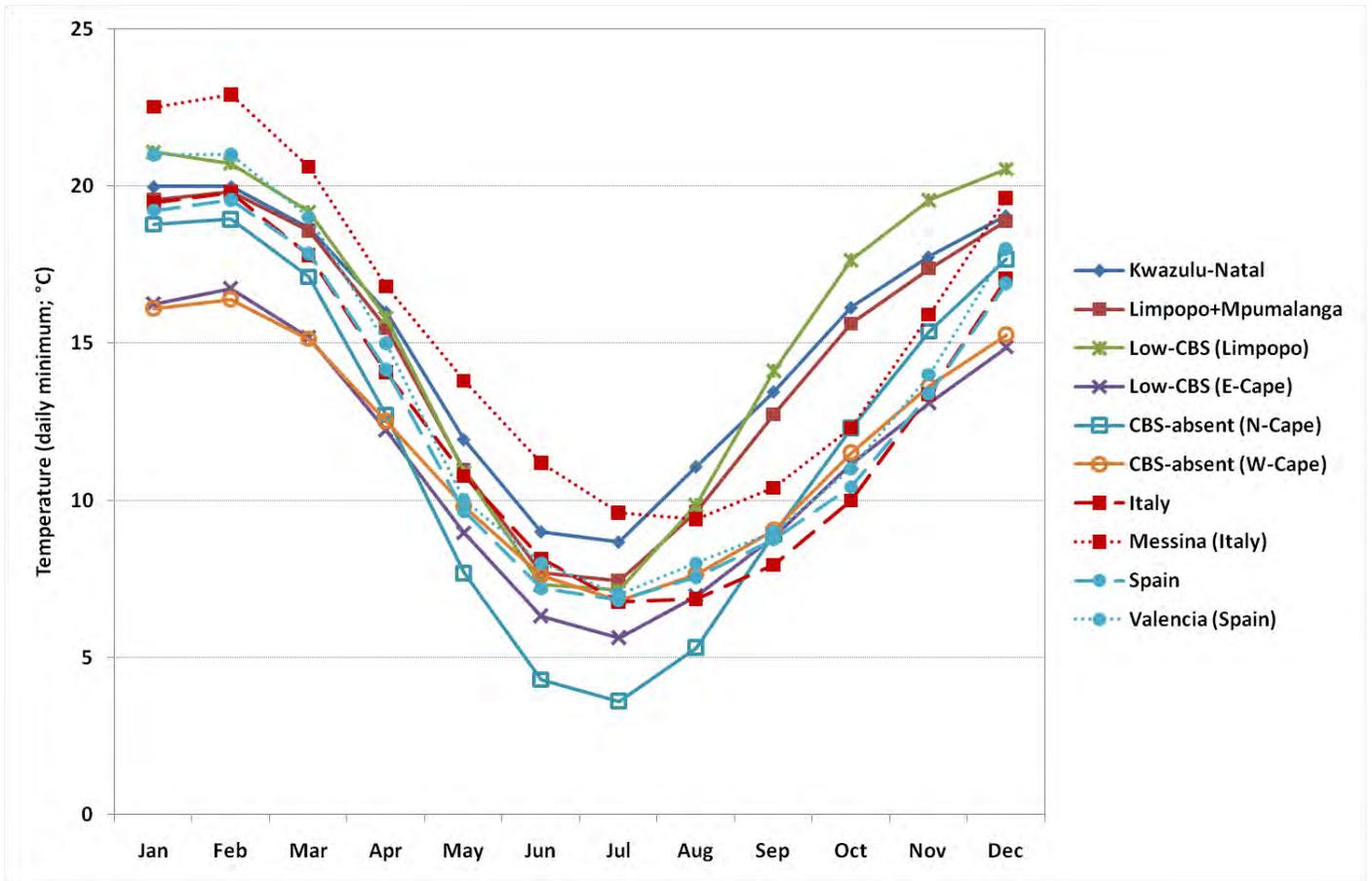


Figure 4. Means of long term monthly averages for minimum daily temperatures for selected citrus producing localities in the European Union (Greece, Italy, Portugal and Spain) compared with localities in South Africa where *Guignardia citricarpa* has established causing moderate to high levels (Kwazulu-Natal, Limpopo and Mpumalanga provinces), or low to moderate levels (Low-CBS in Limpopo and Eastern Cape provinces) of Citrus Black Spot, and localities where it has failed to become established (CBS-absent in Northern and Western Cape province). Data for the European localities were shifted by 6 months.

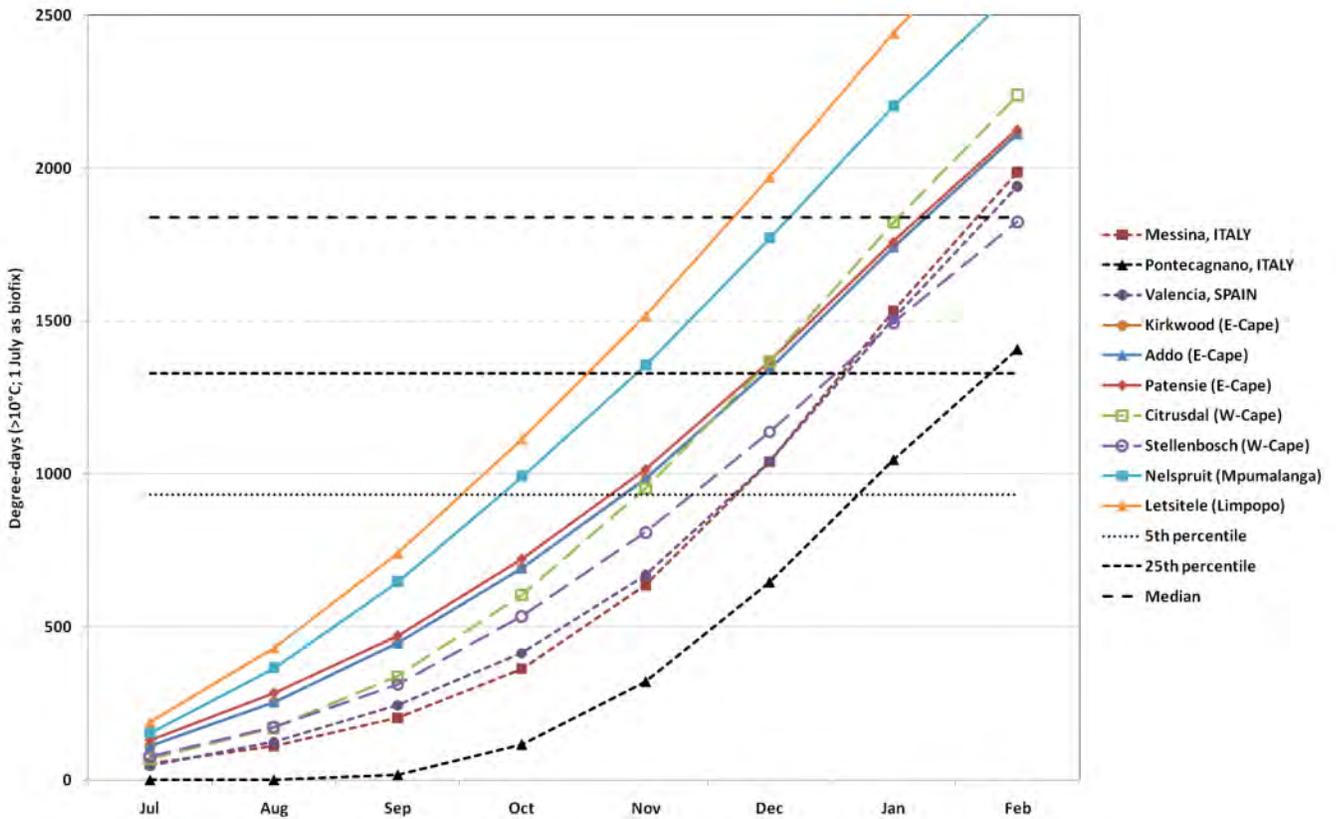


Figure 5. Degree-day accumulation for citrus producing localities in Italy and Spain compared with localities in South Africa where *Guignardia citricarpa* has established causing moderate to high levels (Limpopo and Mpumalanga provinces), or low to moderate levels (Eastern Cape province) of Citrus Black Spot, and localities where it has failed to become established (Western Cape province). Data for the northern hemisphere locations were shifted by 6 months. Horizontal dotted lines indicate the 5th, 25th percentiles and median for degree-days (°C) accumulated from 1 July until days on which ascospores were trapped.

Table 2. Means of rainfall and temperature values for selected citrus producing localities in the European Union (Greece, Italy, Portugal and Spain) compared with localities in South Africa where *Guignardia citricarpa* has established causing moderate to high levels (Kwazulu-Natal, Limpopo and Mpumalanga provinces), or low to moderate levels (Low-CBS in Limpopo and Eastern Cape provinces) of Citrus Black Spot, and localities where it has failed to become established (CBS-absent in Northern and Western Cape province). Data for the European localities were shifted by 6 months.

