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Evaluation of a paper by Guarnaccia et al. (2017) on the first report of *Phyllosticta citricarpa* in Europe

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Abstract

The Plant Health Panel reviewed the paper by Guarnaccia et al. (2017) and compared their findings with previous predictions on the establishment of *Phyllosticta citricarpa*. Four species of *Phyllosticta* were found by Guarnaccia et al. (2017) in Europe. *P. citricarpa* and *P. capitalensis* are well-defined species, with *P. citricarpa* recorded for the first time in Europe, confirming predictions by Magarey et al. (2015) and EFSA (2008, 2014, 2016) that *P. citricarpa* can establish in some European citrus-growing regions. Two new species *P. paracitricarpa* and *P. paracapitalensis* were also described, with *P. paracitricarpa* (found only in Greece) shown to be pathogenic on sweet orange fruits. Genotyping of the *P. citricarpa* isolates suggests at least two independent introductions, with the population in Portugal being different from that present in Malta and Italy. *P. citricarpa* and *P. paracitricarpa* were isolated only from leaf litter in backyards. However, since *P. citricarpa* does not infect or colonise dead leaves, the pathogen must have infected the above living leaves in citrus trees nearby. Guarnaccia et al. (2017) considered introduction to be a consequence of *P. citricarpa* having long been present or of illegal movement of planting material. In the Panel's view, the fruit pathway would be an equally or more likely origin. The authors did not report how surveys for citrus black spot (CBS) disease were carried out, therefore their claim that there was no CBS disease even where the pathogen was present is not supported by the results presented. From previous simulations, the locations where Guarnaccia et al. (2017) found *P. citricarpa* or *P. paracitricarpa* were conducive for *P. citricarpa* establishment, with number of simulated infection events by pycnidiospores comparable to sites of CBS occurrence outside Europe. Preliminary surveys by National Plant Protection Organisations (NPPOs) have not confirmed so far the findings by Guarnaccia et al. (2017) but monitoring is still ongoing.

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1. Introduction

1.1. Background and Terms of Reference as provided by the requestor

The European Commission has been recently informed by EFSA of the availability on line (<http://www.sciencedirect.com/science/article/pii/S0166061617300234>) of an accepted manuscript that will be published in the near future in Studies of Mycology entitled "First report of *Phyllosticta citricarpa* and description of two new species, *P. paracapitalensis* and *P. paracitricarpa*, from citrus in Europe". This document described the identification of *Phyllosticta citricarpa* in Italy, Malta and Portugal and other species found in Europe.

EFSA is requested, pursuant to Article 29 of Regulation (EC) No 178/2002, to prepare an urgent evaluation of this scientific paper, i.e. before the end of September 2017. In particular, EFSA is asked, in view of current scientific knowledge, to review the methodologies used in this paper, as well as the analysis of the results and the related discussion. In addition, EFSA is requested to report and discuss how these findings fit the predictions of the model simulations on the establishment of *P. citricarpa* in the EU.

1.2. Interpretation of the Terms of Reference

Phyllosticta citricarpa (McAlpine) van der Aa is officially absent from the EU and is a harmful organism listed in Directive 2000/29/EC, whereas *Phyllosticta capitalensis* Henn., which is morphologically similar to *P. citricarpa*, is a non-pathogenic endophyte, commonly isolated from citrus tissues and other hosts and is known to occur in the European Union (Wikee et al., 2013). *P. paracapitalensis* Guarnaccia & Crous, sp. nov. and *P. paracitricarpa* Guarnaccia & Crous, sp. nov. are proposed by Guarnaccia et al. (2017) as new fungal species and have never been previously reported in Europe.

In this Scientific Opinion, the Panel has evaluated the paper by Guarnaccia et al. (2017), but it has not assessed the regulatory status of the harmful plant pathogenic fungus *P. citricarpa*. Such activities are within the competence of the National Plant Protection Organisations (NPPOs).

The Terms of reference required EFSA to undertake two distinct tasks, and these are assessed in separate chapters of this opinion:

- 1) the evaluation of the paper by Guarnaccia et al. (2017) (see Section 3.1)
- 2) comparison of the findings by Guarnaccia et al. (2017) with previous model predictions on the establishment of *P. citricarpa* in the European Union (see Section 3.2).

Since the newly described species *P. paracitricarpa* has been shown by Guarnaccia et al. (2017) to induce symptoms of citrus black spot (hereafter referred to as CBS) in artificially inoculated sweet orange (*Citrus sinensis* Osbeck) fruits, for the second task the Panel considered *P. paracitricarpa* as well as *P. citricarpa*.

1.3. Additional information

Before the publication of this Scientific Opinion, EFSA has already provided risk assessment and scientific advice on *P. citricarpa* for the EU territory. The principal EFSA outputs on this topic are listed below:

- EFSA (European Food Safety Authority), 2008. Scientific Opinion of the Panel on Plant Health (PLH) on a request from the European Commission on *Guignardia citricarpa* Kiely. EFSA Journal 2008;7(1):925, 108 pp. <https://doi.org/10.2903/j.efsa.2009.925>
- EFSA PLH Panel (EFSA Plant Health Panel), 2014. Scientific Opinion on the risk of *Phyllosticta citricarpa* (*Guignardia citricarpa*) for the EU territory with identification and evaluation of risk reduction options. EFSA Journal 2014;12(2):3557, 243 pp. <https://doi.org/10.2903/j.efsa.2014.3557>
- EFSA PLH Panel (EFSA Plant Health Panel), 2016. Evaluation of new scientific information on *Phyllosticta citricarpa* in relation to the EFSA PLH Panel (2014) Scientific Opinion on the plant health risk to the EU. EFSA Journal 2016;14(6):4513, 53 pp. <https://doi.org/10.2903/j.efsa.2016.4513>

To reduce the key risk assessment uncertainties, EFSA also outsourced research on the splash dispersal of *P. citricarpa* asexual spores (conidia, hereafter referred to as pycnidiospores) from artificially infected citrus fruit. Such work can be consulted in the following publications:

- Perryman SAM and West JS, 2014. Splash dispersal of *Phyllosticta citricarpa* pycnidiospores from infected citrus. EFSA supporting publication 2014-EN-560, 30 pp.
- Perryman SAM, Clark SJ and West JS, 2014. Splash dispersal of *Phyllosticta citricarpa* conidia from infected citrus fruit. Scientific Reports, 4, 6568.

2. Data and methodologies

2.1. Data

The data used in this Scientific Opinion are provided:

- in the Tables published by Guarnaccia et al. (2017); the geographical locations are given in Table 1 of the paper by Guarnaccia et al. (2017);
- in publications reporting the results of previous model simulations for the establishment of *P. citricarpa* in Europe (EFSA, 2008; Yonow et al., 2013; EFSA PLH Panel, 2014, 2016; Magarey et al., 2015).

In Table C.1 of Appendix C – Data provenance, the locations are shown for the data files on which the maps of this Scientific Opinion are based on. The data files are deposited on <http://zenodo.org>, as 10.5281/zenodo.1003121 (<https://doi.org/10.5281/zenodo.1003121>).

2.2. Methodologies

When conducting this assessment, the Panel also took into consideration the following EFSA horizontal guidance documents:

- Guidance of the Scientific Committee on Transparency in the Scientific Aspects of risk assessments carried out by EFSA. Part 2: General Principles (EFSA, 2009);
- Guidance on the structure and content of EFSA's scientific opinions and statements (EFSA Scientific Committee, 2014);
- Guidance on uncertainty (EFSA Scientific Committee, 2016).

3. Assessment

3.1. Evaluation of a paper by Guarnaccia et al. (2017)

The paper has three principal components that have been evaluated according to: (1) the methodology used; (2) the results; (3) the discussion of the results; additionally, (4) some other considerations relating to the evaluation have been raised. The paper has four distinct but inter-related lines of enquiry: the sampling sites used for isolation of *Phyllosticta* spp.; the identification of known and new species using morphological and DNA-based criteria; the inferences made on the origin of introduction; and the relationships between the isolations made and CBS disease at the sampling sites. The Panel first notes that the Institute involved in the designation of the two new *Phyllosticta* species is at the forefront of fungal taxonomy and the Panel is of the view that the new species designations are well-founded and make a major contribution to the taxonomy of the genus *Phyllosticta*. The Panel also notes that the 'One fungus-one name' principle in nomenclature has been criticised by some mycologists (Gams and Jaklitsch, 2011). As in other PLH Panel opinions, the Panel accepts the principle of 'one fungus-one name'.

3.1.1. Evaluation of Methodology

The paper by Guarnaccia et al. (2017) describes results obtained from samples taken from 95 locations in a wide range of citrus hosts and associated environments such as leaf litter in southern Europe from 2015 to 2017 to assess for the presence of *Phyllosticta* species.

The locations of sample sites were reported with GPS coordinates in decimal degrees to 6 decimal places, allowing them to be mapped and revisited if necessary, but without mention of the method used to record these coordinates. This implies a spatial resolution of approximately 0.1 m. However, to obtain such a level of precision requires professional GPS equipment with differential correction. The

accuracy of the GPS on mobile phones depends on the model and the location. Zandbergen and Barbeau (2011) found that the mobile phones they tested had a median horizontal error of between 5.0 and 8.5 m. Thus, depending on the type of GPS used by Guarnaccia et al. (2017) and the type of GPS available to other teams undertaking the resampling of the locations, it may be difficult to re-identify the precise tree that yielded the positive samples found by Guarnaccia et al. (2017), particularly if several trees are located in the near vicinity. The sampling produced 64 isolates of *Phyllosticta*, of which 52 were selected for further morphological and molecular characterisation. A multilocus phylogenetic analysis, using partial DNA sequences from genes encoding the rDNA internal transcribed spacer region (ITS), actin (*actA*), the translation elongation factor 1- α (*tef1*), glyceraldehyde-3-phosphate dehydrogenase (*gapdh*), the 28S large subunit nrDNA (LSU) and the RNA polymerase II second largest subunit (*rpb2*), was carried out on many *Phyllosticta* species (99 isolates in total, including the 52 selected test isolates). Growth rates at 9–39°C at 3°C intervals were assessed for a subset of isolates of the *Phyllosticta* species collected. The number of isolates of each species in the subset was not given.

Some details of the methods used were either missing or unclear:

- It was not clear from the methodology presented in the paper how the sample locations and sites (95 in total) were chosen as well as how different fruits (approximately 25), twig portions (25), living leaves (50) and litter layer leaves (50) from each *Citrus* host were collected at each site.
- The dates of sampling for the isolations done in 2015 and 2016 were not reported. The number of host plants sampled at each location was not given. It was only stated that a certain number of leaves, twigs or fruit per host was sampled, i.e. we do not know whether the 50 living leaves were from one tree or many trees found at a site or whether leaves from the leaf litter were collected beneath one tree or many trees. The approach of trying to isolate from plant material has been shown to be less effective in detecting the presence of the pathogen than established molecular diagnostic methods using macerated or powdered leaves (Bonants et al., 2003; Meyer et al., 2012; Hu et al., 2014). With the methods they used, symptoms would only be expected to be visible on certain plant tissues at particular times of the year depending on the location, citrus species and cultivar.
- New field visits, in the sites where *P. citricarpa* or *P. paracitricarpa* were found in 2015 and 2016, were made between December 2016 and January 2017 to confirm the initial findings and to assay for the presence of symptoms on fruit, leaves and twigs. However, the Panel notes that this period would have been too early for symptoms to be visible on fruit for most citrus varieties in southern Europe (see Figure 51 in EFSA PLH Panel, 2014). These follow-up visits in December 2016 to January 2017 were simply reported in the methods section but no results were presented in the paper (from personal communication from the corresponding author, the Panel was informed that no samples were taken; for details, please see Other considerations, Section 3.1.4).
- The number of isolation points attempted per sampled unit (leaf, fruit, twig) was not given, although the discussion mentions that *P. citricarpa* was found at a very low frequency in a few of the sites investigated. As *P. citricarpa* was only found in leaf litter leaves and approximately 50 were tested for the presence of *P. citricarpa* at each site, a frequency of the percentage of positive isolations could have been provided.
- In the material and methods, it was stated that a total of 111 *Phyllosticta* (incl. 64 European) isolates were included in the study (Table 2 of Guarnaccia et al., 2017) of which 100 (including the out-group) were used in the phylogenetic analysis. However, the discussion indicated that a subset of 52 European isolates was compared to several reference isolates using partial gene sequences of six different loci, as well as morphological characteristics. This was based on a comparison with sequences retrieved from GenBank of an additional 43 isolates. It is not stated why 12 European isolates were not sequenced (eight *P. citricarpa* isolates and four *P. paracitricarpa* isolates were not used). For the four isolates from Malta that underwent multilocus sequence analysis, it was also not clear whether the isolates used came from both or only one of the two possible locations in Malta, since the isolate location was not given in Table 2.
- No explanation is given for how the CBS disease surveys were conducted:
 - whether the numbers of trees and locations were chosen systematically or randomly;
 - whether disease surveys were conducted in or close to the vicinity of the sampling sites;

- which methods were used to decide on the time the surveys were conducted. Symptoms would only be expected to be visible on certain plant tissues at certain times of the year, varying with location, citrus species and cultivar;
 - whether the second set of field visits conducted in December 2016 and January 2017 was accompanied by a disease survey. It was intended to revisit the sites that had previously tested positive in order to confirm the findings and to assay for the presence of symptoms on fruit, leaves and twigs. However, this period, as explained above, depending on the citrus cultivar, may be not optimal for symptom expression on fruit;
 - why some important citrus production areas and sites at risk of infection, e.g. those identified by Magarey et al. (2015) or EFSA PLH Panel (2014), were not visited. In order to relate the findings to climate-based risk, information on the type of irrigation system used at the sites selected would have been very useful.
- Without properly constituted disease surveys, there is little support for the conclusion that the *P. citricarpa* isolates did not naturally cause disease.
 - The pathogenicity tests did not include a positive control, viz. inoculation with a known pathogenic isolate; the tests were terminated after 25 days, while a relatively longer period might have been needed to obtain typical CBS lesions and subsequent pycnidia (Perryman et al., 2014).
 - A minor technical point is that it is stated that 100 mL of spore suspension was inoculated onto fruit but this must have been 100 μ L.

3.1.2. Evaluation of Results

3.1.2.1. New species/taxonomy

A Bayesian tree based on ITS partitioning constructed for the genus *Phyllosticta* (isolates and/or sequences obtained from 7 European and 12 non-European countries) resolved 15 species, of which two, *P. paracapitalensis* and *P. paracitricarpa* (previously considered a subclade of *P. citricarpa*), named after their close resemblance to *P. capitalensis* and *P. citricarpa*, respectively, were new species. Of the 15 species resolved, only these four were found from the locations in Europe (*P. citricarpa* was isolated from leaf litter in Italy, Malta and Portugal; *P. paracitricarpa* from leaf litter in Greece). Sequence analysis showed the presence of two previously described species, *P. capitalensis* (from asymptomatic living leaves of *Citrus* spp.) in Greece, Italy, Malta, Portugal and Spain, and *P. citricarpa* (from leaf litter of *C. sinensis* and *C. limon*) in Italy, Malta and Portugal. In addition, two new species were described, *P. paracapitalensis* (from asymptomatic living leaves of *Citrus* spp.) in Italy and Spain, and *P. paracitricarpa* (from leaf litter of *C. limon*) in Greece. As indicated above, the Panel is of the view that the new species designations are well-founded.