CBS prevalence	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Rainfall (monthly average; mm)												
Kwazulu-Natal	97.45a	87.38A	61.87bc	37.59cde	16.02d	15.58d	15.20de	19.48def	35.33cde	66.60a	97.79a	85.14b
Limpopo+Mpumalanga	110.46a	103.95A	96.11a	34.52cde	12.25d	7.75d	5.63e	8.63ef	25.74de	50.91ab	91.87a	129.11a
Low-CBS (Limpopo)	72.98ab	61.50ab	37.99cd	21.69de	6.15d	4.75d	2.33e	1.32f	10.33de	22.18bc	54.31bc	56.73c
Low-CBS (E-Cape)	43.08bc	47.38B	53.65bc	38.76cde	22.03d	18.96d	20.27de	31.04de	28.66de	45.78ab	54.94b	48.71c
CBS-absent (N-Cape)	20.39bcd	26.50bc	30.85cd	16.16e	7.44d	3.65d	2.78e	2.87f	3.90e	7.61c	11.16d	16.00d
CBS-absent (W-Cape)	14.62cd	14.56bc	22.13d	55.31bcd	80.57bc	100.55bc	92.56bc	72.83bc	45.72bcd	32.59bc	19.81cd	18.37d
Greece	6.80cd	17.00bc	92.40ab	145.60a	194.60a	196.90a	161.00a	134.30a	98.10a	62.90ab	43.30bcd	15.20d
Italy	17.73cd	24.45bc	60.75bc	110.53a	108.43b	116.13b	105.88b	84.58b	71.38ab	58.38ab	36.95bcd	29.28d
Portugal	1.97d	2.70C	20.00d	59.47bcd	94.60b	87.73c	80.40bc	71.83bc	56.40bc	45.67ab	27.10cd	13.43d
Spain	5.10d	11.80bc	34.25cd	65.65b	64.40c	64.20c	53.00cd	46.55cd	49.55bcd	44.70ab	35.40bcd	18.95d
Temperature (daily average; °C)												
Kwazulu-Natal	24.88abc	24.85abc	23.89abc	21.75ab	18.80a	16.26a	16.15a	18.05a	19.92a	21.54b	22.84b	24.22bcd
Limpopo+Mpumalanga	25.03ab	25.17ab	23.92abc	21.75a	18.72a	15.94a	15.80a	17.71a	20.10a	21.87b	23.12b	24.29bc
Low-CBS (Limpopo)	27.13a	26.54a	25.37a	22.96a	19.27a	16.09a	15.99a	18.51a	21.93a	24.65a	25.87a	26.70a
Low-CBS (E-Cape)	22.99cd	23.32cd	21.73d	19.20cd	16.44ab	13.82b	13.48b	14.56b	16.40bc	18.04c	19.91c	21.72e
CBS-absent (N-Cape)	27.10a	26.78a	24.74ab	20.54abc	15.96bc	12.62bc	12.23bc	14.19bc	17.79b	20.93b	23.97ab	26.06ab
CBS-absent (W-Cape)	23.05bcd	23.35bcd	21.90cd	18.77cd	15.55bc	13.00bc	12.35bc	13.22bc	15.11cd	17.90c	20.37c	22.00e
Greece	24.97ab	24.93abc	22.57bcd	18.67cd	14.43c	11.87c	10.43cd	10.43de	12.03e	15.07de	18.90cd	22.70cde
Italy	24.48abc	24.63abc	22.20cd	18.13de	14.18c	11.33c	9.85d	10.25e	11.75e	14.10e	17.90de	21.75e
Portugal	21.57d	21.90d	20.87d	18.20de	14.77bc	12.43bc	11.73bcd	12.27cd	13.53d	14.80e	17.07e	19.57f
Spain	25.53a	25.93a	23.90abc	19.67bcd	15.43bc	12.30bc	11.60cd	12.83c	14.43d	16.27d	18.90cd	22.57de

Table 2. Continued.

CBS prevalence	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Temperature (average of daily maximum; °C)												
Kwazulu-Natal	30.48bc	30.37b	29.78bc	28.10a	26.68a	24.25a	24.23a	25.66a	26.89b	27.59c	28.49bc	30.02bc
Limpopo+Mpumalanga	30.48bc	30.52b	29.28bc	28.04a	26.44a	24.12a	24.19a	25.77a	27.46b	28.14bc	28.85b	29.68c
Low-CBS (Limpopo)	33.16ab	32.36ab	31.54ab	30.02a	27.64a	24.87a	24.87a	27.14a	29.73a	31.63a	32.25a	32.90ab
Low-CBS (E-Cape)	29.74c	29.84b	28.18c	25.98b	23.74b	21.25b	21.30b	22.12b	23.88c	24.99d	26.69c	28.64cd
CBS-absent (N-Cape)	35.39a	34.63a	32.36a	28.38a	24.21b	20.91b	20.85b	23.08b	26.69b	29.56ab	32.48a	34.46a
CBS-absent (W-Cape)	30.02bc	30.31b	28.66bc	25.01bc	21.29c	18.38c	17.87c	18.78c	21.14d	24.29d	27.10bc	28.74cd
Italy	29.10c	29.38b	26.65d	22.30d	18.23d	14.95d	13.53e	14.03e	15.65f	18.25f	22.30e	26.33e
Spain	30.80bc	31.37b	28.83bc	24.17c	19.17d	16.13d	15.77d	16.87d	18.77e	20.67e	24.43d	27.40de
Temperature (average of daily minimum; °C)												
Kwazulu-Natal	19.98a	19.98a	18.66a	15.99a	11.94a	9.00a	8.68a	11.07a	13.46a	16.14a	17.75ab	19.06ab
Limpopo+Mpumalanga	19.57a	19.82a	18.57a	15.49a	10.96ab	7.72a	7.45ab	9.64ab	12.74a	15.63a	17.38ab	18.88ab
Low-CBS (Limpopo)	21.08a	20.71a	19.18a	15.81a	10.89ab	7.32ab	7.14ab	9.86ab	14.13a	17.65a	19.54a	20.54a
Low-CBS (E-Cape)	16.24b	16.74b	15.18b	12.23b	8.97b	6.33ab	5.64bc	6.98bc	8.88b	11.17b	13.10d	14.88d
CBS-absent (N-Cape)	18.79ab	18.95ab	17.12ab	12.72ab	7.69b	4.29b	3.61c	5.31c	8.93b	12.30b	15.38bc	17.68abc
CBS-absent (W-Cape)	16.11b	16.40b	15.13b	12.54ab	9.82ab	7.61ab	6.82ab	7.66bc	9.07b	11.51b	13.64cd	15.26cd
Italy	19.48a	19.80a	17.80a	14.08ab	10.78ab	8.15a	6.78ab	6.88bc	7.95b	10.00b	13.38cd	17.05bc
Spain	19.23a	19.57a	17.87a	14.17ab	9.67ab	7.20ab	6.83ab	7.57bc	8.77b	10.43b	13.40cd	16.90bc

*For each month and parameter combination, values followed by the same letter do not differ significantly; Fisher's LSD test ($P=0.05$)

Appendix A:

Effect of temperature on *Guignardia pseudothecium* maturation and ascospore dispersal

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Introduction

Ascospore dispersal of *Guignardia citricarpa*, the causal agent of Citrus Black Spot (CBS), has not been modeled, largely due to the lack of long-term empirical data. In the Limpopo province of South Africa, several large citrus estates have contracted the plant pathology service providing company, QMS Agriscience (PO Box 416, Letsitele 0885, South Africa), to conduct *Guignardia* ascospore trapping and weather monitoring to support CBS management as an ongoing service contract. The aim of the present work in progress is to use several seasons' ascospore trapping and weather data to model *Guignardia pseudothecium* maturation and ascospore dispersal. This report summarises through descriptive statistics the temperature-related variables associated with ascospore dispersal events.

Material and Methods

As per the methods described by Reis *et al.* (2006), *Guignardia* ascospores were trapped during September through March in several localities. A Quest volumetric spore trap system (Quest Developments, Pretoria, South Africa) with a capacity of 20 L/h with the orifice 0.5 m above soil level was used for ascospore monitoring. Spores were trapped onto a circular spore trap disc that was divided into octants, which represented 8 days. Each day-section was divided into 8 sections (10 × 8 × 8.5 mm), representing 3-hourly intervals. Prior to placing the disc in the spore trap, it was sprayed with a fine film petroleum jelly spray (PJS, Quest Development, Pretoria, South Africa). After 7 days in the orchard, the disc was replaced with a clean disc and brought into the laboratory for further processing. In the laboratory, discs were stained with cotton blue in lactic acid (1:600 weight/volume) and the total number of *Guignardia* ascospores counted in 6 separate microscope fields on a compound microscope at 400× magnification. Ascospore identification of *G. citricarpa* was based on their morphology as described by Sutton and Waterston (1966). Unfortunately, ascospores of *G. citricarpa* and *G. mangiferae* could not be distinguished (Baayen *et al.*, 2002). Weather stations (Adcon Telemetry Inc., Santa Rosa, California USA) in close proximity (<1 km) from the spore traps made hourly recordings of rainfall (mm), temperature (°C) and relative humidity (%) throughout the spore trapping period and the preceding months.

Effect of temperature on ascospore dispersal. The hourly temperature data and concomitant 3-hourly ascospore trap data were acquired for three localities, Letaba (near Letsitele), Mahela (near Tzaneen) and Portsgate (near Hoedspruit) for the 2006/7, 2007/8 and 2008/9 seasons. Hourly temperature data were transformed to 3-hourly data as means of temperature. The total numbers of ascospore events

(i.e. a 3-hour interval in which *Guignardia* ascospores were trapped) were compared using descriptive statistics (XLSTAT Version 2009.3.01). The Portsgate dataset for 2006/7 was not considered due to missing weather data during September through November.

Effect of temperature on pseudothecium maturation and ascospore dispersal. The datasets with 3-hourly temperature and spore trap data were transformed to daily data as mean daily temperature (minimum, average and maximum). Additionally, means were also calculated for the 7 days leading up to a spore event.

For pseudothecium maturation, degree-day accumulation was calculated from the daily weather data sets using 1 July as biofix and 10°C as base temperature, using the formula $[(maxtemp + mintemp)/2] - basetemp$ to calculate the daily degree-day contribution (Lovell *et al.*, 2003). As complete weather data sets were required for the degree-day calculation, the data sets for Mahela in 2008/9 (missing weather data in winter) and Portsgate in 2006/7 were not considered due to missing data. Descriptive statistics were conducted using XLSTAT Version 2009.3.01.

Results and Discussion

Effect of temperature on ascospore dispersal. In total, 2000× 3-hourly ascospore events were recorded. Apart from the Portsgate 2006/7-data, which were not included, the weather data were acceptably complete with only 30 missing data points (Table 1). Mean minimum temperature (T_{min}) at which ascospores were trapped was 13.3°C, but should not be seen as indicative of the cardinal T_{min} , as the mean 2nd and 5th percentiles were 16.7 and 18.0°C, respectively. This clearly indicates that the vast majority of ascospore dispersal events occur at temperatures above 18°C. The mean maximum temperature (T_{max}) at which ascospores were trapped was 37.2°C, but the 75th percentile was 10°C lower at 27.5°C, indicating that ascospore dispersal occurs during temperatures that would allow germination, i.e. 15-29.5°C when combined with 15-38 hours of wetness (Kotze, 1963). In accordance with the optimum temperature reported for *G. citricarpa* (Kotze, 1963; Lee and Huang, 1973), the average temperature (T_{avg}) at which ascospores were dispersed was 24.4°C.

Effect of temperature on pseudothecium maturation and ascospore dispersal. Descriptive statistics of the daily temperature data (Tables 2) showed slightly lower 5th and 25th percentile values for daily T_{min} (15.3 and 18.2°C, respectively) as was observed for the 3-hourly intervals (18.0 and 20.9°C, respectively). Likewise, mean daily T_{min} (11.4°C) was lower than the respective mean observed for 3-hourly intervals (13.3°C). The 5th and 25th percentile values for mean daily T_{min} of the 7 days leading up to ascospore dispersal events (15.9 and 18.4°C, respectively; Table 3) were similar to the mean T_{min} recorded on the day of ascospore dispersal. One can therefore deduce that actual ascospore dispersal mostly occurs at >18°C (Table 1), although daily or weekly T_{min} might be slightly lower as a result of intervals during or prior to those days with cooler temperatures (Table 2). The 5th and 25th percentile values for daily T_{avg} were 19.2 and 22.6°C, respectively (Table 2), while the respective values for T_{avg} in the 7 days leading up to ascospore dispersal events were 21.2 and 22.9°C (Table 3), indicating the mild conditions prior to and during ascospore dispersal. The longterm monthly T_{min} values for October – November for Lestitele (Letaba and Mahela) and Hoedspruit (Portsgate) were 15.8 – 17.8°C and 16.3 – 17.8°C, respectively; and

T_{avg} 20.3 – 22.0°C and 20.6 – 22.5°C, respectively. This indicates that temperatures during these months should be high enough to favour ascospore dispersal as well subsequent germination and infection.

Mean degree-day accumulation (Table 4) until the 5th percentile of ascospore events was 932.5°C, ranging from 820.7 to 1138.4°C. The 25th percentile and median values were 1329.9 (± 214.80) and 1839.6 (± 193.22)°C, respectively. This fairly uniform degree-day accumulation to first and peak ascospore dispersal clearly demonstrates the importance of mild to warm conditions ($T_{avg} > 10^\circ\text{C}$) required from mid-winter (1 July) onward for pseudothecium maturation.

These data are being analysed in combination with rainfall and relative humidity in an attempt to predict first and subsequent ascospore dispersal events (work in progress).

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Table 1. Descriptive statistics for temperatures (°C) during 2000 *Guignardia* ascospore dispersal events measured at 3-hour intervals at three localities over 3 seasons.

Locality	Season	N	Min	Mean	Max	Percentiles				
						2nd%	5th%	25th%	Median	75th%
Letaba	2006/7	204	15.80	26.43	38.90	17.81	19.50	22.57	25.98	30.19
Letaba	2007/8	260	10.27	22.77	35.23	13.97	16.09	19.07	22.13	25.83
Letaba	2008/9	165	15.67	24.58	36.23	16.86	18.87	21.37	23.40	27.47
Mahela	2006/7	280	13.83	25.81	39.33	17.21	18.35	22.17	24.93	29.63
Mahela	2007/8	321	12.73	22.68	36.70	14.92	16.23	19.23	22.20	25.71
Mahela	2008/9	276	12.23	24.52	36.43	18.24	18.87	21.59	23.62	27.05
Portsgate	2007/8	227	12.00	23.69	36.63	16.13	16.69	19.93	22.97	27.32
Portsgate	2008/9	267	14.10	24.57	37.73	18.51	19.18	21.48	23.75	26.96
Overall means	2000		13.33	24.38	37.15	16.71	17.97	20.93	23.62	27.52

Table 2. Descriptive statistics for daily temperatures measured during 635 *Guignardia* ascospore dispersal events at three localities over 3 seasons.

Locality	Season	N	Min	Mean	Max	Percentiles			
						5th%	25th%	Median	75th%
Temperature (daily minimum; °C)									
Letaba	2006/7	79	10.37	20.24	24.13	15.98	18.88	20.80	21.75
Letaba	2007/8	102	10.27	18.45	27.00	13.89	16.67	18.65	20.29
Letaba	2008/9	72	14.37	20.20	23.87	14.96	19.13	20.70	21.53
Mahela	2006/7	95	12.17	20.14	24.60	16.26	18.48	20.27	22.10
Mahela	2007/8	109	12.73	18.71	23.40	15.35	17.10	18.57	20.60
Portsgate	2007/8	84	12.00	19.16	23.93	15.57	17.57	19.28	20.89
Portsgate	2008/9	94	8.17	20.33	25.60	15.26	19.21	20.80	22.23
Overall means		635	11.44	19.60	24.65	15.32	18.15	19.87	21.34
Temperature (daily average; °C)									
Letaba	2006/7	79	17.59	25.43	29.92	20.68	24.26	25.92	26.97
Letaba	2007/8	102	15.05	22.95	27.83	17.63	21.54	23.35	24.97
Letaba	2008/9	72	17.15	24.53	28.90	19.83	23.24	24.81	26.37
Mahela	2006/7	95	17.68	24.87	29.93	19.16	23.12	25.36	26.89
Mahela	2007/8	109	15.18	23.00	28.92	17.83	20.69	23.63	25.24
Portsgate	2007/8	84	15.77	23.33	29.23	18.69	22.04	23.68	25.27
Portsgate	2008/9	94	16.93	24.63	30.10	20.51	23.28	24.73	26.34
Overall means		635	16.48	24.11	29.26	19.19	22.60	24.50	26.01
Temperature (daily maximum; °C)									
Letaba	2006/7	79	17.93	31.57	38.90	24.12	30.48	32.33	34.07
Letaba	2007/8	102	16.37	28.34	35.67	19.77	26.53	28.70	31.45
Letaba	2008/9	72	19.43	29.95	38.00	21.89	27.47	30.22	33.13
Mahela	2006/7	95	18.07	30.39	39.33	22.66	27.48	31.10	33.50
Mahela	2007/8	109	16.60	28.05	36.70	19.67	24.77	28.77	31.50
Portsgate	2007/8	84	17.60	28.12	36.63	19.97	25.72	28.63	30.82
Portsgate	2008/9	94	21.10	29.65	37.73	23.40	26.95	29.52	32.63
Overall means		635	18.16	29.44	37.57	21.64	27.06	29.90	32.44

Table 3. Descriptive statistics for daily temperatures measured during and preceding 635 *Guignardia* ascospore dispersal events at three localities over 3 seasons.

Locality	Season	N	Min	Mean	Max	Percentiles			
						5th%	25th%	Median	75th%
Temperature (average of daily minimum for 7 days leading up to event; °C)									
Letaba	2006/7	79	14.06	19.81	22.94	15.85	18.78	19.68	21.37
Letaba	2007/8	102	13.19	18.50	28.39	15.31	17.28	18.50	19.84
Letaba	2008/9	72	12.38	19.78	22.50	15.45	19.12	20.18	21.37
Mahela	2006/7	95	12.49	20.15	23.08	16.58	19.09	20.44	21.60
Mahela	2007/8	109	14.68	18.78	21.56	16.19	17.62	18.90	20.10
Portsgate	2007/8	84	15.79	19.15	21.93	16.40	17.97	19.24	20.42
Portsgate	2008/9	94	13.30	20.08	22.84	15.37	19.19	20.73	21.59
Overall means		635	13.70	19.46	23.32	15.88	18.43	19.67	20.90
Temperature (daily average for 7 days leading up to event; °C)									
Letaba	2006/7	79	22.13	25.26	27.66	22.90	23.98	25.31	26.63
Letaba	2007/8	102	19.44	23.06	29.05	20.23	21.59	23.16	24.49
Letaba	2008/9	72	20.53	24.41	27.05	21.41	23.31	24.79	25.56
Mahela	2006/7	95	19.01	25.40	27.96	21.77	24.14	25.94	26.95
Mahela	2007/8	109	19.33	23.26	26.37	20.44	21.77	23.43	24.82
Portsgate	2007/8	84	19.48	23.44	25.93	20.43	22.33	23.88	24.74
Portsgate	2008/9	94	20.12	24.40	27.44	21.44	23.31	24.75	25.70
Overall means		635	20.01	24.17	27.35	21.23	22.92	24.47	25.56

Table 4. Descriptive statistics for degree-day calculations based on temperatures measured during and preceding 635 *Guignardia* ascospore dispersal events at three localities over 3 seasons.

Locality	Season	N	Min	Mean	Max	Percentiles			
						5th%	25th%	Median	75th%
Degree-days (>10°C; 1 July as biofix)									
Letaba	2006/7	79	957.35	1992.45	2889.62	1138.38	1558.35	1977.90	2471.73
Letaba	2007/8	102	667.10	1780.99	3286.07	820.73	1064.95	1586.17	2458.24
Letaba	2008/9	72	763.02	1875.06	2932.52	927.52	1476.59	1859.17	2282.75
Mahela	2006/7	95	527.28	2155.12	3605.29	1016.88	1536.59	2116.56	2700.40
Mahela	2007/8	109	750.52	1755.29	3315.62	835.03	1115.92	1609.47	2177.62
Portsgate	2007/8	84	820.20	1765.37	3172.55	890.06	1138.61	1795.49	2301.83
Portsgate	2008/9	94	747.96	1904.75	3012.87	898.55	1418.31	1932.56	2370.69
Overall means		635	747.63	1889.86	3173.51	932.45	1329.90	1839.62	2394.75

**VALIDATION STUDY and RISK ASSESSMENT: *Guignardia citricarpa*,
(Citrus black spot),**

USDA-APHIS-PPQ-CPHST-PERAL/ NCSU

October, 2009

Scientific name:

Guignardia citricarpa

Order: Dothideales, Family: Botryosphaeriaceae

Common Name: Citrus black spot *Guignardia citricarpa* Kiely

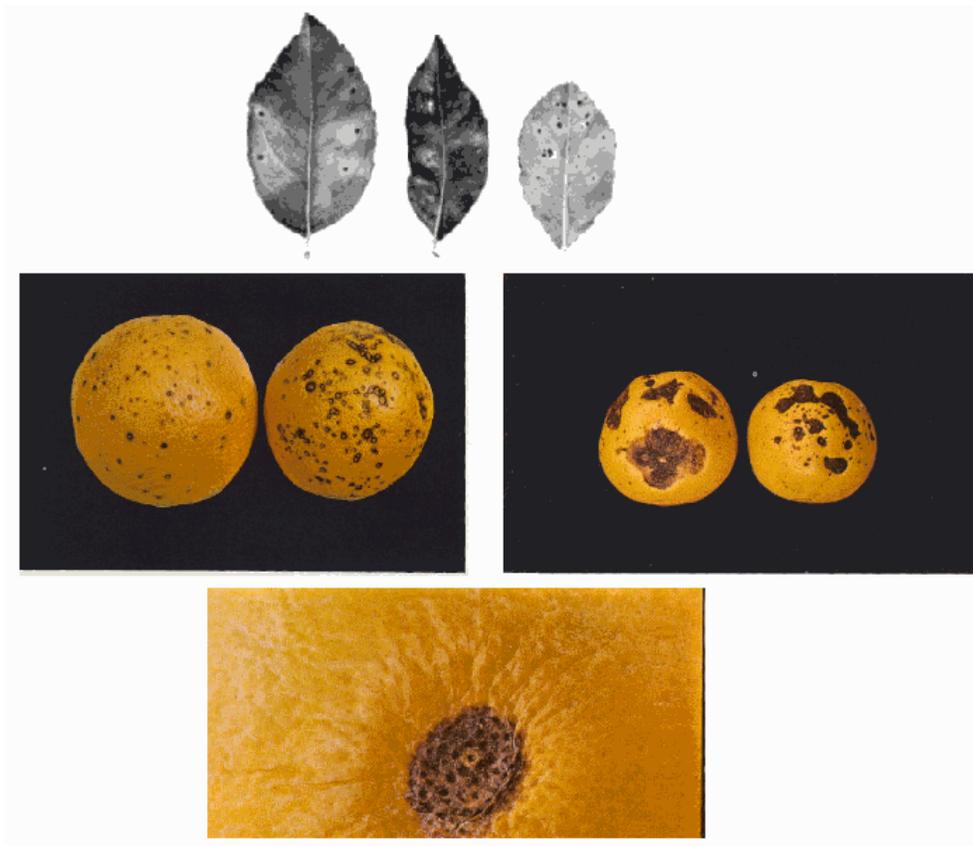


Figure 1. Typical Symptoms of Black Spot Disease on Citrus Fruit and Leaves. (L. C. Knorr, 1965).

Validation study

A validation study of *Guignardia citricarpa* a pathogen of citrus was conducted with observations from South Africa and Australia using the NAPFAST modeling system. Predictions from an infection model were compared to observations of disease prevalence in major citrus districts. Projections of likely disease prevalence based on the infection model are made for the United States and Europe where the pathogen is known not to occur.

Description

CBS is primarily a disease of fruit (Kotzé, 2000), although leaves and stems are also infected (CABI, 2002). 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). Symptomatic citrus fruit may display four types of symptoms: hard spot, melanose spot, freckle spot, and virulent spot (Kiely, 1948; Kotzé, 1963, 2000). The type of symptoms that develop is a result of 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, dependant on environmental conditions (Kiely, 1948; Kotzé, 1963, 2000).

Hosts of CBS are exclusively in the genus *Citrus* (Baayen et al., 2002). Leaves are susceptible up to 10 months of age (Truter et al., 2007), while fruit are susceptible for four to five months after petal fall fruit, independent of rainfall, temperature, or inoculum levels (Kotzé, 1963, 2000). Younger trees appear to be less susceptible to CBS disease (Kiely, 1948); in trees up to 10 years of age the susceptible period is limited to 3 months, and CBS disease is more easily controlled (Kiely, 1969). Typically the infection in leaves 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 remains in the quiescent stage until the fruit matures. Ascospores are produced in infected leaf debris from pseudothecia that develop 40-180 days after infection (CABI, 2002). Approximately 12 to 15 months separates the initiation of the primary infection (ascospore development in leaves) and the development of pycnidiospores for secondary infection (Kiely,

1948).

Life History:

The epidemiology of black spot of citrus is influenced by the presence of inoculum, optimum climatic conditions for infection, growth cycle of the tree, and age of the fruit in relation to susceptibility to infection and development of symptoms (Kiely, 1950). The causal organism of citrus black spot has two stages: a sexual stage represented by the ascospores of *G. citricarpa* and an asexual stage represented by the pycnidiospores of *Phyllosticta citricarpa*. Ascospores are produced in infected leaf debris from pseudothecia that develop 40-180 days after infection (CABI, 2002). Alternate rainy and dry periods aid in disease development. Wetting and drying of leaves is essential for ascocarp 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). 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 eradicating the CBS casual agent (Lee and Huang, 1973). In addition, excess rain prevents pseudothecia formation as the leaves become colonized by competing saprobes (CABI, 2002).

During rainfall mature ascospores are forcibly ejected up to a centimeter high (Kiely, 1948; Kotzé, 1963). Ascospores are subsequently spread by wind and water (Kiely, 1948; Kotzé, 1963). Wind can spread ascospores over short distances (Whiteside, 1965). Upon depositing on attached 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 in between the cuticle and epidermal wall to form a quiescent infection (Kotzé, 2000).

Ascospores develop in infected leaf debris. Typically the infection in leaves 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). Dead leaves may produce ascospores for several months, even when the leaves are in an advanced stage of decomposition (Kiely, 1948). In fruit, the infection remains in the quiescent stage until the fruit matures. Upon fruit maturation the infection grows into the skin, producing leaf spots and pycnidia (the asexual phase of the fungus). Under favorable environmental conditions, pycnidiospore production is continuous (Kiely, 1948). Ascospores have never been observed to develop on fruit attached to the tree (Kiely, 1948; Kotzé, 1963,

1981).

Adequate rainfall, temperatures, and inoculum must be present simultaneously for infection to occur (Huang and Chang, 1972; Kotzé, 1981; Lee and Huang, 1973). Temperature affects several aspects of CBS epidemiology. 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, independent of rainfall, temperature, or inoculum levels (Kotzé, 1963, 2000).

The most important factors promoting epidemics are summer rains and proximity of lemon orchards. Citrus black spot occurs in subtropical regions with summer rainfall. Countries with Mediterranean climates such as Spain and Portugal are thought to be unfavorable for the pathogen. The epidemic development during the growing season primarily depends upon sufficient moisture and favorable temperature conditions for infection, sporulation and dispersal.

Methods

Notes on Selection of Citrus districts

A validation study of *Guignardia citricarpa* was conducted using observations from South Africa and Australia. We focused on specific citrus districts in order to calibrate the model, although all locations can be seen in the map products. These districts are shown in Fig. 1. In Australia, Mildura was included as an example of a semi-arid production district. In South Africa, individual citrus districts are designated by the nearest big city location. For example, Addo, Patensie, and Kirkwood South Africa are incorporated into the Port Elizabeth location. In Europe, we selected citrus districts with more than 10,000 ha of citrus plantations. This did not include Pontecagnago which was included in the EFSA report (EFSA, 2009). Messina, Italy is included in the Sicilia observations. Projections of likely disease prevalence are made for California and Florida in the United States where the pathogen is known not to occur.

Prediction model

The prediction model was created using the NCSU-APHIS Plant Pest Forecast System (NAPPFASST). NAPPFASST is a tool used for weather-based mapping of exotic plant pests (Magarey et al. 2007) and includes a generic infection model. The NAPPFASST 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.

The NAPPPFAST infection model uses a temperature response function to estimate daily infection risk (Magarey et al. 2005). The inputs to the model are daily average temperature and leaf wetness hours per day. The models parameters are the cardinal temperatures and minimum wetness hours to achieve infection. The parameters for the infection model were obtained from the EFSA report (EFSA 2008), a comprehensive study of pathogen epidemiology. Models were created for both pycnidiospores and ascospores.

Susceptible period

In Florida, the months of April, May and June represent the first three months after fruit set and the period of greatest fruit susceptibility (Mosser and Aerts, 2007; Kotze, 1981). We chose to use a more extensive 5 month window of susceptibility. Consequently, in the United States the period of April to August was used as the susceptible period. In Europe, fruit set in the Mediterranean occurs around the beginning of May (Agustí, 2000). However the maturation of pseudothecia and ascospores in the leaf litter (Kiely, 1948; Kotzé, 1981; Lee and Huang, 1973) may be delayed by cool winter temperatures potentially causing asynchrony between the pathogen and the host. Fruit set in South Africa is around the beginning of October (Villiers and Joubert, 2006). Based on these data, the susceptible period was assumed to be October through February in South Africa and in Australia, and June through October in Europe.

Results

Continental maps of infection level were created to assist in model validation (Fig. 2, 3). The predicted number of infection days for citrus by *Guignardia citricarpa* pycnidiospores and ascospores in different citrus districts worldwide is organized by rank score (Table 1) and by country (Table 2). For ascosporic infection, all districts where the disease was endemic had an infection score above 12 (Fig. 4A), while those districts with a score below 12 the disease was absent. For pycnidiosporic infection, the results were similar with a critical infection score of 15 (Fig. 4B). Probability maps showing the frequency of years were these thresholds were met or exceeded were also created for each continent (Fig. 3).

Discussion

This report improves upon an earlier NAPPPFAST report (Magarey and Borchert, 2003) by including international validation data. Unlike a previous study, the NAPPPFAST infection model did not include wetness interruption (EFSA, 2009). At this point in time wetness interruptions are not available in the NAPPPFAST infection model template but might be included in future versions of NAPPPFAST. In addition, the 32 k grid data set is relatively coarse and may not perform as well in areas with mountainous topography.

In addition, the model does not account for the influence of the environment on the host, for example citrus is not cultivated widely throughout Europe despite a favorable climate for the pathogen.

Conclusions

In the United States, we conclude that *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. In Europe, *G. citricarpa* is not expected to have an impact in areas with commercial citrus production.