Nucleotide variation was observed when comparing *P. paracapitalensis* and *P. capitalensis* (20 fixed nucleotide changes) and between *P. paracitricarpa* and *P. citricarpa* (seven fixed nucleotide changes). One nucleotide change distinguishing *P. capitalensis* from *P. paracapitalensis*, position 628, was wrongly marked in bold (Table 3) but it was not counted as one of the 20 nucleotide changes. Morphological observations also supported the distinction between the two known species and their sister novel species. The species *P. capitalensis* and *P. paracapitalensis* grew faster than *P. citricarpa* and *P. paracitricarpa* in the mycelial growth rate study, although all four species had similar cardinal temperatures. Formal descriptions of the two new species are provided, including the designation of holotypes and culture ex-types. Mating type identifications were made for *P. citricarpa* but not for *P. paracitricarpa*. The *P. citricarpa* isolates found at sites in Italy and Malta were identical multilocus genotypes (MLG) with mating type MAT1-2-1, while additionally isolates from sites in Portugal were a different single MLG (with mating type MAT1-1-1). At each site, only a single mating type was found.

3.1.2.2. Pathogenicity tests

Lesions developed on sweet orange fruits inoculated with two isolates of *P. citricarpa*. The lesions were considered by the authors to be atypical compared to those seen in CBS disease. The pathogen was consistently re-isolated from these lesions. The Panel, however, observes that these lesions resembled some of those in the plates of the EPPO/OEPP (2009) and ISPM 27 diagnostic protocols and therefore were not atypical. Known pathogenic *P. citricarpa* isolates from other continents were not included in the pathogenicity tests; under the experimental conditions used, known pathogenic isolates could have induced similar or more typical lesions compared to the European isolates. No symptoms

were observed by the authors on fruits inoculated with two isolates of *P. capitalensis* or two of *P. paracapitalensis*; however, this is not immediately obvious from their Figures 6C and 6D. Moreover, the water control was not shown. No attempt was made to re-isolate from asymptomatic fruit (i.e. water controls or inoculated with *P. capitalensis* or *P. paracapitalensis*). The two isolates of *P. paracitricarpa* included in the pathogenicity tests were able to induce lesions (necrosis) in artificially inoculated mature sweet orange fruit and were consistently re-isolated from these lesions.

3.1.2.3. Genotypic evaluation/connectivity of *P. citricarpa*

A distinction needs to be made between the isolates obtained and the populations from which the samples were taken. The isolate sampled may not be representing the diversity of the populations, especially when the number of isolates (64 across all sampling sites) is small. For this reason, as recognised by the authors, the populations can only be described as putative. The 20 *P. citricarpa* isolates obtained from the four localities in the three countries (as shown in Table 2 of Guarnaccia et al., 2017) were genotyped and two MLG were identified. The isolates from Malta (2) and from Italy (1) shared one MLG, the isolates from Portugal had a distinct MLG. The isolates from Portugal shared an MLG with three isolates from Australia. The MLG shared between Malta and Italy was different from those of isolates obtained from the other countries.

3.1.2.4. Sampling results

According to the sampling and isolation results, 64 *Phyllosticta* isolates were recovered from 11 sites. Of these, 32 were obtained from fresh leaves, 28 were associated with leaf litter and 4 with leaf spot symptoms. Table 2 of Guarnaccia et al. (2017) shows that all 20 'pathogenic' *Phyllosticta* isolates (12 isolates of *P. citricarpa* from *C. limon* and *C. sinensis* and 8 isolates of *P. paracitricarpa* from *C. limon*) originated from leaf litter and the 4 'leaf spot' isolates were associated with *P. capitalensis* on *C. medica* var. *sarcodactylis* (Wikee et al., 2013 suggest that *P. capitalensis* can be associated with lesions caused by insects) at a site in Italy.

Guarnaccia et al. (2017) indicated that during the periods when samples were taken from the different locations, no CBS symptoms were observed. However, the basis for this statement is not given. The methodology for disease surveys has been clearly described for epidemiological studies, including where first detection is the objective of the survey (Weltzien, 2012), and in the particular case where the results are to conform to IPPC requirements (Kalaris et al., 2014). Guarnaccia et al. (2017) describe their sampling procedures for isolating *Phyllosticta*; however, no survey methodology for detecting disease is described. The symptoms of CBS are variable in appearance and often resemble those caused by other citrus pathogens or by insects, mechanical or cold damage, particularly in the case of freckle spot (EPPO/OEPP, 2009; ISPM 27, 2016). Symptoms that might be confused with those of CBS have previously been reported in the Mediterranean Basin, but these were caused by other pathogens, pests or abiotic disorders (Amat, 1988; Agustí et al., 2004; Vacante and Calabrese, 2009; Agustí, 2012).

After the initial samplings in 2015 and 2016, Guarnaccia et al. (2017) revisited during December 2016 and January 2017 the sites where *P. citricarpa* or *P. paracitricarpa* had been found. Details and results of the isolations eventually performed in this confirmatory sampling were not reported in the paper. However, from a personal communication by the corresponding author of the paper (see Other considerations, Section 3.1.4), the Panel was informed that no samples were taken. Guarnaccia et al. (2017) provided an overall statement saying that during the 'surveys' no CBS symptoms had been observed. Presumably this also applied to the second set of visits. However, as in 2015 and 2016, no information was provided on how these disease surveys were conducted.

Table 1 below summarises the 95 locations investigated by Guarnaccia et al. (2017) in Greece, Italy, Malta, Portugal and Spain. In Figure 1, the 95 locations are shown over a European map showing the density of citrus production areas. The countries with the highest number of locations investigated were Italy (36), Greece (22) and Spain (21). Portugal and Malta were represented by 10 and 6 locations, respectively. A total of 63 orchards were investigated and 22 backyards.¹ Two botanical gardens and three experimental orchards were investigated in Spain. Five nurseries were investigated in Italy. Regarding the condition of the investigated locations, 48 orchards were cultivated and 15 were 'unkept' (defined as 'plants abandoned'). From the 22 backyards investigated, 10 were cultivated and 12 were 'unkept'. One botanical garden was cultivated and another 'unkept'. The three

¹ When the locations sampled by Guarnaccia et al. (2017) were examined in Google Earth or by field visits by the NPPOs, those locations described as backyards appear to vary from large hotel gardens to relatively few citrus trees in a small garden.

experimental orchards and five nurseries investigated were all cultivated. Most of the locations investigated (69) had trees between 10 and 30 years old. Only 14 locations had trees younger than 10 years and 12 locations were older than 30 years. Based on this summary, it would appear that Guarnaccia et al. (2017) followed a stratified if uneven sampling scheme. For instance, some site types such as nurseries, botanical gardens and experimental orchards, were only sampled in two countries. Likewise, the number of backyards sampled was highly variable, from 1 out of 21 in Spain to 4 out of 6 in Malta.

Table 1: Summary of locations and characteristics of the investigated sites as from Table 1 by Guarnaccia et al. (2017)

Country	Total	Number of sites investigated by Guarnaccia et al. (2017)				
		Backyards	Botanical gardens	Experimental orchards	Nurseries	Orchards
Greece	22	2	0	0	0	20
Italy	36	11	0	0	5	20
Malta	6	4	0	0	0	2
Portugal	10	4	0	0	0	6
Spain	21	1	2	3	0	15
Total	95	22	2	3	5	63

Guarnaccia et al. (2017) found *P. citricarpa* and *P. paracitricarpa* only in leaf litter samples. In Malta, *P. citricarpa* was detected in leaf litter samples in two backyards, one cultivated site with 60–70 years old trees at Gozo and another 'unkept' location with 15–25 years old trees at Zurrieq. In Italy, *P. citricarpa* was detected in leaf litter in a cultivated backyard with 20–30 years old trees at Trebisacce. In Portugal, *P. citricarpa* was detected at Monchique, in an 'unkept' backyard with 30–40 years old trees. The newly described species, *P. paracitricarpa* was detected in Arta, Greece, in a leaf litter sample from an 'unkept' backyard with 30–40 years old trees. Photos of the backyards and smallholdings from the sites corresponding to the geographical coordinates indicated by Guarnaccia et al. (2017) for its findings of *P. citricarpa* are shown in Figure 2.

P. citricarpa and *P. paracitricarpa* were found by Guarnaccia et al. (2017) in 4.2% and 1%, respectively, of the locations investigated. In Italy, *P. citricarpa* was found in 2.8% of all sites investigated (9% when considering only the backyards). Equivalent figures for Malta were 33.3% overall and 50% in the backyards. In Portugal, the findings were 10% overall and 25% in the backyards. The overall percentages for *P. paracitricarpa* in Greece were 4.5% overall and 50% when considering only the backyards. Despite the relative low number of sampled sites, the overall percentage of backyard sites where *P. citricarpa* was found was relatively high, particularly in Malta. The NPPOs are making follow up investigations at these locations.

As indicated in Section 3.1.1, the evaluations by Guarnaccia et al. (2017) were conducted by sampling fruits, twigs, living leaves and leaf litter from each citrus host present at each site investigated. *P. citricarpa* or *P. paracitricarpa* were only detected by Guarnaccia et al. (2017) in the leaf litter but not in living leaves in the canopy of the trees nearby. Isolations from the leaf litter are much more difficult to make than those from living leaves in the canopy because of the presence of soil microorganisms that may contaminate the samples (Frankland et al., 1990; Waller et al., 1998). Moreover, Truter et al. (2007) indicated that *P. citricarpa* pycnidiospores were not able to infect and colonise freshly detached green leaves or natural leaf litter. Therefore, leaf infections by *P. citricarpa* are restricted to those on living leaves in the canopy. Infected leaves will eventually fall down and form the leaf litter on the soil surface; therefore, it is unclear why no isolations were obtained from the living leaves.

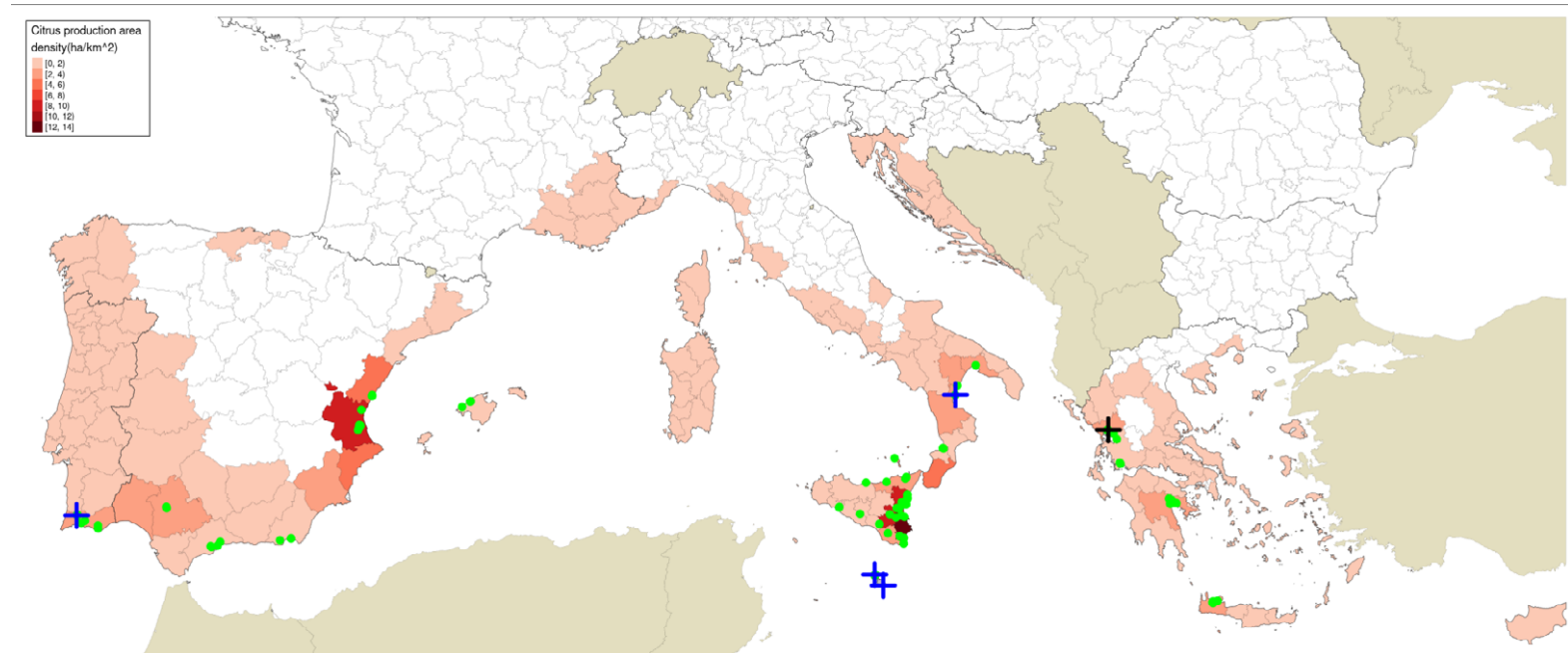


Figure 1: Locations sampled by Guarnaccia et al. (2017): blue crosses indicate sites where *P. citricarpa* was reported in the leaf litter; a black cross indicates the site where *P. paracitricarpa* was reported in leaf litter in Greece; green dots indicate sites of sampling without positive finding. The map provides in the background the density of citrus production areas (in ha of citrus production areas per km² of total area) for the European Union NUTS3 regions (as extracted from national statistical databases of Portugal, Spain, France, Italy, Malta, Croatia, Greece and Cyprus for year 2007; for details, see Appendix F of EFSA PLH Panel, 2014)