Authors

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Table 1. Parameters for *Guignardia citricarpa* infection model based upon EFSA, 2008

Model	Minimum Temperature Tmin	Optimum Temperature Topt	Maximum Temperature Tmax	Minimum Wetness Wmin	Maximum Wetness Wmax	Minimum Precipitation
Ascospores	15°C	27°C	35°C	15h	38h	2mm
Pycnidiospores	10°C	25°C	35°C	12h	35h	2mm

Table 2. Predicted infection of citrus by *Guignardia citricarpa* in districts worldwide organized by Score

Country	District	GC Presence	Period Start	Period End	Score
Ascospores					
Australia	Darwin, NT	Endemic	1-Oct	28-Feb	60
United States	Southern Florida	Unknown	1-Apr	31-Aug	60
South Africa	Durban	Endemic	1-Oct	28-Feb	37.5
South Africa	Nelspruit	Endemic	1-Oct	28-Feb	37.5
Australia	SE Queensland	Endemic	1-Oct	28-Feb	25
Australia	Emerald, Qld	Endemic	1-Oct	28-Feb	25
South Africa	Messina	Low	1-Oct	28-Feb	25
Australia	Patterson, NSW	Endemic	1-Oct	28-Feb	12.5
South Africa	Port Elizabeth	Endemic	1-Oct	28-Feb	12.5
South Africa	Upington	Absent	1-Oct	28-Feb	10
Italy	Sicilia	Unknown	1-Jun	31-Oct	7.5
Italy	Calabria	Unknown	1-Jun	31-Oct	7.5
Malta	Malta	Unknown	1-Jun	31-Oct	7.5
Portugal	Algarve	Unknown	1-Jun	31-Oct	7.5
Greece	Peloponese	Unknown	1-Jun	31-Oct	5
Australia	Mildura, Vic	Absent?	1-Oct	28-Feb	2.5
South Africa	Cape Town	Absent	1-Oct	28-Feb	2.5
Spain	Andalucia	Unknown	1-Jun	31-Oct	2.5
Spain	Valencia	Unknown	1-Jun	31-Oct	2.5
Spain	Murcia	Unknown	1-Jun	31-Oct	2.5
United States	Central Valley CA	Unknown	1-Apr	31-Aug	2.5
Pycnidiospores					
Australia	Darwin, NT	Endemic	1-Oct	28-Feb	60
United States	Southern Florida	Unknown	1-Apr	31-Aug	60
South Africa	Durban	Endemic	1-Oct	28-Feb	37.5
South Africa	Nelspruit	Endemic	1-Oct	28-Feb	37.5
Australia	SE Queensland	Endemic	1-Oct	28-Feb	25
Australia	Emerald, Qld	Endemic	1-Oct	28-Feb	25
South Africa	Messina	Low	1-Oct	28-Feb	25
Australia	Patterson, NSW	Endemic	1-Oct	28-Feb	17.5
South Africa	Port Elizabeth	Endemic	1-Oct	28-Feb	17.5
Italy	Calabria	Unknown	1-Jun	31-Oct	12.5
Portugal	Algarve	Unknown	1-Jun	31-Oct	12.5
South Africa	Upington	Absent	1-Oct	28-Feb	12.5
Australia	Mildura, Vic	Absent	1-Oct	28-Feb	7.5
Greece	Peloponese	Unknown	1-Jun	31-Oct	7.5
Italy	Sicilia	Unknown	1-Jun	31-Oct	7.5
Malta	Malta	Unknown	1-Jun	31-Oct	7.5
Spain	Valencia	Unknown	1-Jun	31-Oct	7.5
Spain	Andalucia	Unknown	1-Jun	31-Oct	7.5
South Africa	Cape Town	Absent	1-Oct	28-Feb	2.5
Spain	Murcia	Unknown	1-Jun	31-Oct	2.5
United States	Central Valley CA	Unknown	1-Apr	31-Aug	2.5

Table 3 Predicted infection of citrus by *Guignardia citricapiae* in districts worldwide organized by country.

Country	District	GC Presence	Period Start	Period End	Score
Ascospores					
Australia	Darwin, NT	Endemic	1-Oct	28-Feb	60
Australia	SE Queensland	Endemic	1-Oct	28-Feb	25
Australia	Emerald, Qld	Endemic	1-Oct	28-Feb	25
Australia	Patterson, NSW	Endemic	1-Oct	28-Feb	12.5
Australia	Mildura, Vic	Absent	1-Oct	28-Feb	2.5
Greece	Peloponese	Unknown	1-Jun	31-Oct	5
Italy	Sicilia	Unknown	1-Jun	31-Oct	7.5
Italy	Calabria	Unknown	1-Jun	31-Oct	7.5
Malta	Malta	Unknown	1-Jun	31-Oct	7.5
Portugal	Algarve	Unknown	1-Jun	31-Oct	7.5
South Africa	Durban	Endemic	1-Oct	28-Feb	37.5
South Africa	Nelspruit	Endemic	1-Oct	28-Feb	37.5
South Africa	Messina	Low	1-Oct	28-Feb	25
South Africa	Port Elizabeth	Endemic	1-Oct	28-Feb	12.5
South Africa	Upington	Absent	1-Oct	28-Feb	10
South Africa	Cape Town	Absent	1-Oct	28-Feb	2.5
Spain	Andalucia	Unknown	1-Jun	31-Oct	2.5
Spain	Valencia	Unknown	1-Jun	31-Oct	2.5
Spain	Murcia	Unknown	1-Jun	31-Oct	2.5
United States	Southern Florida	Unknown	1-Apr	31-Aug	60
United States	Central Valley CA	Unknown	1-Apr	31-Aug	2.5
Pycnidiospores					
Australia	Darwin, NT	Endemic	1-Oct	28-Feb	60
Australia	SE Queensland	Endemic	1-Oct	28-Feb	25
Australia	Emerald, Qld	Endemic	1-Oct	28-Feb	25
Australia	Patterson, NSW	Endemic	1-Oct	28-Feb	17.5
Australia	Mildura, Vic	Absent?	1-Oct	28-Feb	7.5
Greece	Peloponese	Unknown	1-Jun	31-Oct	7.5
Italy	Calabria	Unknown	1-Jun	31-Oct	12.5
Italy	Sicilia	Unknown	1-Jun	31-Oct	7.5
Malta	Malta	Unknown	1-Jun	31-Oct	7.5
Portugal	Algarve	Unknown	1-Jun	31-Oct	12.5
South Africa	Durban	Endemic	1-Oct	28-Feb	37.5
South Africa	Nelspruit	Endemic	1-Oct	28-Feb	37.5
South Africa	Messina	Low	1-Oct	28-Feb	25
South Africa	Port Elizabeth	Endemic	1-Oct	28-Feb	17.5
South Africa	Upington	Absent	1-Oct	28-Feb	12.5
South Africa	Cape Town	Absent	1-Oct	28-Feb	2.5
Spain	Valencia	Unknown	1-Jun	31-Oct	7.5
Spain	Andalucia	Unknown	1-Jun	31-Oct	7.5
Spain	Murcia	Unknown	1-Jun	31-Oct	2.5
United States	Southern Florida	Unknown	1-Apr	31-Aug	60
United States	Central Valley CA	Unknown	1-Apr	31-Aug	2.5

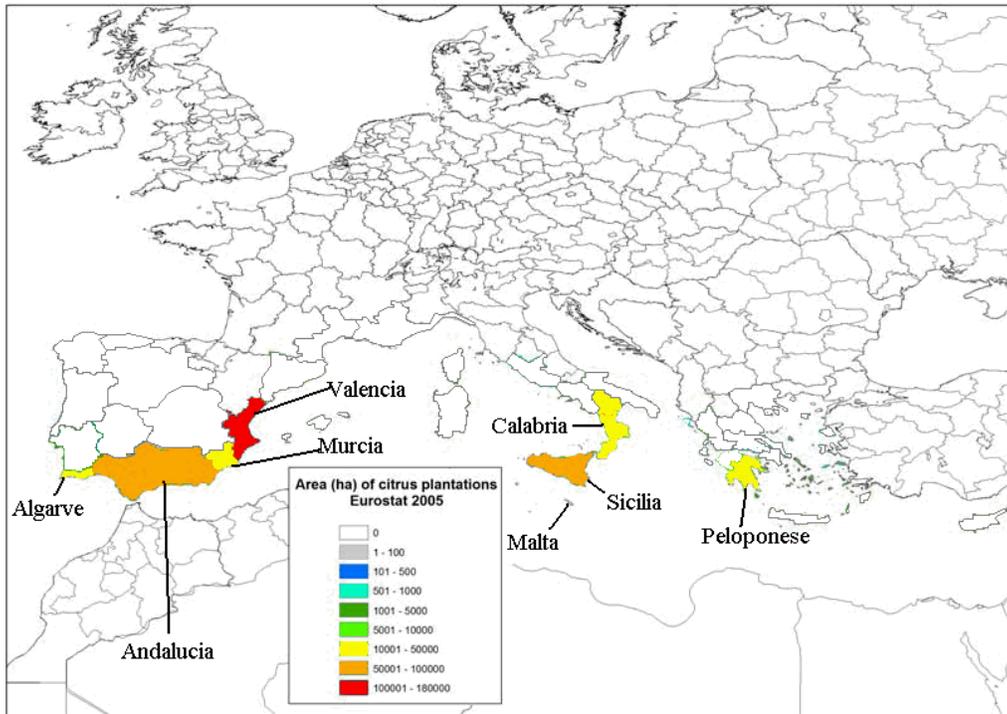


Fig 1A. Distribution of major citrus growing areas in Europe. There is no *G. citricarpa* prevalence in reported in Europe due to pathogen absence.

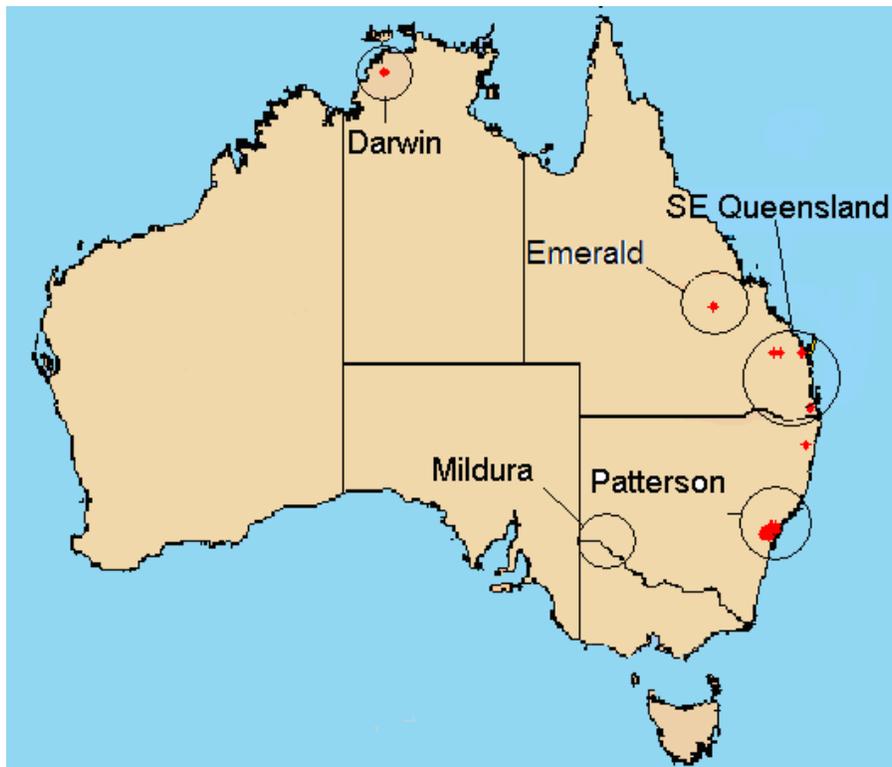


Fig. 1B Selected citrus production areas in Australia. Areas with *G. citricarpa* prevalence are shown in red.

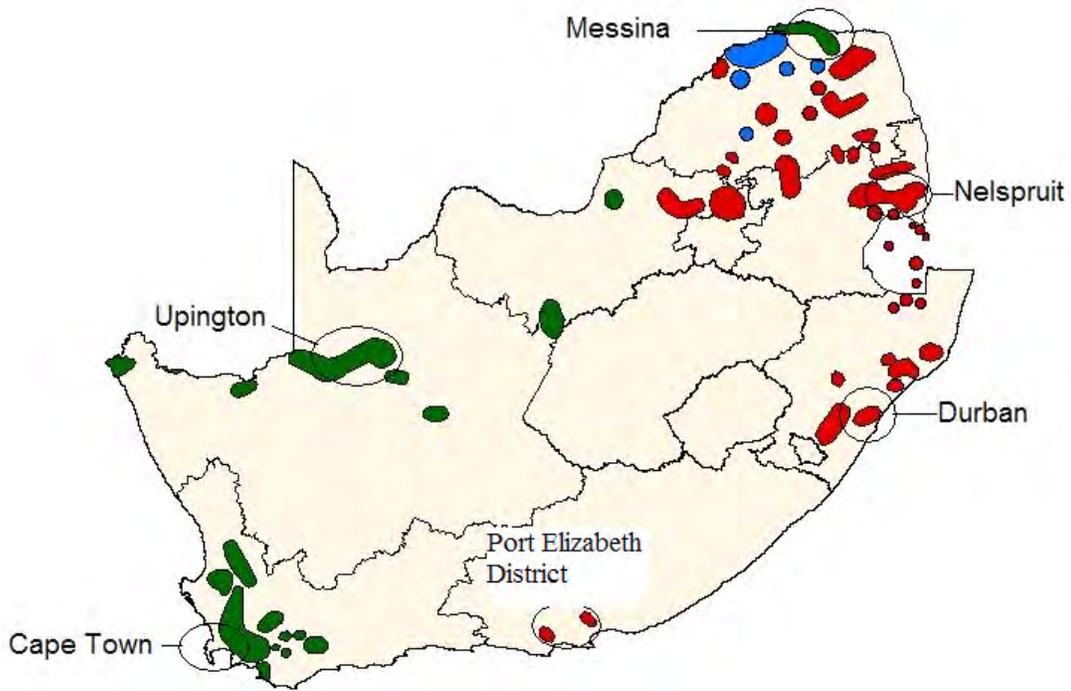


Fig 1C. Citrus production areas in South Africa. Areas with high prevalence of *G. citricarpa* prevalence are shown in red, low in blue and absent in green.

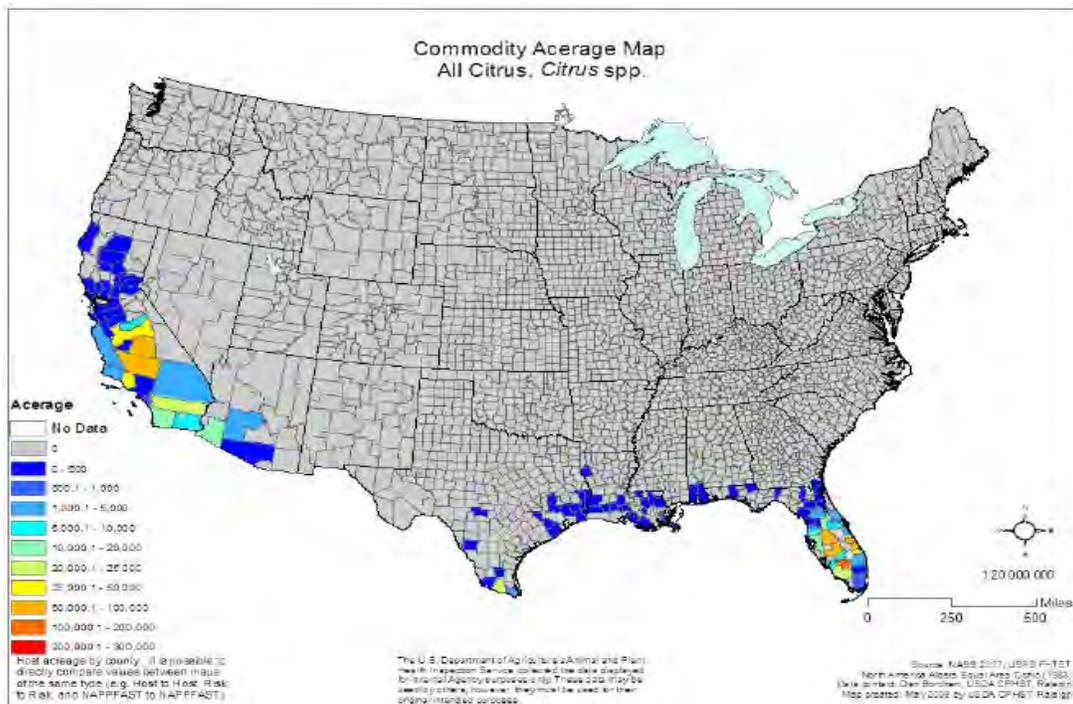


Fig 1D. Citrus production areas in United States. There is no *G. citricarpa* prevalence reported in all areas due to pathogen absence.

Accum. Infection Level

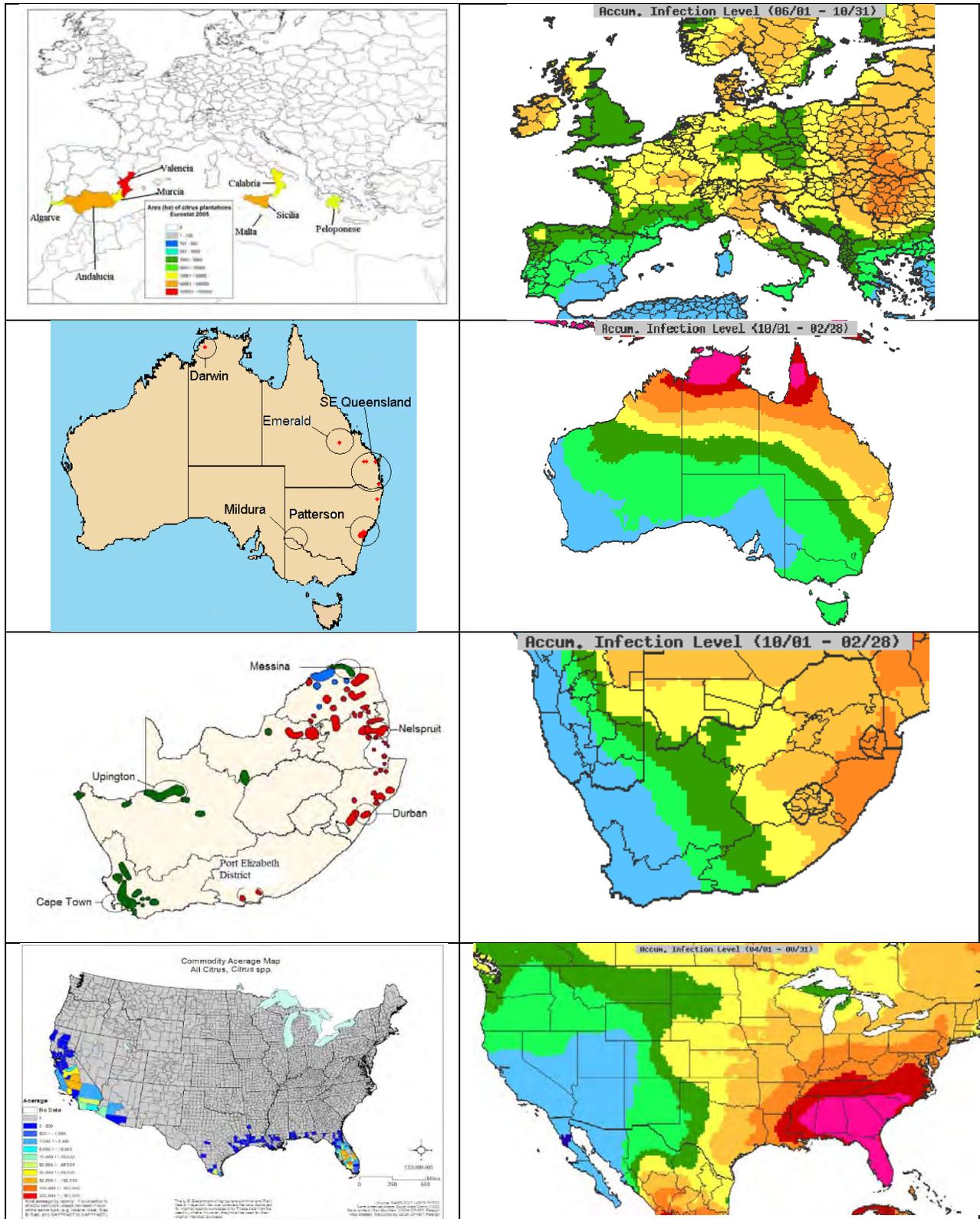
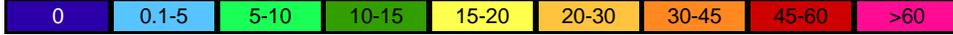


Figure 2A. The accumulated number of days suitable for *G. citricarpa* ascospore infection by continent based on 10 years of climate data.