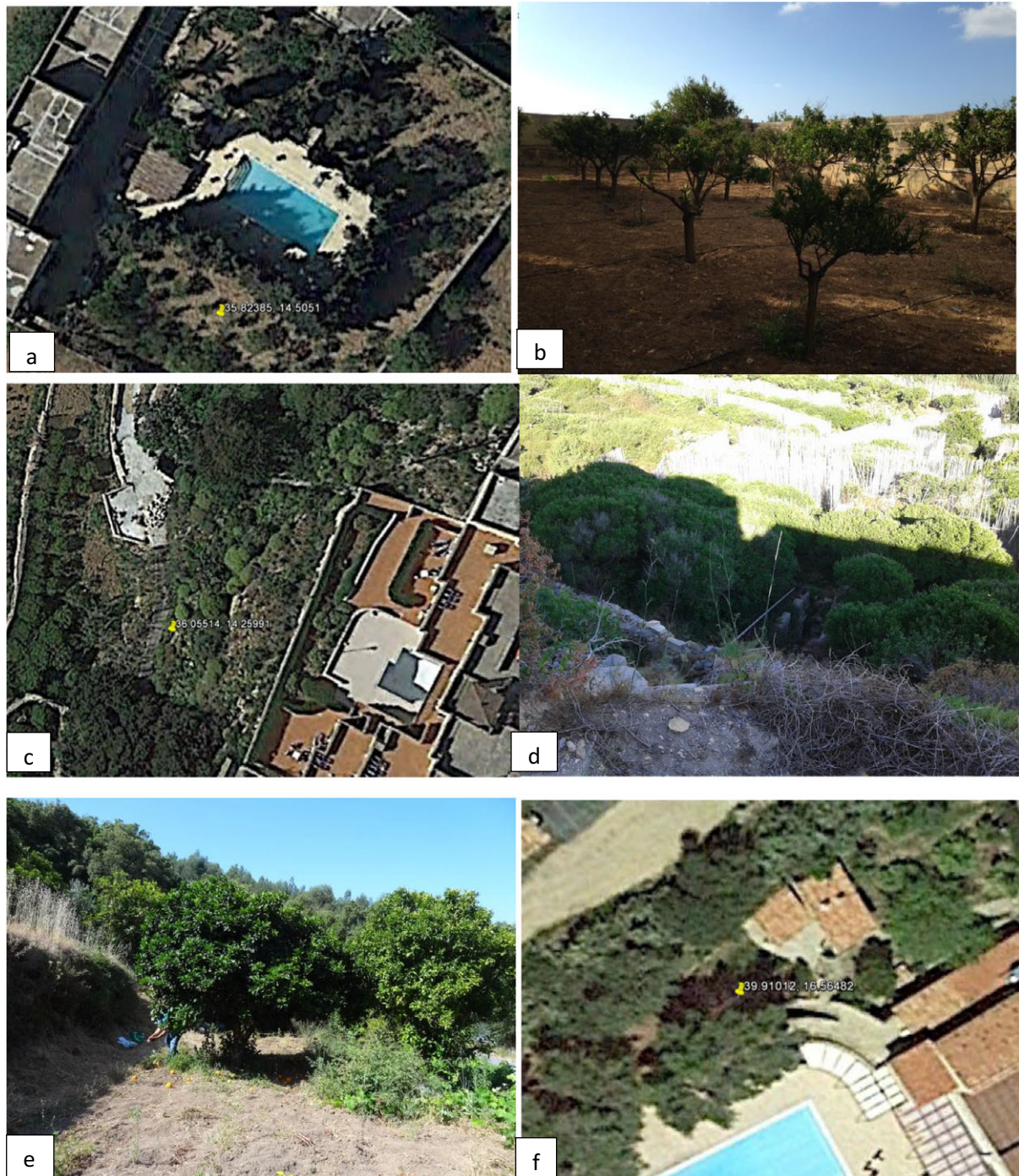


Figure 2: Photos of backyards and smallholdings from sites corresponding to the geographical coordinates indicated in Guarnaccia et al. (2017) for their findings of *P. citricarpa*. Top: (a) site in Zurrieq, Malta (Source: Google Earth, 5.823845 N, 14.505099 W, Eye altitude 200 m, Image: CNES/Airbus 2017 with approximate scale 1: 250); and (b) site at Zurrieq, Malta (by courtesy of Malta NPPO); Middle: (c) site at Zhagra, Gozo (Source: Google Earth, 36.055138 N, 14.259907 W, Eye altitude 200 m, Image: CNES/Airbus 2017 scale 1: 250); and (d) site at Zhagra, Gozo, (by courtesy of Malta NPPO); bottom; (e) site at Monchique, Algarve (by courtesy of Portugal NPPO); (f) site at Trebisacce, Calabria (Source: Google Earth, 39.910122 N, 16.564824 W, Eye altitude 80 m, Image: TerraMetrics 2017 with approximate scale 1:250)

3.1.3. Evaluation of Discussion

This study is a result of fresh collections of several *Phyllosticta* isolates from citrus in Europe and of a comprehensive DNA sequence analysis of *Phyllosticta* isolates from all continents; however, as discussed in Section 3.1.2 above, it does not represent a disease survey. This study is the first report of *P. citricarpa* in Europe and the first description of the new species *P. paracitricarpa* from eight isolates from Greece and two others from China, previously identified as *P. citricarpa*. The discussion by Guarnaccia et al. (2017) demonstrates the importance of this study and the need for further research, particularly on the relevance of *P. paracitricarpa* as a citrus pathogen.

The *P. citricarpa* isolates from Portugal and Malta/Italy represent two distinct putative clones based on MLGs and mating types. They differed also in their degree of 'connectedness' with isolates from other countries. Populations from Portugal and Australia were closely connected but those from Malta/Italy appeared distinct from other populations. The authors claim that this study establishes the presence of *P. paracitricarpa* only in Asia and Europe; however, the Panel notes the lack of any systematic investigation in citrus-producing areas worldwide on this newly described species.

No statistically based sampling procedure was employed by Guarnaccia et al. (2017), so the representativeness of the samples and the resulting population structure of *P. citricarpa* and *P. paracitricarpa* in Europe are not conclusive. Indeed, Guarnaccia et al. (2017) refer to 'putative' populations due to the low number of isolates obtained and the sampling strategy employed. Moreover, as indicated in Section 3.1.1, no information was provided about the number of isolation points in each sample and the corresponding association of the isolates obtained with them. For instance, isolates obtained from the same pycnidium in a dead leaf are likely to belong to the same mating type and MLG.

The species *P. citricarpa* and *P. paracitricarpa* were detected only in leaf litter samples, whereas they were apparently not detected on living leaves at the same location. It is known that CBS leaf symptoms are seldom seen on living leaves except on lemon leaves, a very susceptible host species. Nevertheless, isolations from leaf litter are usually less successful with respect to isolation from living plant material due to the presence of soil microorganisms and contaminants (Frankland et al., 1990; Waller et al., 1998). Moreover, Truter et al. (2007) indicated that pycnidiospores of *P. citricarpa* cannot infect and colonise detached leaves or leaf litter. Consequently, it can be assumed that, in order for the leaf litter to be colonised by *P. citricarpa*, the leaves in the canopy should have been first infected by the pathogen before falling down to form the leaf litter. This raises the questions as to how *P. citricarpa* persisted over time in these locations and how it was able to colonise other substrates as a saprophyte, a life style that has not previously been described for this species. Nevertheless, the use of detection methods based on polymerase chain reaction (PCR) (Meyer et al., 2012; Hu et al., 2014) applied to a broader sample of living leaves would have improved the sensitivity of the analyses to clarify this issue. *Phyllosticta* species are often associated with endophytic infections (Johnston, 1998) in other plant species but it is not clear whether the presence of a fungus in a citrus plant is truly endophytic or symptomless infection of a pathogen, which may still reduce yield and sporulate, without causing visible symptoms (as in the case of *Rhynchosporium commune*² on barley reported by Looseley et al., 2012). This raises a question over the final statement made in the abstract, that *Phyllosticta* species did not cause disease.

The authors indicated that the putative 'populations' of *P. citricarpa* from Italy and Malta represented a single clone of mating type MAT1-2-1, and that from Portugal another of mating type MAT1-1-1. The presence of only one mating type in each country suggests that *P. citricarpa* would reproduce only asexually through pycnidiospores in these sites. These asexual spores are formed in fruit and twig lesions as well as on leaf litter (Kotzé, 1981; Kotzé, 2000; Silva et al., 2016), although Guarnaccia et al. (2017) only detected *P. citricarpa* in the leaf litter.

Pycnidiospores of *P. citricarpa* are dispersed by water splash and they have typically been considered as a short-range dispersal mechanism of minor epidemiological relevance when compared with the airborne ascospores (Kotzé, 1981; Kotzé, 2000). However, more recent studies under laboratory conditions demonstrated that *P. citricarpa* pycnidiospores could reach longer distances than previously thought (Perryman and West, 2014; Perryman et al., 2014). In addition, infected leaf litter leaves could be dispersed by wind over relatively long distances. Furthermore, pycnidiospores are believed to play a major epidemiological role in Florida, where the population of *P. citricarpa* is clonal and only one mating type (MAT1-2) is present (Wang et al., 2016; Hendricks et al., 2017). Studies conducted in Brazil also suggested that pycnidiospores are of major importance in CBS epidemics

² Current name according to Index Fungorum (Anonymous, 2016) is *Rhynchosporium graminicola* Heinsen (syn. *R. commune* used by Looseley et al., 2012).

(Spósito et al., 2007; Spósito et al., 2008), even in the presence of complementary mating types allowing for sexual reproduction through ascospores (Amorim et al., 2016).

Some evidence worldwide also illustrates the importance of pycnidiospores during the early stages of invasion. At the beginning of the epidemics in Zimbabwe, it was shown that most infections originated from pycnidiospores, while ascospores were only found in very small numbers (Whiteside, 1967). Later in 1978, when epidemics were well established, Kotzé (1981) found that ascospores were abundant in the same area. In Argentina, Garrán (1996) indicated that attempts to detect the sexual stage by weekly sampling of dead leaves in plots affected by CBS were unsuccessful. In Florida, CBS was first observed in 2010 (Schubert et al., 2012). As indicated above, disease establishment and spread in this area were attributed to pycnidiospores (Wang et al., 2016; Hendricks et al., 2017).

Citrus regions in Florida and Brazil are characterised by having high rainfall amounts during the growing season, creating conditions very favourable for splash dispersal and subsequent infections by pycnidiospores. On the contrary, citrus regions in the Mediterranean Basin are characterised by semi-arid climates with much lower rainfall amounts than Florida or Brazil. The ways in which *P. citricarpa* has been able to establish and persist with asexual water-splashed pycnidiospores under the particular environmental conditions of the Mediterranean Basin should be further studied. In any case, as Guarnaccia et al. (2017) pointed out, a broader sampling and a larger number of isolates would be needed to determine whether other complementary mating types are present in these areas.

Guarnaccia et al. (2017) suggested that *P. citricarpa* has probably been introduced in the Mediterranean basin with ancient importations of citrus plants, as early as the 5th century BC. However, the putative clonal populations of *P. citricarpa* found over all three area sites by the authors do not support the conclusion made in the paper about the hypothesis of ancient introductions. Many studies have documented clonal population structures throughout the entire introduced range. Shifts to clonality and loss of sexual reproduction are frequent in the life-history traits of introduced fungi (Gladieux et al., 2015). Bottlenecks and founder effects may hamper the coexistence of complementary mating types so that the pathogen can only reproduce asexually. However, the persistence of isolated clonal populations in the long term would be possible only in the absence of subsequent introductions of the pathogen, potentially carrying other complementary mating types leading to sexual reproduction. Assuming their hypothesis of introduction with ancient citrus plant imports, it is unlikely that no further introductions of *P. citricarpa* occurred in such a long period of time. Although clonal populations of pathogens such as *Phytophthora infestans* (Mont.) de Bary may persist over centuries, this was associated with a large pathogen population, leading to devastating outbreaks of late blight disease. Clonal populations of *P. citricarpa*, in the claimed absence of CBS disease, could be indicative of more recent introductions.

Infected fruit was not considered a pathway for *P. citricarpa* by Guarnaccia et al. (2017), although the potential of this pathway as a means of introduction was thoroughly documented in the pest risk assessment by EFSA (EFSA PLH Panel, 2014). Relatively large amounts of citrus fruit are imported every year from CBS-affected areas into the EU. These imports are subjected to specific phytosanitary measures to provide a level of confidence that fruits are free from CBS symptoms, but *P. citricarpa* interceptions are reported by border inspections in the EU (EFSA PLH Panel, 2014). Guarnaccia et al. (2017) indicated that compost heap waste disposal and fruit processing plants were not observed near the sites where *P. citricarpa* was detected. However, compost heaps and fruit processing plants could have been present in the area before 2015, when the samplings were initiated. Even within the 2015–2017 sampling period, the frequency and duration of the visits to the locations investigated might not have been sufficient to ensure that infected fruits or peel were not deposited in close proximity to the plots. Interestingly, *P. citricarpa* was detected only in backyard trees, which are more likely to come in close contact with household fruit refuse than commercial orchards.

The Panel considers that citrus fruit will be equally or more likely to introduce a single mating type clonal genotype of *P. citricarpa*, compared to an infected plant for planting. This is because *P. citricarpa* only reproduces on fruit through asexual pycnidiospores (Kotzé, 2000). Moreover, fruit is short-lived compared to an infected plant and the chance of an infection establishing by splash dispersal from a single lesion of a transient fruit would be lower (EFSA PLH Panel, 2014), leading to a greater likelihood that only one genotype would successfully establish a founding population. Tran et al. (2017) suggest only one mating type being present in a single disease lesion in aboveground parts. A plant-for-planting pathway would be in general a more likely route of introduction for both mating types from a citrus production area with both mating types, as the plant will be a persistent source of inoculum and sexual ascospores can be produced on the citrus leaves in the leaf litter.

As indicated in Section 3.1, the methods described for disease survey by Guarnaccia et al. (2017) raised some concerns. No information about the citrus cultivars present in the investigated locations was provided. No information was provided on the number of trees assessed for CBS symptoms or how they were selected for assessment (e.g. systematically or at random). The dates when the locations were visited were not reported, except for the second field visit that was conducted during December 2016 and January 2017 at sites where *P. citricarpa* or *P. paracitricarpa* were found during the initial samplings. Leaf symptoms of CBS are rare and infected leaves remain generally asymptomatic (Kotzé, 2000). In fruit, CBS is characterised by a relatively long incubation period, and fruit symptoms become visible several months after infection. Lesion formation is driven by phenology and environmental factors and CBS symptoms are visible when fruit mature and reach the ripening stage (Timmer, 1999; Spósito et al., 2004; Sousa and de Goes, 2010). As shown in the harvesting calendar in Figure 51 by EFSA PLH Panel (2014), citrus fruit in the Mediterranean Basin may ripen from September to July depending on the cultivar. Therefore, fruit symptoms may be easily overlooked if surveys were not conducted to coincide with the specific ripening season for each cultivar.

The CBS disease is characterised by a long lag phase and so the initial symptoms of the disease may remain undetected during the early stages of introduction. As Kotzé (1981) indicated, the causal fungus may be present for many years in a particular area before symptoms are observed. Inoculum build-up from the time of introduction to the onset of an epidemic may take decades. It may take 5–30 years from the time the first symptoms are noticed until the disease reaches epidemic proportions, depending on climate conditions and host susceptibility (Kotzé, 1981). Indeed, CBS symptoms in South Africa were observed for over 30 years before control measures became necessary (Kotzé, 1981). Likewise, experiences from Zimbabwe and Argentina are also indicative of the long lag phase of CBS. In Zimbabwe, CBS was first discovered in 1961 and in 1967 was still considered rare and very localised (Whiteside, 1967). It was not until 1978 that an epidemic was considered to be properly established in this country (Kotzé, 1981). In the north-east of Entre Rios, Argentina, CBS was first reported in 1986, but in 1996, it was not yet prevalent in the area with only a few foci showing slight to moderate disease severity (Garrán, 1996). The report of *P. citricarpa* in some European citrus locations by Guarnaccia et al. (2017) and the presumed absence of CBS symptoms could be explained by the known long lag phase of the CBS disease and might be indicative of recent introduction events.