Accum. Infection Level

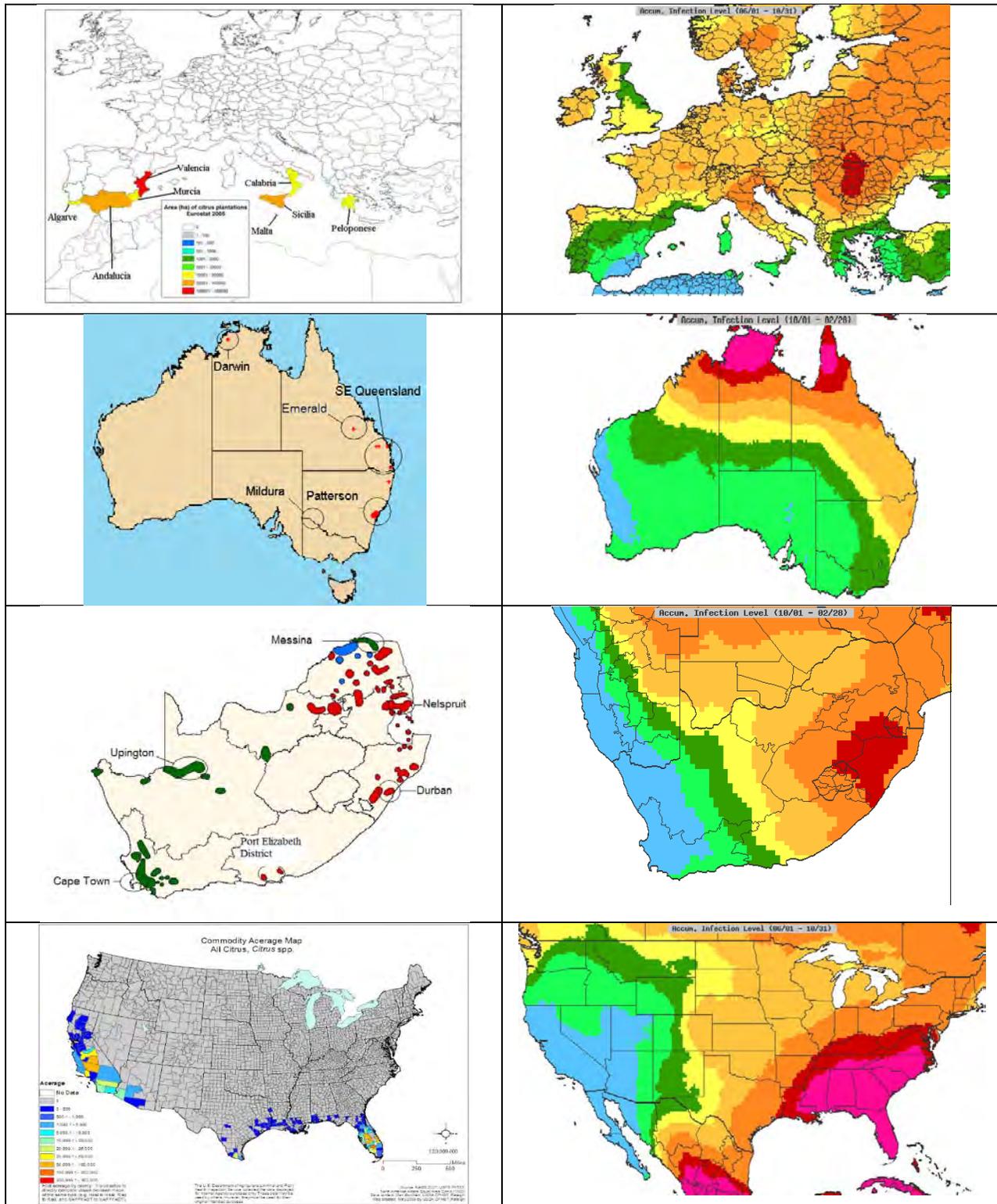
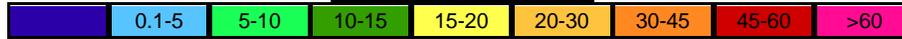


Figure 2B. The accumulated number of days suitable for *G. citricapiae* pycnidiospore infection by continent based on 10 years of climate data.

Accum. Infection Level

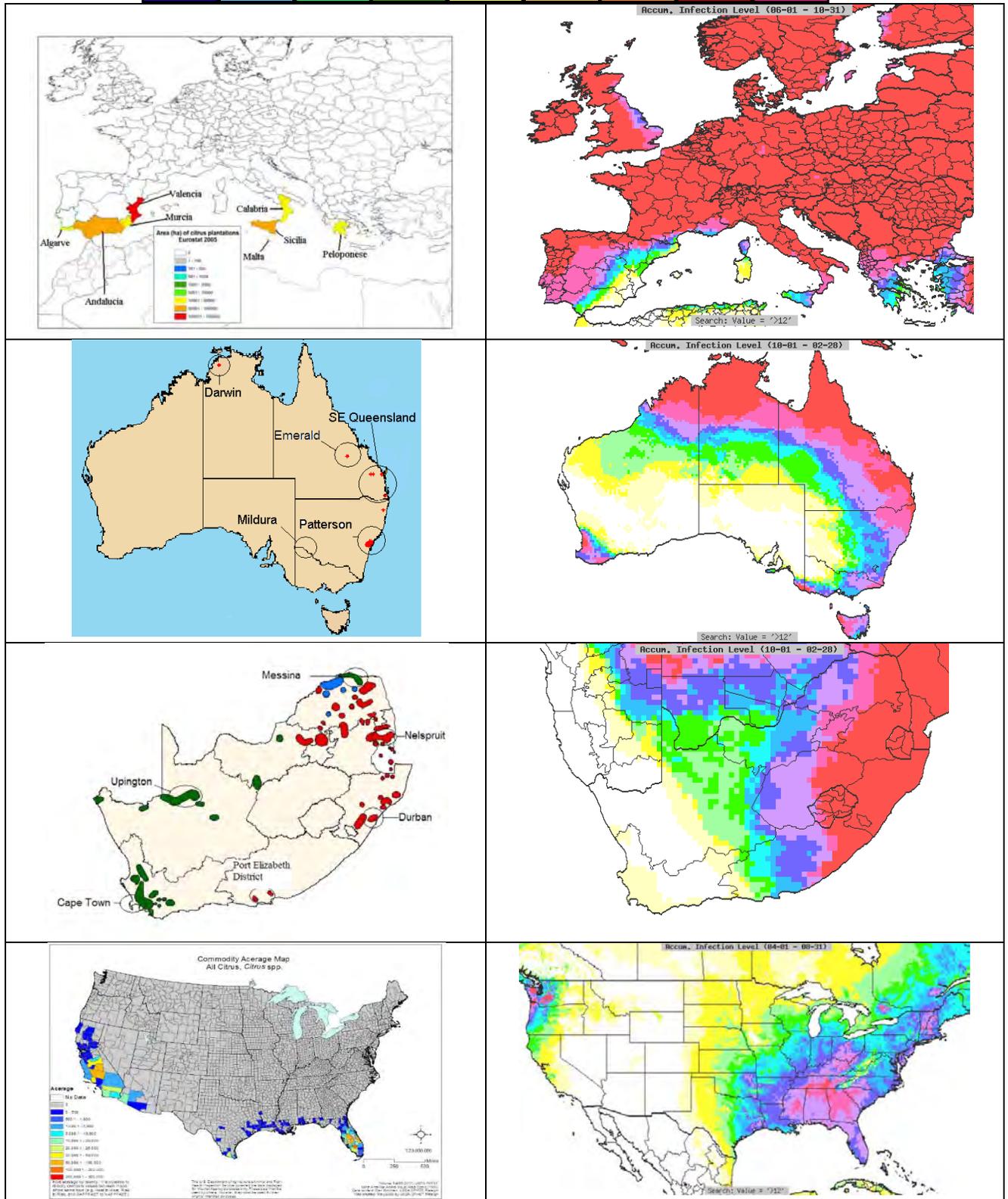


Figure 3A. The probability of more than 12 days suitable for *Guignardia citricarpa* ascospore infection by continent.

Accum. Infection Level

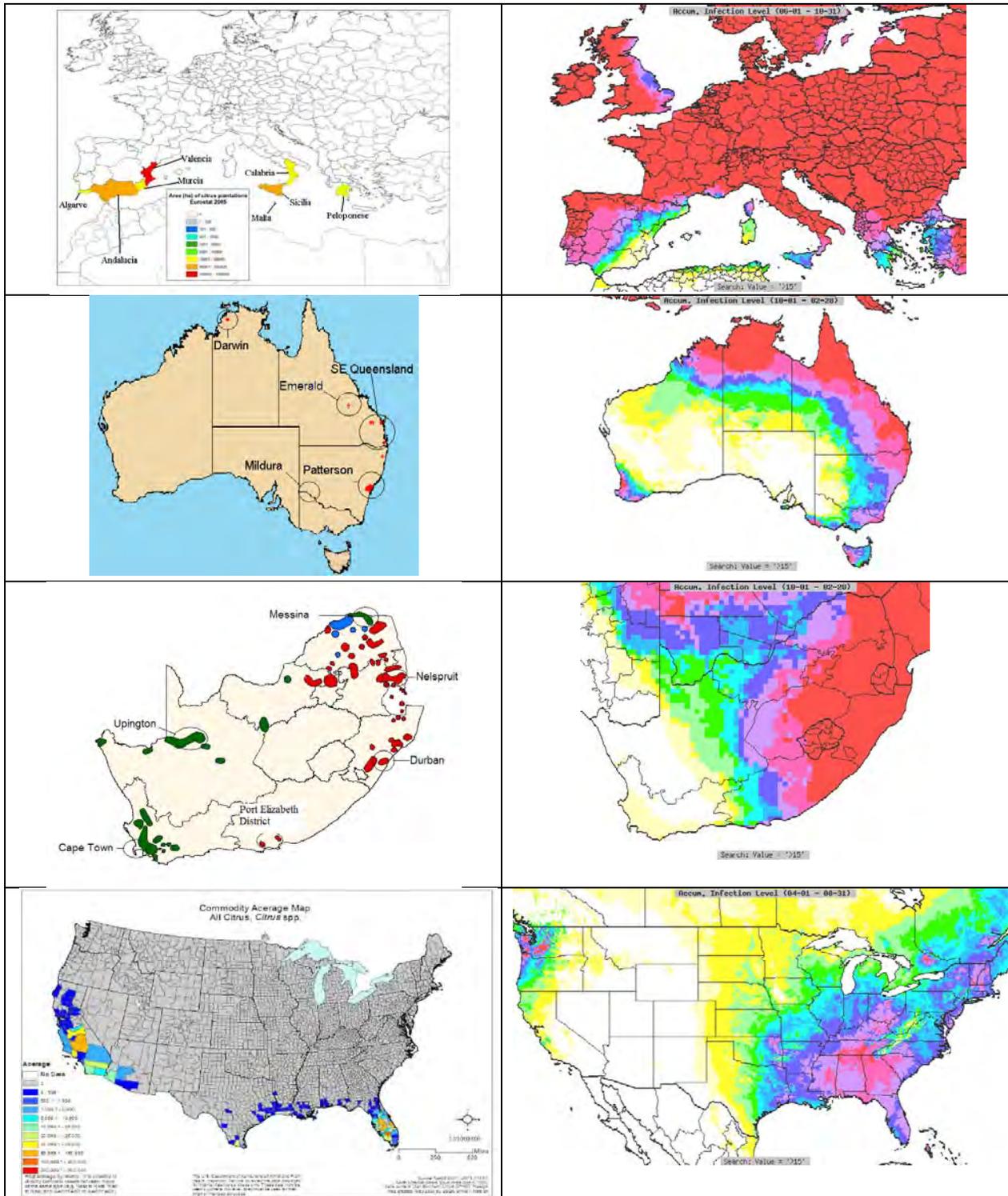


Figure 3B. The probability of more than 15 days suitable for *Guignardia citricarpa* pycnidiospore infection by continent.

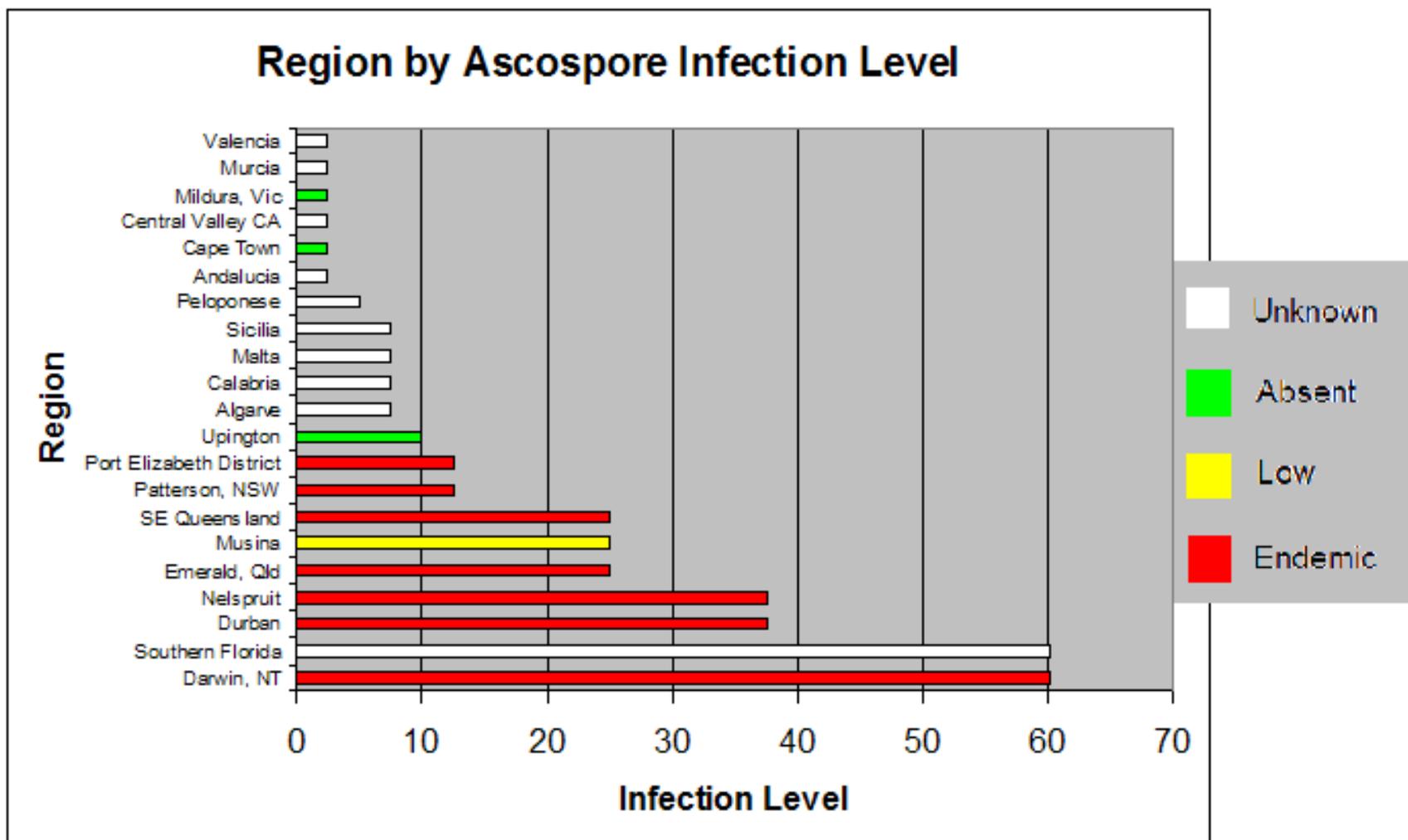


Figure 4A. The accumulated number of days suitable for *Guignardia citricarpa* ascospore infection arranged in descending value and colors corresponding to the observed incidence of disease in the citrus growing district.

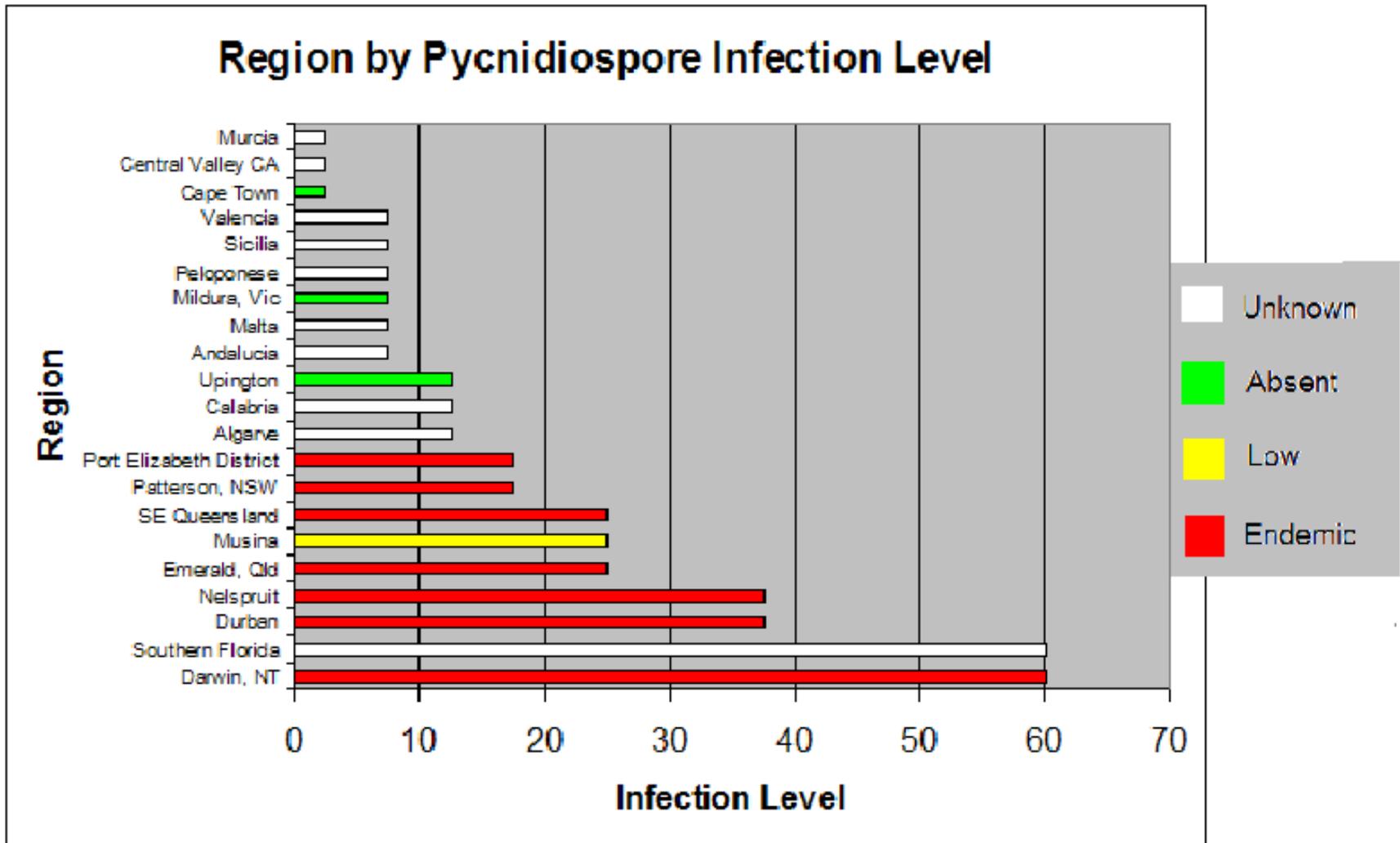


Figure 4B. The accumulated number of days suitable for *Guignardia citricarpa* pycnidiosporic infection arranged in descending value and colors corresponding to the observed incidence of disease in the citrus growing district.