The penultimate sentence of the paper emphasises that the climate modelling studies by Paul et al. (2005), Yonow et al. (2013) and Magarey et al. (2015) were parameterised according to the presence, absence, distribution and severity of CBS, and not the potential presence of the fungus in the absence of disease. With the exception of this paper by Guarnaccia et al. (2017), the known geographical distribution of *P. citricarpa* worldwide matches with that of CBS. Consequently, *P. citricarpa* and CBS distributions cannot be disaggregated within the datasets used by these previous climate model studies (Paul et al., 2005; Yonow et al., 2013; Magarey et al., 2015). Paul et al. (2005) indicated that areas of CBS presence were mapped where *P. citricarpa* has been isolated from symptomatic citrus fruit and/or leaves, or where the disease has been observed by field specialists. Likewise, Yonow et al. (2013) indicated that the distribution of *P. citricarpa* in South Africa was based on the data reported by Paul et al. (2005) revised in accordance with recent surveys, quoting E. Carstens, pers. comm. Sampling and isolations for *P. citricarpa* are described by Carstens et al. (2012) and are further reviewed by the EFSA PLH Panel (2016). Based on Carstens et al. (2012), *P. citricarpa* absence in South Africa was defined in citrus areas without CBS symptoms. For Taiwan and Australia, Yonow et al. (2013) used official sources and reports for *P. citricarpa*. Disease prevalence data from South Africa and Australia were used by Magarey et al. (2015) but data of *P. citricarpa* distribution by Carstens et al. (2012) and Yonow et al. (2013) were used for model validation. Moreover, as indicated above, in these two countries the current geographical distributions on *P. citricarpa* and CBS are coincident.

3.1.4. Other considerations

The Panel notes that notifications of the findings of the quarantine plant pathogen³ *Phyllosticta citricarpa* by Guarnaccia et al. (2017) were not sent to the NPPOs of the respective

³ As required by Directive 2000/29/EC as last amended, in Articles 15a and 16, and addressed in national plant health legislations (for Italy, see the Decreto Legislativo 19 agosto 2005, n. 214, art. 8; for Malta, see the Maltese Subsidiary Legislation 433.03 Regulation 5b; for Portugal, see the Portuguese Plant Health Law - Decree no 154/2005 and its amendments, article 30). Moreover, the species *P. citricarpa* is a listed harmful organism by Directive 2000/29/EC. Therefore, the conditions under which *P. citricarpa* may be moved within the EU for scientific purposes are clearly established by Commission Directive 2008/61/EC.

countries and that the NPPOs of Italy, Malta and Portugal became aware of these findings only by the publication online of the paper by Guarnaccia et al. (2017) on 29 May 2017.⁴ Hence, measures, in the sites of the findings by Guarnaccia et al. (2017), could only be taken by NPPOs starting from summer 2017.

Following a request for clarifications, the corresponding author of the paper has informed EFSA that, during the field visits undertaken in December 2016 and January 2017 to confirm the initial findings and to assay the presence of symptoms on fruit, leaves and twigs, as there were no symptoms, no samples were collected.⁵ Samples are currently being taken by the NPPOs of Italy, Malta and Portugal, at the sites where Guarnaccia et al. (2017) reported the finding of *P. citricarpa*. Preliminary results of surveys conducted by the NPPOs during the summer 2017 in Malta⁶ and Portugal,⁷ using an established DNA-based diagnostic protocol and repeating the methods by Guarnaccia et al. (2017), did not confirm the findings. Further samplings will be continued in the following seasons.

3.2. Comparisons of findings by Guarnaccia et al. (2017) with previous model predictions

As explained in Sections 1.1 and 1.2, EFSA is requested to report and discuss how the findings of *P. citricarpa* by Guarnaccia et al. (2017) in Europe fit the predictions of previous model simulations on the establishment of *P. citricarpa*.

The potential of *P. citricarpa* to establish in Europe has been studied previously (Paul et al., 2005; EFSA, 2008; Yonow et al., 2013; EFSA PLH Panel, 2014, 2016; Magarey et al., 2015). The EFSA PLH Panel (2014) combined previous modelling work on *P. citricarpa* ascospore maturation and release (Fourie et al., 2013) and a generic infection model (Magarey et al., 2005), to predict ascospore maturation, release and infection in EU citrus-growing areas. The EFSA PLH Panel (2014) primarily focused on the sexual reproduction cycle, as this was expected to be the principal way for this pathogen to establish and spread in the risk assessment area. However, this work also included the modelling of pycnidiospore infections taking into account experimental studies on pycnidiospore splash dispersal (Perryman and West, 2014; Perryman et al., 2014).

The geographical coordinates of the findings by Guarnaccia et al. (2017) of *P. citricarpa* and *P. paracitricarpa* were superimposed on the results for those sites from previous model simulations for the establishment of *P. citricarpa* in Europe. The models considered were the applications of the generic infection model for foliar fungal pathogens by Magarey et al. (2005), as extended for ascospore infections simulations by the ascospore maturation and release model of Fourie et al. (2013) (EFSA, 2008; EFSA PLH Panel, 2014, 2016; Magarey et al., 2015). The CLIMEX model by Yonow et al. (2013), further implemented by the EFSA PLH Panel (2014) with interpolated climate datasets of higher spatial and temporal resolution, was not the most appropriate for *P. citricarpa* but is also considered here as its outcomes were discussed by Guarnaccia et al. (2017).

3.2.1. Review of models previously applied to simulate *P. citricarpa* establishment

Models to simulate *P. citricarpa* establishment have been thoroughly reviewed by EFSA (EFSA, 2008; EFSA PLH Panel, 2014, 2016). A summary of results taken from these reviews is presented here.

Yonow et al. (2013) used CLIMEX, a model which predicts an organism's potential distribution based on climatic conditions in its current distribution and any known climatic responses (Table 2). The overall suitability of the location is given by the ecoclimatic index (EI), which is the product of growth and stress indices. The application of CLIMEX for the modelling of *P. citricarpa* establishment in Europe was evaluated in Section 2.1.3 of EFSA (2008) and in Section 3.3.2.2 of EFSA PLH Panel (2014). The key shortcoming noted in CLIMEX was that the calculation of the EI to provide an indicator of the overall suitability of the location does not take into account the periods of the year when the host is at a susceptible stage and inoculum is potentially available. Moreover, it cannot directly take into account

⁴ Personal communication from Paula Cruz de Carvalho, Direcção-Geral de Alimentação e Veterinária (DGAV), Lisboa (PT), 7 June 2017; Personal communication from Sharlo Camilleri, Veterinary and Phytosanitary Regulation Department, Plant Health Directorate, Lija (MT), 8 June 2017; Personal communication from Michele Ghezzi, Direzione Generale dello Sviluppo Rurale, Ufficio DISR V, Servizio Fitosanitario Centrale, Roma (IT), 16 June 2017.

⁵ Personal communication from Pedro Crous, Westerdijk Fungal Biodiversity Institute, Utrecht (NL), 9 November 2017.

⁶ Note dated 11 September 2017 of the Plant Health Directorate, Lija (MT).

⁷ Note dated 18 September 2017 of the DGAV, Lisboa (PT).

the effect of leaf wetness, a critical microclimatic environmental variable for the successful infection of most fruit and foliage fungal pathogens including *P. citricarpa*. Nevertheless, the Panel found that the potential for establishment represented by the EI in some EU citrus-growing areas varied according to both the spatial resolution and the time period covered by the input climate data with the maximum EI ranging from 3 (1961–1990, 0.5° resolution) to 4 (1961–1990 0.1° resolution) and 11 (1998–2007, 25 km resolution). These EI values were similar to those obtained for Addo in the Eastern Cape province of South Africa where *P. citricarpa* persists but according to Yonow et al. (2013) 'does not flourish'. Nevertheless, fungicide sprays are applied for CBS control in this area (Schutte, 1995; Korf, 1998; Grout, 2015).

Table 2: Summary of differences in the spatial and temporal factors among the three models

Models, locations and climatic data	Yonow et al. (2013) and its applications by EFSA PLH Panel (2014)	EFSA PLH Panel (2014)	Magarey et al. (2015)
Models	CLIMEX Compare Locations	Ascospore maturation and release Fourie et al. (2013) Ascospore/pycnidiospore infection Magarey et al. (2005)	Ascospore maturation and release Fourie et al. (2013) Ascospore/pycnidiospore infection Magarey et al. (2005)
Locations modelled, spatial resolution and extent	Global 0.5° latitude × 0.5° longitude grid cells. The EFSA PLH Panel (2014) used the same model also for 1518 25 × 25 km citrus grid cells and for 0.1° latitude and 0.1° latitude grid cells for the EU.	One thousand five hundred and eighteen 25 × 25 km grid cells in Europe covering all European NUTS Level 3 regions with citrus production	36 locations in Europe: Cyprus (1), France (2), Greece (7), Italy (15), Malta (1), Portugal (5) and Spain (5)
Source of weather/climatic data	CRU CL1.0 New et al. (1999). The EFSA PLH Panel (2014) used the same model with MCYFS climatic data and with CRU CL1.0 1961–1990 climatology for 0.1° latitude and 0.1° latitude grid cells (New et al., 2002).	The JRC Mars Crop Yield Forecasting System (MCYFS) grid cells interpolated to 25 × 25 km based on the mean agricultural altitude Agri4castWiki (2015)	NCEP CFSR global database interpolated to 38 × 38 km based on the mean grid elevation
Time period of weather/climatic data	1961–1990. The EFSA PLH Panel (2014) used the same model with data from 1998 to 2007	1989–2009	2003–2011
Temporal resolution of weather/climatic period utilised	Monthly. The EFSA PLH Panel (2014) used the same model with daily MCYFS data.	Daily for MCYFS data. Bregaglio et al. (2010) was used to generate hourly values of relative humidity. Hourly for 10 Italian and 14 Spanish agro-meteorological stations	Hourly

In order to overcome these limitations of CLIMEX, process-based models were later used to simulate the potential establishment of *P. citricarpa*. Models by Fourie et al. (2013) were used to simulate the maturation and release of *P. citricarpa* ascospores (i.e. inoculum availability) and the generic infection model by Magarey et al. (2005) to simulate infections by ascospores or pycnidiospores (EFSA PLH Panel, 2014; Magarey et al., 2015). Sections 3.1.1.2 and 4.2, and Table 1 of EFSA PLH Panel (2016) provided a detailed comparison of the data inputs and modelling approaches adopted by EFSA PLH Panel (2014) and Magarey et al. (2015) for ascospore maturation, release and infection as well as pycnidiospore infection. They found a poor correlation between the models, although both studies simulated infection by ascospores or pycnidiospores with essentially the same equations and parameter values and also utilised the Gompertz equation (Fourie et al., 2013) to describe the dynamics of ascospore production. However, there were a number of differences in the weather and climatic data as well as in model assumptions (Table 2). Actually, the models were shown

to be highly sensitive to these assumptions and more precise definitions of the model components are needed to allow direct comparisons to be made.

Magarey et al. (2015) published a rule to classify the suitability of sites for ascospore or pycnidiospore infections based on the average predicted infection period score at the lowest ranked site with a moderate observed disease prevalence (Addo, South Africa). Magarey et al. (2015) stated that the infection score represents the total number of predicted ascospore or pycnidiospore infection periods over a 9-year period. However, because they presented the model outputs as averages with a standard deviation, the logical interpretation is that they were actually providing the average number of infection periods per year.

3.2.2. Applications of the models to the locations sampled by Guarnaccia et al. (2017)

3.2.2.1. CLIMEX model

Regarding the CLIMEX growth index and EI for the grid cells where the sites investigated by Guarnaccia et al. (2017) are located, the results presented are from the model by Yonow et al. (2013) run over the 1961–1990 climatic dataset by New et al. (2002) (Appendix A Figures A.1 and A.2) and JRC Mars 1989–2009 interpolated climatic dataset (Appendix A Figures A.3 and A.4) for the cells of the EU citrus-growing area. Table A.1 in Appendix A shows that none of the 95 locations sampled by Guarnaccia et al. (2017) in southern Portugal, southern and eastern Spain, Majorca, Sicily, southern Italy, Malta, mainland Greece and Crete were in 25 km grid cells with EIs greater than 4 and 26 cells had zero EIs, as in Yonow et al. (2013). Samples were not taken from the Ebro Delta Region of eastern Spain, where the EFSA PLH Panel (2014) found the highest EI values.

Guarnaccia et al. (2017) found *P. citricarpa* in Zurrieq, Gozo, Trebisacce and Monchique, and *P. paracitricarpa* in Arta (Figure 1). The grid cells with the five locations where Guarnaccia et al. (2017) found *P. citricarpa* either had EIs of 1 or 2 (Table 3 below and Figures A.1, A.2, A.3 and A.4 in Appendix A). However, the EFSA PLH Panel (2014) noted that the CLIMEX growth index, the combination of the temperature index and moisture index, may be a more appropriate indicator of climate suitability for *P. citricarpa* since these show the extent to which suitable temperatures and moistures coincide in the spring and autumn and this ranged from 2 to 7 in the 25 km grid cells where Guarnaccia et al. (2017) found *P. citricarpa* (Table 3). Although the EFSA PLH Panel (2014) has already shown that CLIMEX has limited applicability in determining the area of potential establishment for *P. citricarpa*, it can be concluded that nearly 75% of the samples taken by Guarnaccia et al. (2017), including the five positive samples, were in 25 km grid cells shown to be marginally suitable following the methods used by Yonow et al. (2013).

Table 3: Model outputs from Yonow et al. (2013) for grid cells corresponding to the locations in Europe where Guarnaccia et al. (2017) found *Phyllosticta citricarpa* or *P. paracitricarpa*, run with different spatial and temporal resolution interpolated climate datasets: 0.5° climate dataset applied by Yonow et al. (2013), with the 0.1° and JRC Mars 25 km

Locations where <i>P. citricarpa</i> or <i>P. paracitricarpa</i> were found by Guarnaccia et al. (2017)				Yonow et al. (2013), run on 0.1° climatic dataset		Yonow et al. (2013), run on 25 km JRC Mars climatic dataset	
Country	Location	Latitude	Longitude	Growth Index	Ecoclimatic Index	Growth Index	Ecoclimatic Index
Malta	Zurrieq	35.823845	14.505099	n.a.	n.a.	4	2
Malta	Gozo	36.055138	14.259907	3	1	5	2
Italy	Trebisacce	39.910122	16.564824	3	0	6	1
Portugal	Monchique	37.326195	−8.526232	1	0	2	1
Greece	Arta	39.160465	20.918257	6	1	7	2

3.2.2.2. Models for ascospore maturation, release and infection (EFSA PLH Panel, 2014; Magarey et al., 2015)

The simulations by Magarey et al. (2015) for ascospore maturation and infection did not include the sites investigated by Guarnaccia et al. (2017); therefore, the values reported in Table 4 for ascospore

infection scores are from the nearest location in the site dataset of Magarey et al. (2015): i.e. for Zurrieq and Gozo, the values are from Luqa in Malta; for Trebisacce the values are from Marina di Ginosa; for Monchique, the values are from Sagres; for Arta, the values are from Kerkyra (Figure 3). The lowest score of 0.4 was recorded for Trebisacce in Italy and the highest of 11 at Arta in Greece. The threshold for a site predicted to be suitable for ascospore infection by Magarey et al. (2015) was 13, based on the score reported for Addo, South Africa. Nevertheless, different conclusions would have been reached if other sites (EFSA PLH Panel, 2016) or periods (see paragraph below) had been chosen as the benchmark (EFSA PLH Panel, 2016). Magarey et al. (2015) considered Addo as of moderate disease prevalence, however fungicide sprays are applied for CBS control there (Korf, 1998; Grout, 2015).

Table 4: Model outputs from Magarey et al. (2015) for locations in Europe near to sites where Guarnaccia et al. (2017) found *Phyllosticta citricarpa* or *P. paracitricarpa*

Locations where <i>P. citricarpa</i> or <i>P. paracitricarpa</i> were found by Guarnaccia et al. (2017)				Near location from Table 1 of Magarey et al. (2015)	From Table 2 of Magarey et al. (2015)					
					Ascospores			Pycnidiospores		
					Infection on days with PAT			Infection		
Country	Location	Latitude	Longitude	Location, Country	Mean	Std. Dev.	% suitable years	Mean	Std. Dev.	% suitable years
Malta	Zurrieq	35.823845	14.505099	Luqa, Malta	4.8	2.2	0	9.4	5.3	0
Malta	Gozo	36.055138	14.259907	Luqa, Malta	4.8	2.2	0	9.4	5.3	0
Italy	Trebisacce	39.910122	16.564824	Marina di Ginosa, Italy	0.4	0.9	0	15.9	9.3	0
Portugal	Monchique	37.326195	-8.526232	Sagres, Portugal	6.3	3.2	0	15.7	9.2	0
Greece	Arta	39.160465	20.918257	Kerkyra, Greece	11	5.6	22	21.6	7.9	0

PAT: proportion of ascospores trapped.

Table 5 shows the outputs from the application of the model by Fourie et al. (2013) for ascospore maturation and release and the generic infection model (Magarey et al., 2005) for *P. citricarpa* ascospores (EFSA PLH Panel, 2014) for the locations in Europe where Guarnaccia et al. (2017) found *P. citricarpa* or *P. paracitricarpa*. The outputs from the locations where neither species was found are given in Appendix B. The results are shown as the number of infections accumulated from May to September considering a fruit susceptibility of 5 months (as per Magarey et al., 2015); accumulated from May to November taking into account studies indicating a period of fruit susceptibility of at least 6–7 months (Reis et al., 2003; Baldassari et al., 2006; Brentu et al., 2012; Lanza et al., 2017); and accumulated for the whole year considering that susceptible leaves are always present in the citrus canopy (Truter et al., 2004a,b; Truter, 2010). Values for the 25 × 25 km grid cells of the JRC Mars 1989 interpolated climatic dataset for the cells of the EU citrus-growing area are shown in Figures 4–6, including the sites where Guarnaccia et al. (2017) found *P. citricarpa* or *P. paracitricarpa*.

The mean number of ascospore infections was higher during the period from May to November compared with those from May to September. The highest mean number of ascospore infections from May to November was 15 at Monchique, Portugal, and the lowest was 9 at Zurrieq, Malta, and Trebisacce, Italy. From May to November, the mean number of ascospore infections ranged from 10 at Trebisacce, Italy, to 30 at Monchique, Portugal. Numbers of ascospore infections from January to December and from May to November did not differ. The percentage of suitable years for ascospore infections ranged from 62% at Monchique, Portugal, for the period May–September to 100% at Gozo, Malta, for the periods May–November or January–December.

In conclusion, simulations by EFSA PLH Panel (2014) and Magarey et al. (2015) suggested that climatic conditions at the locations in Europe where Guarnaccia et al. (2017) found *P. citricarpa* or *P. paracitricarpa* may sustain to some extent infections by *P. citricarpa* ascospores.

3.2.2.3. Models for pycnidiospore infection (EFSA, 2008; EFSA PLH Panel, 2014; Magarey et al., 2015)

As for ascospores, the simulations for pycnidiosporic infection by Magarey et al. (2015) did not include the sites investigated by Guarnaccia et al. (2017); therefore, the values reported in Table 3 are for the nearest locations in each case (Figure 7). The scores ranged from 9.4 at Luqa (nearest location to Zurrieq and Gozo) in Malta to 21.6 at Kerkyra (nearest location to Arta) in Greece. The threshold set by Magarey et al. (2015) for pycnidiosporic infection was 48, based on the average score for Addo, South Africa. The score values obtained for the locations where Guarnaccia et al. (2017) found *P. citricarpa* or *P. paracitricarpa* were somewhat lower than the threshold set by Magarey et al. (2015). However, Guarnaccia et al. (2017) only found one mating type of *P. citricarpa* in each location; thus, the pathogen is likely to thrive through asexual reproduction by pycnidiosporic infection. Nevertheless, as indicated above, different conclusions may be reached by choosing other thresholds.

Table 6 shows the model outputs from the application of the generic infection model (Magarey et al., 2005) for pycnidiospores (EFSA PLH Panel, 2014) for the locations in Europe where Guarnaccia et al. (2017) found *P. citricarpa* or *P. paracitricarpa*. The outputs from the locations where neither species was found are given in Appendix B. As for ascospores, the results are shown as the number of infections accumulated from May to September considering a fruit susceptibility of 5 months (as per Magarey et al., 2015); accumulated from May to November taking into account studies indicating a period of fruit susceptibility of at least 6–7 months (Reis et al., 2003; Baldassari et al., 2006; Brentu et al., 2012; Lanza et al., 2017); and accumulated for the whole year considering that susceptible leaves are always present in the citrus canopy (Truter et al., 2004a,b; Truter, 2010). This is particularly important in the case of *P. citricarpa* pycnidiospores, because significant numbers of infection events were predicted by the model in October and November and from December to May (see Figures 29, 32–35 in EFSA (2008) and Figure 19 in EFSA PLH Panel (2014)).

When considering the 5-month period from May to September, the mean number of pycnidiospore infections ranged from 25 at Zurrieq, Malta, to 55 at Monchique, Portugal (Table 6, Figure 8). Mean values increased for the 7-month period of May–November, with the lowest value of 91 at Gozo, Malta, and the highest of 150 at Monchique, Portugal (Table 6, Figure 9). The highest values were obtained for the 12-month period, ranging from 61 at Trebisacce, Italy, to 241 at Monchique, Portugal (Table 6, Figure 10). The percentage of suitable years for pycnidiospore infections was 95% for the period May–September and 100% for the periods May–November or January–December.

Taking into account the 5-month period of May–September, relatively lower number of pycnidiospore infection events was reported by Magarey et al. (2015) (Table 4) when compared with those by EFSA PLH Panel (2014) in Table 6. Both studies used the same generic infection model (Magarey et al., 2005), the same parameter values: $T_{min} = 10^{\circ}\text{C}$, $T_{opt} = 25^{\circ}\text{C}$, $T_{max} = 35^{\circ}\text{C}$ and $D_{50} = 3$ hours, and incorporated a rain threshold to account for the splash requirement in pycnidiospore dispersal. However, in Magarey et al. (2015), this threshold was set to a default value of 0.2 mm per hour, whereas the presence of at least one rain event per day was considered by EFSA PLH Panel (2014). Moreover, different underlying climatic datasets were used and locations drawn from EFSA PLH Panel (2014) were just proxies of those in Magarey et al. (2015).

EFSA (2008) simulated infections by *P. citricarpa* pycnidiospores for 12-month periods using the same generic infection model, parameter values, and rain threshold for the splash dispersal. The number of potential infection events obtained by EFSA (2008) for locations in Portugal, Greece, Spain and Italy (Figure 25 of EFSA, 2008) were in line with those by EFSA PLH Panel (2014) from January to December for the locations in Europe where Guarnaccia et al. (2017) found *P. citricarpa* or *P. paracitricarpa* (Table 6). Likewise EFSA (2008) concluded, the number of infection events by EFSA PLH Panel (2014) for these locations in Europe (Table 6) was comparable to those from sites of current CBS occurrence (Figure 25 of EFSA, 2008). These results indicated that climatic conditions at these sites are favourable for *P. citricarpa* to complete its asexual cycle through infections by pycnidiospores.

Table 5: Model outputs for *Phyllosticta citricarpa* ascospore infections from EFSA PLH Panel (2014) ($D_{50} = 3$; $T_{min} = 15$) for locations in Europe where Guarnaccia et al. (2017) found *P. citricarpa* or *P. paracitricarpa*, considering different periods of host susceptibility: five months (May–September) of fruit susceptibility; seven months (May–November) of fruit susceptibility; 12 months (January–December) of presence of susceptible leaves

Locations and coordinates where <i>P. citricarpa</i> (Zurrieq, Gozo, Trebisacce and Monchique) or <i>P. paracitricarpa</i> (Arta) were found by Guarnaccia et al. (2017)				Ascospores ($D_{50} = 3$ h; $T_{min} = 15^{\circ}\text{C}$)								
				Number of ascospore infections in the period May–September (5 months fruit susceptibility) in days with PAT			Number of ascospore infections in the period May–November (7 months fruit susceptibility) in days with PAT			Number of ascospores infections in the period January–December (12 months leaf susceptibility) in days with PAT		
Country	Location	Latitude	Longitude	Mean	Std. Dev.	% suitable years	Mean	Std. Dev.	% suitable years	Mean	Std. Dev.	% suitable years
Malta	Zurrieq	35.823845	14.505099	9	11	76	22	25	95	22	25	95
Malta	Gozo	36.055138	14.259907	10	9	90	29	33	100	29	33	100
Italy	Trebisacce	39.910122	16.564824	9	15	67	10	16	81	10	16	81
Portugal	Monchique	37.326195	–8.526232	15	32	62	30	49	81	30	49	81
Greece	Arta	39.160465	20.918257	12	20	71	18	26	76	18	26	76

PAT: proportion of ascospores trapped.

Table 6: Model outputs for *Phyllosticta citricarpa* pycnidiospore infections from EFSA PLH Panel (2014) ($D_{50} = 3$; $T_{min} = 10$) for the locations in Europe where Guarnaccia et al. (2017) found *P. citricarpa* or *P. paracitricarpa*, considering different periods of host susceptibility: 5 months (May–September) of fruit susceptibility; 7 months (May–November) of fruit susceptibility; 12 months (January–December) of presence of susceptible leaves

Locations and coordinates where <i>P. citricarpa</i> (Zurrieq, Gozo, Trebisacce and Monchique) or <i>P. paracitricarpa</i> (Arta) were found by Guarnaccia et al. (2017)				Pycnidiospores ($D_{50} = 3$ h; $T_{min} = 10^{\circ}\text{C}$)								
				Number of infections in the period May–September (5 months fruit susceptibility, as per Magarey et al., 2015)			Number of infections in the period May–November (7 months fruit susceptibility, as per EFSA PLH Panel 2014, 2016)			Number of infections in the period January–December (12 months leaf susceptibility, as per EFSA PLH Panel 2014, 2016)		
Country	Location	Latitude	Longitude	Mean	Std. Dev.	% suitable years	Mean	Std. Dev.	% suitable years	Mean	Std. Dev.	% suitable years
Malta	Zurrieq	35.823845	14.505099	25	17	95	92	59	100	152	75	100
Malta	Gozo	36.055138	14.259907	26	18	95	91	67	100	145	76	100
Italy	Trebisacce	39.910122	16.564824	34	29	95	56	40	100	61	43	100
Portugal	Monchique	37.326195	–8.526232	55	51	95	150	93	100	241	128	100
Greece	Arta	39.160465	20.918257	36	33	95	96	54	100	112	63	100

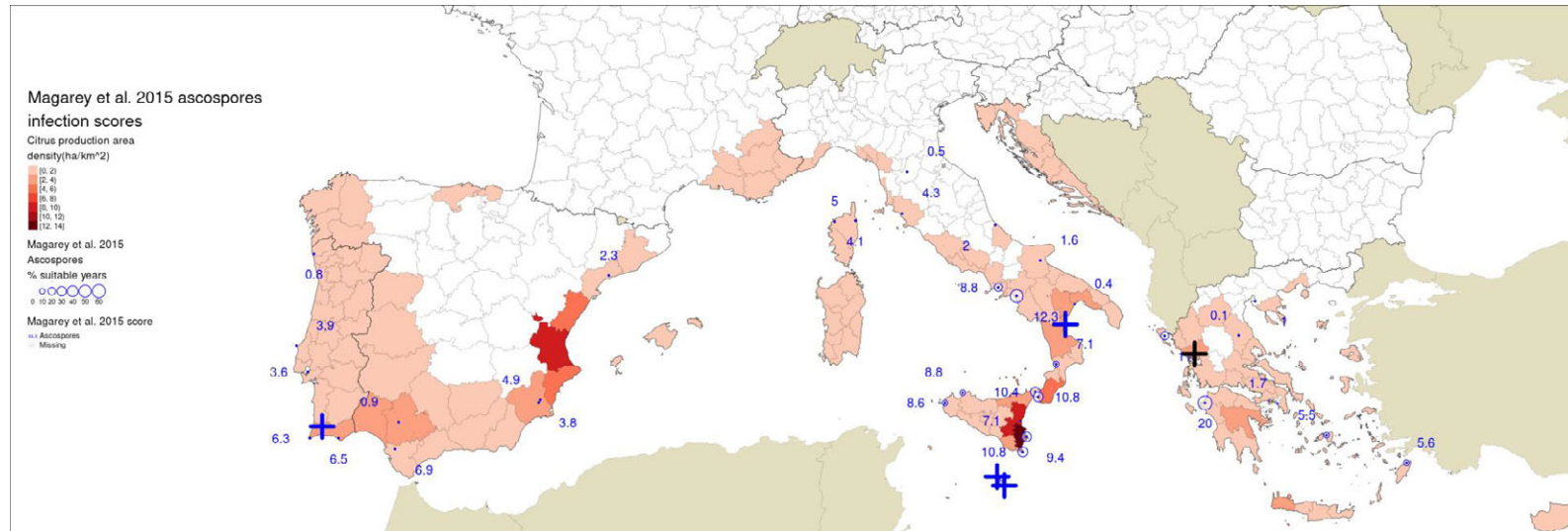


Figure 3: Locations indicated by Guarnaccia et al. (2017) for their samplings: blue crosses indicate sites where *Phyllosticta citricarpa* was reported in the leaf litter; a black cross indicates the site where *P. paracitricarpa* was reported in leaf litter in Greece. The map provides the ascospores infection scores for *P. citricarpa* as calculated by Magarey et al. (2015) over EU NUTS3 citrus production area density (as extracted from national statistical databases of Portugal, Spain, France, Italy, Malta, Croatia, Greece and Cyprus for year 2007; for details, see Appendix F of EFSA PLH Panel, 2014)

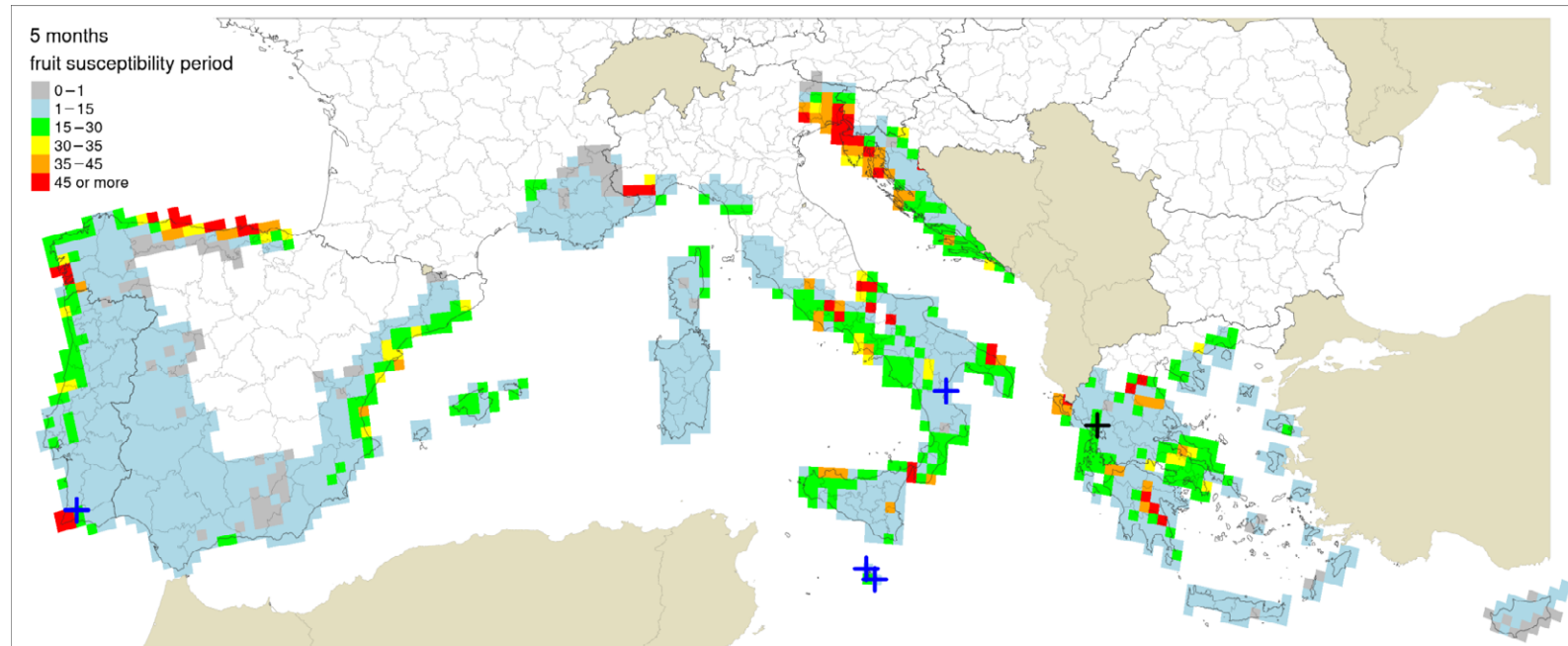


Figure 4: Locations indicated by Guarnaccia et al. (2017) for their samplings: blue crosses indicate sites where *Phyllosticta citricarpa* was reported in the leaf litter; a black cross indicates the site where *P. paracitricarpa* was reported in leaf litter in Greece. The map provides the ascospores infection as calculated by EFSA PLH Panel (2014) for $D_{50} = 3$ h and $T_{min} = 15^{\circ}\text{C}$ (Fourie 0.7), considering a 5-month fruit susceptibility period (from May to September)

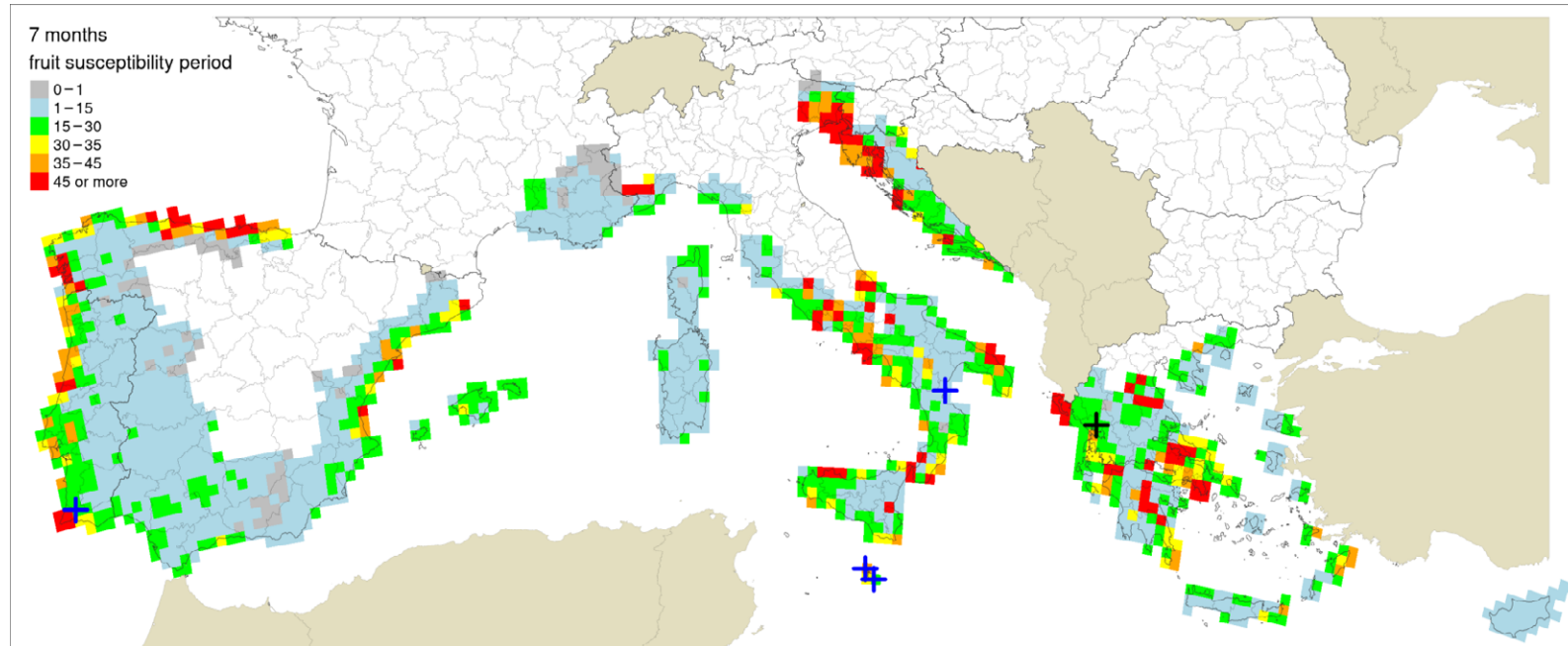


Figure 5: Locations indicated by Guarnaccia et al. (2017) for their samplings: blue crosses indicate sites where *Phyllosticta citricarpa* was reported in the leaf litter; a black cross indicates the site where *P. paracitricarpa* was reported in leaf litter in Greece. The map provides the ascospores infection by EFSA PLH Panel (2014) for $D_{50} = 3$ h and $T_{min}=15^{\circ}\text{C}$ (Fourie 0.7), considering a 7-month fruit susceptibility period (from May to November)

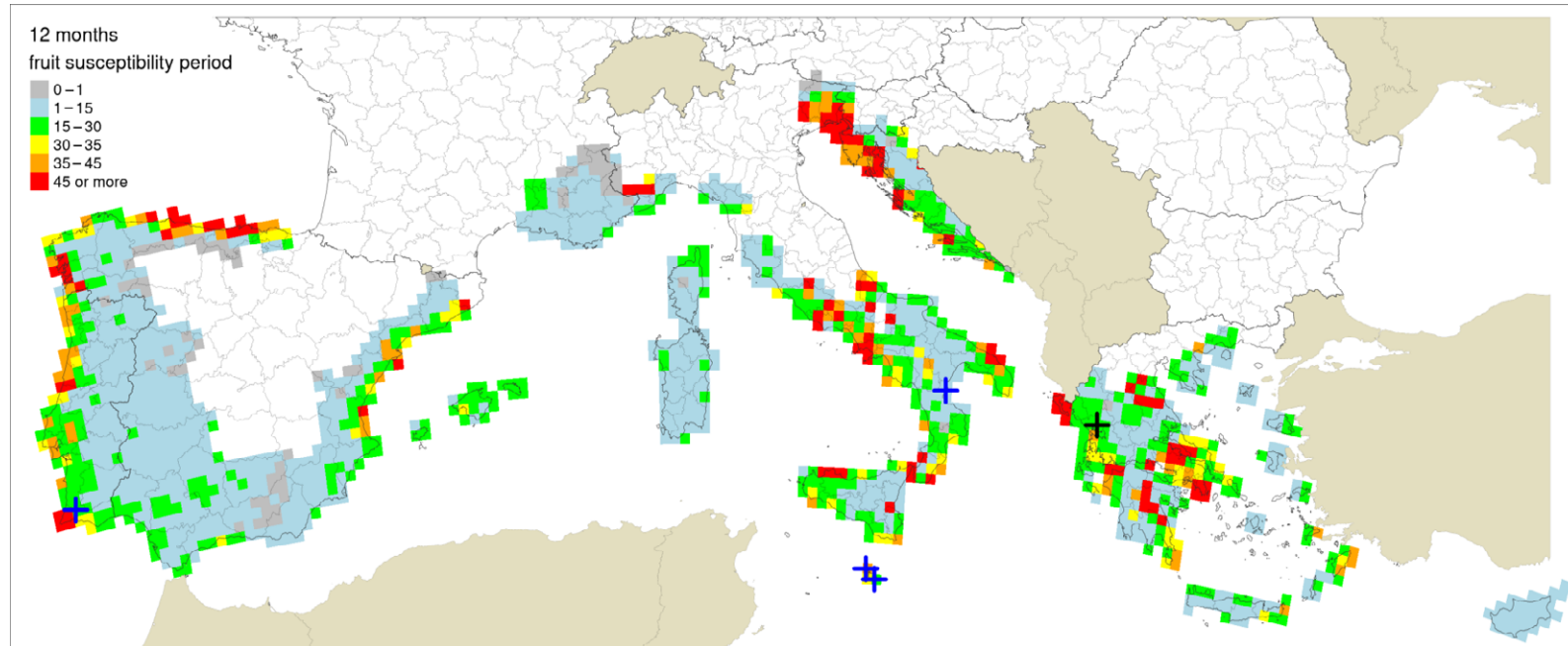


Figure 6: Locations indicated by Guarnaccia et al. (2017) for their samplings: blue crosses indicate sites where *Phyllosticta citricarpa* was reported in the leaf litter; a black cross indicates the site where *P. paracitricarpa* was reported in leaf litter in Greece. The map provides the ascospores infection by EFSA PLH Panel (2014) for $D_{50} = 3$ h and $T_{min}=15^{\circ}\text{C}$ (Fourie 0.7), considering a 12-month leaves susceptibility period

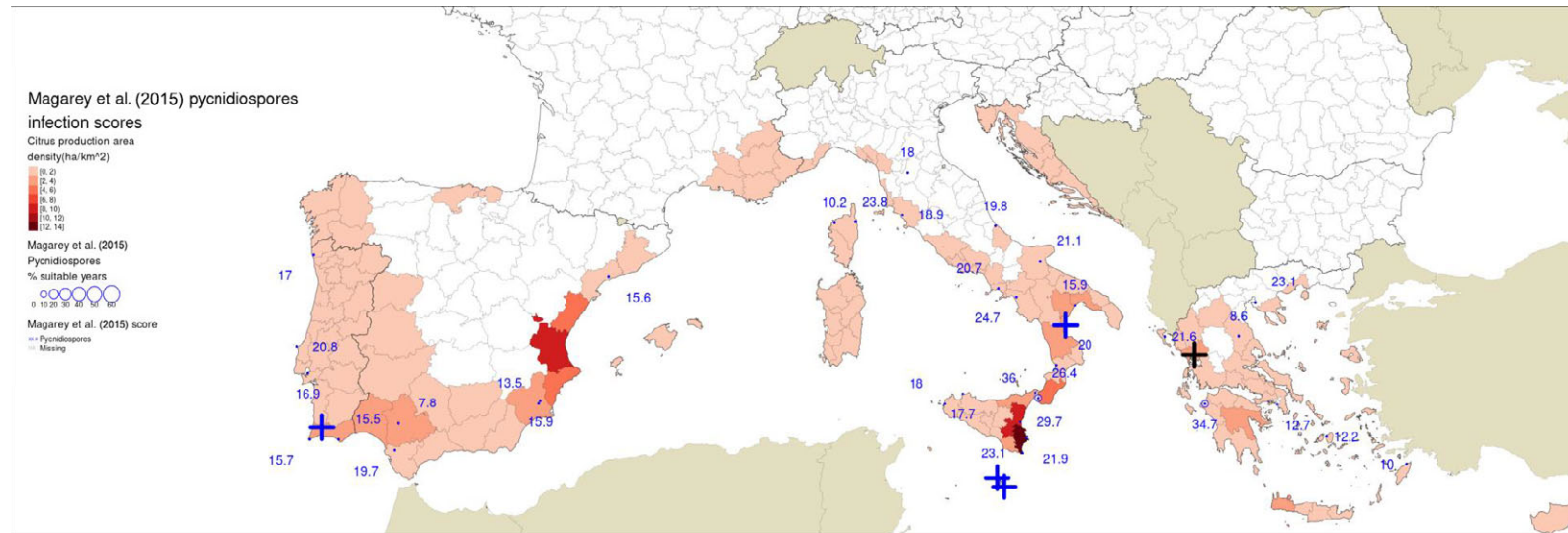


Figure 7: Locations indicated by Guarnaccia et al. (2017) for their samplings: blue crosses indicate sites where *Phyllosticta citricarpa* was reported in the leaf litter; a black cross indicates the site where *P. paracitricarpa* was reported in leaf litter in Greece; green dots indicate sites of sampling without positive finding. The map provides the pycnidiospores infection scores for *P. citricarpa* as calculated by Magarey et al. (2015) over EU NUTS3 citrus production area density (as extracted from national statistical databases of Portugal, Spain, France, Italy, Malta, Croatia, Greece and Cyprus for year 2007; for details, see Appendix F of EFSA PLH Panel, 2014)

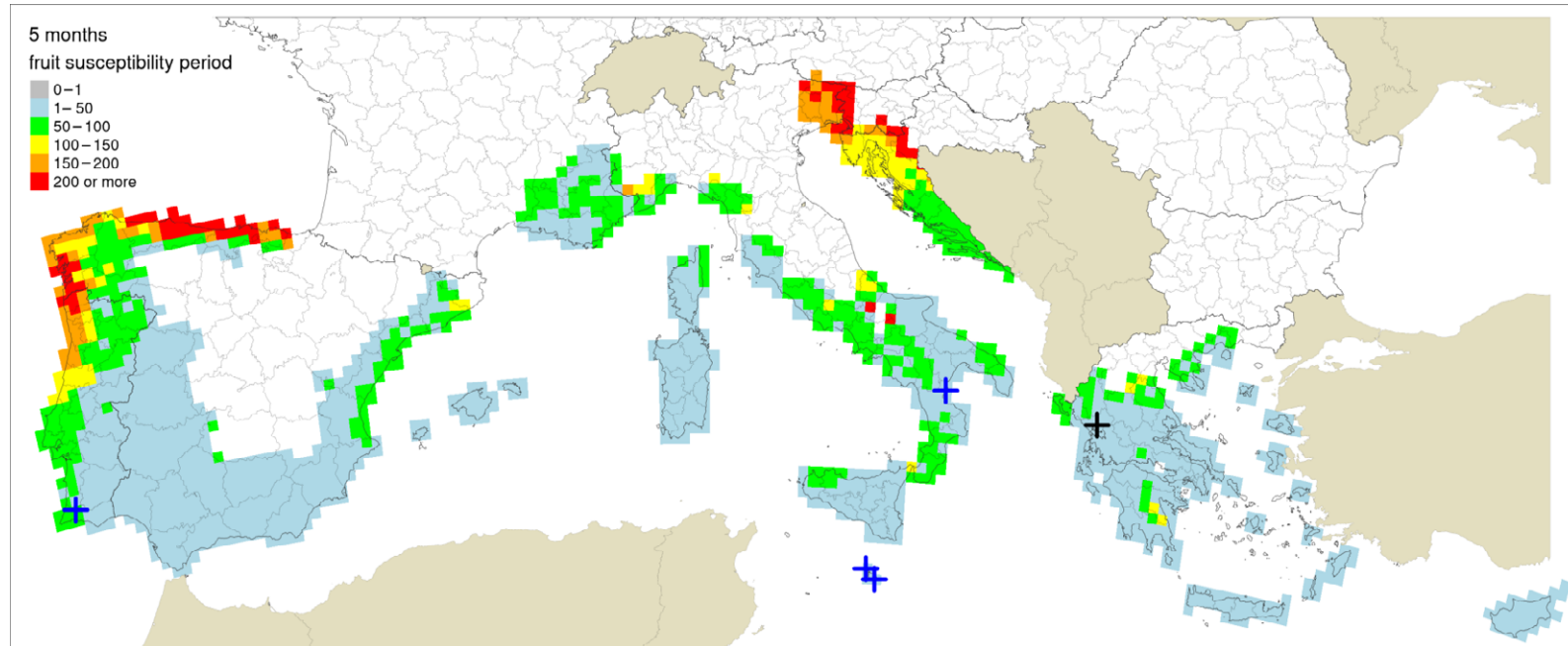


Figure 8: Locations indicated by Guarnaccia et al. (2017) for their samplings: blue crosses indicate sites where *Phyllosticta citricarpa* was reported in the leaf litter; a black cross indicates the site where *P. paracitricarpa* was reported in leaf litter in Greece. The map provides the pycnidiospores infection by EFSA PLH Panel (2014) for $D_{50} = 3$ h and $T_{min} = 10^{\circ}\text{C}$, considering a 5-month fruit susceptibility period (from May to September)

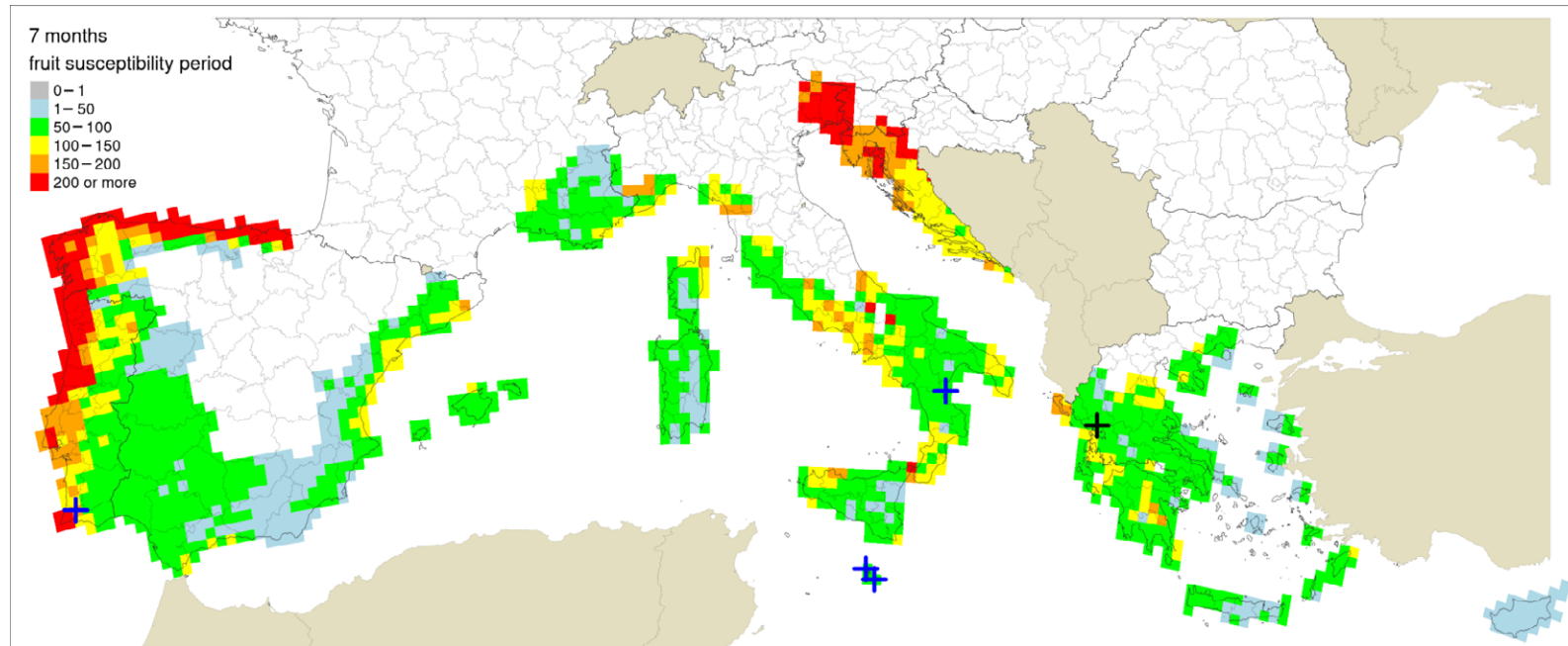


Figure 9: Locations indicated by Guarnaccia et al. (2017) for their samplings: blue crosses indicate sites where *Phyllosticta citricarpa* was reported in the leaf litter; a black cross indicates the site where *P. paracitricarpa* was reported in leaf litter in Greece. The map provides the pycnidiospores infection by EFSA PLH Panel (2014) for $D_{50} = 3$ h and $T_{min} = 10^{\circ}\text{C}$, considering a 7-month fruit susceptibility period (May to November)

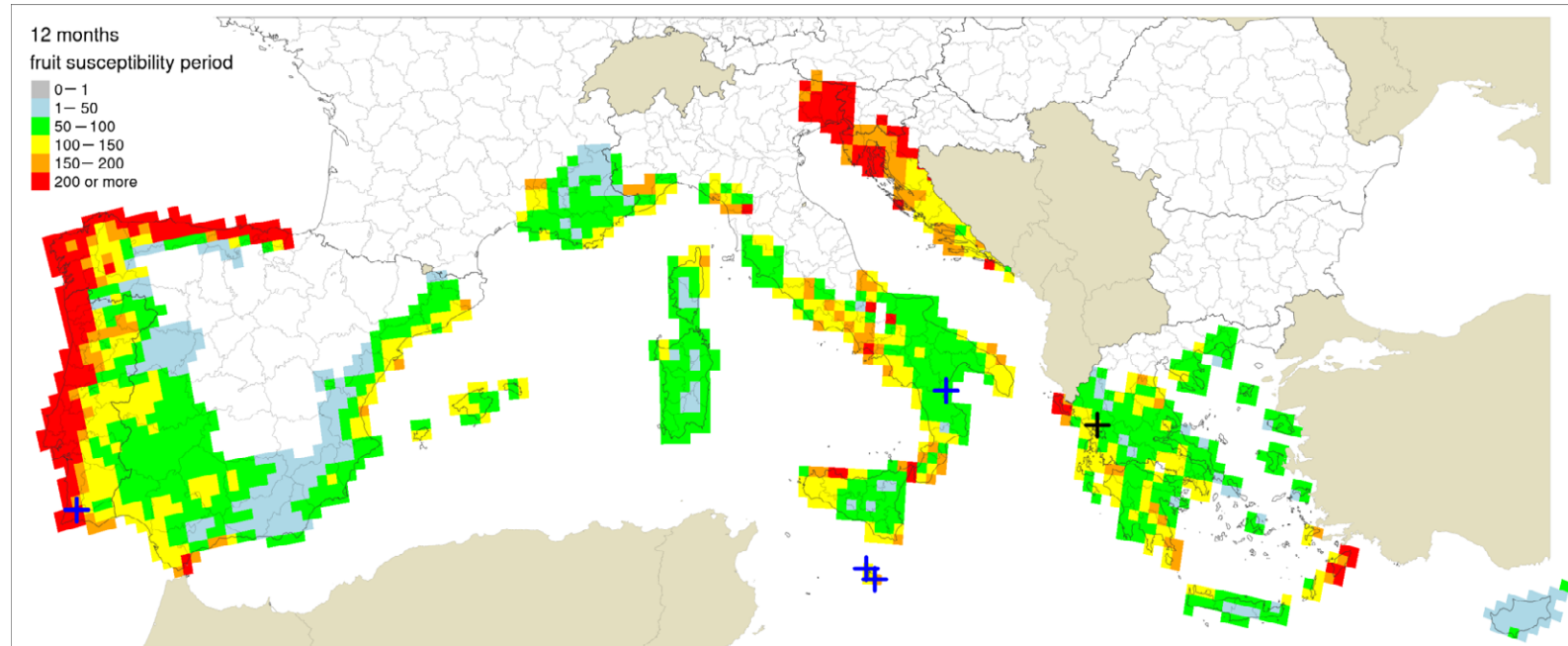


Figure 10: Locations indicated by Guarnaccia et al. (2017) for their samplings: blue crosses indicate sites where *Phyllosticta citricarpa* was reported in the leaf litter; a black cross indicates the site where *P. paracitricarpa* was reported in leaf litter in Greece. The map provides the pycnidiospores infection by EFSA PLH Panel (2014) for $D_{50} = 3$ h and $T_{min} = 10^{\circ}\text{C}$, considering a 12-month leaves susceptibility period

3.3. Uncertainties

3.3.1. Uncertainties identified in the evaluation of the paper by Guarnaccia et al. (2017)

The main uncertainties identified in the evaluation of the paper by Guarnaccia et al. (2017) are:

- the apparent lack of a CBS disease survey by the authors;
- the fact that the NPPOs were not alerted at the time of the findings and as a consequence a confirmation of the findings by Guarnaccia et al. (2017) by the NPPO is still pending, although preliminary results are not positive;
- how to reconcile the novelty of the findings by Guarnaccia et al. (2017) (all findings are in leaf litter and backyards only, all reporting only one mating type) with the known life cycle of *P. citricarpa*;
- the representativeness of the isolates to the populations from which they were sampled;
- the origin of *P. citricarpa* in Europe;
- the ecology and pathogenicity of *P. citricarpa* in the field, if confirmed in the Mediterranean area;
- *P. paracitricarpa* distribution worldwide and its pathogenicity under field conditions.

3.3.2. Uncertainties on the fitting of the previous model simulations to the findings by Guarnaccia et al. (2017)

There are general uncertainties on the comparison of previous model simulations to the findings of *P. citricarpa* in Europe by Guarnaccia et al. (2017):

- These findings present some atypical aspects which were not considered in the previous models for *P. citricarpa*: in all samplings, *P. citricarpa* was only found in the leaf litter but never on living plant tissues; in each location, all isolates belong to a single mating type, hence, if representative of the fungal population in each site, they should not be able to produce ascospores but only pycnidiospores.
- In addition, *P. citricarpa* was found by Guarnaccia et al. (2017) only in backyard landscapes (two backyards near a swimming pool, one in a vegetable garden on the road side and one on a terraced slope) where water splashes and runoff might happen more likely than in commercial orchards; however, all previous models did not account for the effect of irrigation.
- All model predictions (EFSA, 2008; Yonow et al., 2013; EFSA PLH Panel 2014, 2016; Magarey et al., 2015) were parameterised and conducted for the fungus *P. citricarpa*. For this reason, the extrapolation of the results of these simulations to the newly described species *P. paracitricarpa* is highly uncertain, because nothing is known about the biology and ecology of *P. paracitricarpa*.
- There are uncertainties on the model parameters for the application to *P. citricarpa* of the generic infection model for foliar fungal pathogens by Magarey et al. (2005). Such uncertainties are due to the lack of epidemiological studies on *P. citricarpa* ascospore and pycnidiospore infection and are discussed in details in previous EFSA opinions (EFSA, 2008; EFSA PLH Panel, 2014).

4. Conclusions

The study reported in Guarnaccia et al. (2017) has identified four species of *Phyllosticta* present on citrus in Europe. Two, *P. citricarpa* and *P. capitalensis*, are well-defined species. *P. citricarpa* is recorded for the first time in Europe, but only in citrus leaf litter, and confirms the predictions made by Magarey et al. (2015) and by EFSA (2008) and EFSA PLH Panel (2014, 2016) that *P. citricarpa* can establish in some citrus-growing regions of Europe. Two new species *P. paracitricarpa* and *P. paracapitalensis* are described using a combination of morphological and molecular phylogeny techniques. *P. paracitricarpa* is shown to have similar pathogenicity to *P. citricarpa* on fruits of *C. sinensis*.

Genotyping of the *P. citricarpa* isolates, assuming they are representative of the population sampled, suggests at least two independent introductions, with the population in Portugal being different from that present in Malta and Italy, based on MLG and mating type idiomorph. The sampling protocols as described in the paper are not explained clearly. Although most of the major citrus-growing areas in Europe were covered, access to the locations seemed to be a key feature

determining the choice of locations. All findings of *P. citricarpa* were in backyards and in a high percentage of these locations there were positive identifications (positive identifications were overall in 18% of the sampled backyards, ranging from 9% of the Italian backyards sampled to 25% in Portugal and 50% in Malta).

The authors only report findings of *P. citricarpa* and *P. paracitricarpa* from leaf litter, but no positive detection from living plants (fruit, leaves or twigs) was obtained in the same plants. However, since leaf litter only originates from living leaves and since *P. citricarpa* has not been shown to infect or colonise dead leaves (Truter et al., 2007), the *P. citricarpa* must have infected the above living leaves.

The origin of the introductions remains obscure. Guarnaccia et al. (2017) considered introduction to be a consequence of *P. citricarpa* having been present for a considerable time through ancient plants or of illegal movement of planting/propagating material. In the Panel's view, there are problems with the first of these interpretations. If the pathogen had been present for a considerable time, then it can only have persisted if there were repeated asexual cycles involving living leaves and possibly other tissues (particularly twigs) and the resulting leaf litter. Assuming only a single mating type population was present at each location, as suggested by the isolates obtained, then no sexual reproduction involving ascospores would have occurred. Persistence of the pathogen over extended time involving only mycelium and pycnidiospores in an endophytic/saprophytic cycle would be highly problematic, especially as no *P. citricarpa* isolates were obtained from living leaves from plants.

In the case of illegal movement of planting/propagating material, it would be more likely that the two mating types would be present on the intact plants than would occur with a sporadic occurrence on fruits which, with only asexual reproduction, would involve more probably a single mating type. Tran et al. (2017) suggest only one mating type being present in a single disease lesion in aboveground parts. In the Panel's view, the fruit pathway would be an equally or more likely origin of these introductions.

It was not reported how surveys for CBS disease were carried out by the authors, and whether these were in or close to the locations where samples were taken and positive identifications made. Thus, the claim that there was no CBS disease even where the pathogen was present is not supported by the results presented. The persistence of *P. citricarpa* in leaf litter also means that the requirement for conducive climatic conditions over a sequence of years would no longer apply as a persistent endophytic/saprophytic cycle could result in CBS disease being manifest in a single year if conditions were favourable.

Results from the simulation models indicated that climatic conditions at the locations where Guarnaccia et al. (2017) found *P. citricarpa* or *P. paracitricarpa* were conducive for *P. citricarpa* establishment. The number of infection events by *P. citricarpa* pycnidiospores obtained for these locations in Europe was comparable to those from sites of current CBS occurrence outside Europe. This is particularly relevant considering that only one mating type of *P. citricarpa* was found by Guarnaccia et al. (2017) in each site; thus, only asexual reproduction by pycnidiospores would be expected. Further epidemic development and impacts would be boosted by the concurrence of complementary mating types of *P. citricarpa* in the same location, leading to sexual reproduction by ascospores, subsequent inoculum build up and airborne dispersal.

The results by Guarnaccia et al. (2017) are based on the sampling conducted during the initial field visits. Following a request for clarifications, the corresponding author of the paper has informed EFSA that, during the second set of field visits undertaken in December 2016 and January 2017 to confirm the initial findings, no symptoms were observed and therefore no confirmatory samples were collected by the authors. Preliminary results of surveys conducted during the summer 2017 in Malta and Portugal by the NPPOs, using an established DNA-based diagnostic protocol and repeating the methods by Guarnaccia et al. (2017), did not so far confirm the findings by Guarnaccia et al. (2017). Further samplings will be continued in all sites in the following seasons.

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Abbreviations

actA	actin
CBS	citrus black spot
CLIMEX	Software to predict the effect of climate on species
EI	ecoclimatic Index
gapdh	glyceraldehyde-3-phosphate dehydrogenase
GI	growth index
GPS	Global Positioning System
ITS	internal transcribed spacer region
LSU	large subunit nrDNA
Mars	Monitoring Agriculture Resources System
MLG	multilocus genotype
MCYFS	Mars Crop Yield Forecasting System
NPPO	National Plant Protection Organisation
PCR	polymerase chain reaction
PLH	EFSA Plant Health Panel
rpb2	RNA polymerase II second largest subunit
tef1	translation elongation factor 1- α

Appendix A – CLIMEX results for locations sampled by Guarnaccia et al. (2017)

Table A.1: Locations sampled for investigation of presence of *Phyllosticta* spp. by Guarnaccia et al. (2017) compared with model results according to Yonow et al. (2013), who parameterised a CLIMEX model to predict the potential global distribution of the citrus black spot disease and the risk posed to Europe. The table provides the CLIMEX model outputs for Ecoclimatic index and Growth index for the investigated sites indicated in Table 1 of Guarnaccia et al. (2017). The CLIMEX model was run with climate data at 25 km resolution for the time period 1998–2007

City	Country	e	n	Type	Grid 0.1°	G. I. 0.1°	E.I. 0.1°	Grid 25 km JRC Mars	G.I 25 Km JRC Mars	EI 25 Km JRC Mars
Arta-1 ^(a)	Greece	20.92959	39.16172	<i>P. paracitricarpa</i>	17753	6	1	52147	7	2
Gozo-1 ^(b)	Malta	14.25991	36.05514	<i>P. citricarpa</i> .	12142	3	1	36125	5	2
Monchique-1 ^(b)	Portugal	-8.52623	37.3262	<i>P. citricarpa</i>	213	1	0	49044	2	1
Trebisacce-1 ^(b)	Italy	16.56482	39.91012	<i>P. citricarpa</i>	14311	3	0	54132	6	1
Zurrieq-1 ^(b)	Malta	14.5051	35.82385	<i>P. citricarpa</i>	NA	NA	NA	35126	4	2
Acitrezza-1	Italy	15.16109	37.56108	Other	12886	2	0	43128	1	1
Agia-1	Greece	23.92124	35.46598	Other	20794	1	0	38160	2	1
Algemesi-1	Spain	-0.44977	39.20764	Other	3928	2	2	52073	2	2
Algemesi-2	Spain	-0.47082	39.1969	Other	3928	2	2	52073	2	2
Alginet-1	Spain	-0.45803	39.26007	Other	3928	2	2	52073	2	2
Alginet-2	Spain	-0.41642	39.25141	Other	3928	2	2	52073	2	2
Alhaurin_El_Grande-1	Spain	-4.67709	36.64537	Other	1648	2	0	43057	2	1
Alhaurin_El_Grande-2	Spain	-4.69818	36.66469	Other	1648	2	0	43057	2	1
Alikianos-1	Greece	23.90863	35.45666	Other	20794	1	0	38160	2	1
Alikianos-2	Greece	23.90437	35.46238	Other	20794	1	0	38160	2	1
Alikianos-3	Greece	23.9198	35.44644	Other	20794	1	0	37160	1	0
Alikianos-4	Greece	23.94556	35.46622	Other	20794	1	0	38160	2	1
Almeria-1	Spain	-2.40293	36.83464	Other	2846	0	0	43065	0	0
Almeria-2	Spain	-2.40289	36.82883	Other	NA	NA	NA	43065	0	0
Alzira-1	Spain	-0.49072	39.15696	Other	3927	2	2	52073	2	2
Amfilochia-1	Greece	21.17164	38.96138	Other	18035	3	0	51148	7	2
Argo-1	Greece	22.74218	37.62865	Other	19593	2	1	46154	1	0
Argo-2	Greece	22.73931	37.65556	Other	19593	2	1	46154	1	0
Argos-1	Greece	22.66172	37.68659	Other	19411	1	0	46154	1	0
Arta-2	Greece	20.90379	39.15566	Other	17753	6	1	52147	7	2

City	Country	e	n	Type	Grid 0.1°	G. I. 0.1°	E.I. 0.1°	Grid 25 km JRC Mars	G.I 25 Km JRC Mars	EI 25 Km JRC Mars
Arta-3	Greece	20.91826	39.16047	Other	17753	6	1	52147	7	2
Barcellona P.G.-1	Italy	15.13679	38.11056	Other	12889	2	0	45127	0	0
Brucoli-1	Italy	15.11052	37.29482	Other	12884	2	1	42128	1	1
Canicattí-1	Italy	13.8409	37.35843	Other	11855	0	0	42123	1	0
Carruba-1	Italy	15.19094	37.68463	Other	13039	4	1	43128	1	1
Castello-1	Spain	-0.0862	39.90392	Other	4097	3	3	55075	1	1
Castello-2	Spain	-0.08823	39.88386	Other	4097	3	3	55075	1	1
Castello-3	Spain	-0.09095	39.88401	Other	4097	3	3	55075	1	1
Cefalú-1	Italy	14.01227	38.02948	Other	NA	NA	NA	45124	6	2
Chania-1	Greece	24.05114	35.49315	Other	20965	1	0	38161	2	1
Chania-2	Greece	23.94806	35.47789	Other	20794	1	0	38160	2	1
citricarpa-1	NA	-8.51451	37.33241	Other	213	1	0	49044	2	1
Comiso-1	Italy	14.63716	36.94398	Other	12430	1	0	40126	1	0
Conceicao-1	Portugal	-7.91693	37.04848	Other	447	1	0	47046	2	1
Curiglia-1	Italy	16.20376	38.76773	Other	13985	4	0	48131	12	2
El_Ejido-1	Spain	-2.71999	36.79521	Other	2669	0	0	43064	0	0
Estellencs-1	Spain	2.481876	39.6535	Other	5090	2	1	53083	1	1
Faro-1	Portugal	-7.91681	37.10846	Other	447	1	0	48046	1	1
Faro-2	Portugal	-7.91743	37.06264	Other	447	1	0	47046	2	1
Giarratana-1	Italy	14.97442	36.88344	Other	12726	1	0	40127	3	1
Gouria-1	Greece	21.25765	38.45498	Other	18032	4	1	49149	7	2
Gozo-2	Malta	14.2593	36.04907	Other	12142	3	1	36125	5	2
Gozo-3	Malta	14.26012	36.03753	Other	12142	3	1	36125	5	2
Gozo-4	Malta	14.27936	36.04965	Other	12142	3	1	36125	5	2
Gozo-5	Malta	14.28445	36.05817	Other	12142	3	1	36125	5	2
Grotte-1	Italy	15.17701	37.67993	Other	13039	4	1	43128	1	1
Guardia-1	Italy	15.17592	37.66271	Other	NA	NA	NA	43128	1	1
Kirtomados-1	Greece	23.91666	35.47875	Other	20794	1	0	38160	2	1
Leni-1	Italy	14.59752	38.04442	Other	12437	2	0	45126	1	1
Leni-2	Italy	14.82713	38.55289	Other	NA	NA	NA	47126	1	0

City	Country	e	n	Type	Grid 0.1°	G. I. 0.1°	E.I. 0.1°	Grid 25 km JRC Mars	G.I 25 Km JRC Mars	EI 25 Km JRC Mars
Lentini-1	Italy	15.0209	37.32058	Other	12884	2	1	42127	1	0
Malaga-1	Spain	-4.42706	36.76176	Other	1801	1	0	44058	1	0
Mascali-1	Italy	15.1925	37.76768	Other	13039	4	1	44128	1	1
Mascali-2	Italy	15.19464	37.76826	Other	13039	4	1	44128	1	1
Massafra-1	Italy	17.14411	40.54476	Other	14761	1	0	57134	3	1
Mastro-1	Greece	21.28054	38.43029	Other	18032	4	1	49149	7	2
Mesquita-1	Portugal	-8.28949	37.21367	Other	328	1	0	48045	2	1
Mesquita-2	Portugal	-8.29781	37.20453	Other	328	1	0	48045	2	1
Mineo-1	Italy	14.69086	37.35072	Other	12580	2	1	42126	0	0
Moncada-1	Spain	-0.39458	39.58855	Other	3930	2	2	54074	3	2
Monchique-2	Portugal	-8.50369	37.33623	Other	214	1	0	49044	2	1
Monchique-3	Portugal	-8.49223	37.33224	Other	270	1	0	49044	2	1
Motta_S._Anastasia-1	Italy	14.88602	37.4821	Other	12729	2	2	42127	1	0
Motta_S._Anastasia-2	Italy	14.95416	37.46971	Other	12729	2	2	42127	1	0
Nafplio-1	Greece	22.78527	37.58931	Other	19593	2	1	46155	0	0
Nafplio-2	Greece	22.69559	37.5751	Other	19593	2	1	46154	1	0
Nafplio-3	Greece	22.6968	37.58229	Other	19593	2	1	46154	1	0
Nafplio-4	Greece	22.87484	37.5888	Other	19771	1	0	46155	0	0
Nicolosi-1	Italy	15.02948	37.61127	Other	12886	2	0	43127	0	0
Nissemi-1	Italy	14.3934	37.13978	Other	12287	1	0	41125	1	0
Noto-1	Italy	15.09545	36.8465	Other	12882	2	1	40128	7	3
Pachino-1	Italy	15.08699	36.72003	Other	12881	3	1	39128	9	4
Pachino-2	Italy	15.08941	36.72233	Other	12881	3	1	39128	9	4
Pedara-1	Italy	15.06654	37.60871	Other	12886	2	0	43127	0	0
Pizzo Calabro-1	Italy	16.22601	38.76039	Other	13985	4	0	48131	12	2
Ribera-1	Italy	13.24185	37.49711	Other	11271	2	1	42121	5	2
Ribera-2	Italy	13.25207	37.50442	Other	11272	1	0	42121	5	2
Riposto-1	Italy	15.19935	37.69647	Other	13039	4	1	43128	1	1
Rocca Imperiale-1	Italy	16.61795	40.10839	Other	14312	3	0	54132	6	1
San_Gregorio-1	Italy	15.10097	37.5623	Other	12886	2	0	43127	0	0

City	Country	e	n	Type	Grid 0.1°	G. I. 0.1°	E.I. 0.1°	Grid 25 km JRC Mars	G.I 25 Km JRC Mars	EI 25 Km JRC Mars
Scordia-1	Italy	14.86915	37.28153	Other	12728	2	1	42127	1	0
Seville-1	Spain	-5.96282	37.50854	Other	1207	3	1	48053	4	3
Seville-2	Spain	-5.95491	37.48298	Other	1206	4	2	48053	4	3
Sikoula-1	Greece	21.0834	39.08593	Other	17891	5	1	52148	6	1
Silves-1	Portugal	-8.39084	37.16415	Other	269	1	0	48044	2	1
Soller-1	Spain	2.709609	39.76453	Other	5211	3	0	53084	1	1
Soller-2	Spain	2.7266	39.77012	Other	5211	3	0	53084	1	1
Terme_Vigliatore-1	Italy	15.16324	38.1458	Other	12889	2	0	45128	0	0
Torremolinos-1	Spain	-4.50413	36.67272	Other	1721	2	0	43057	2	1
Trebisacce-2	Italy	16.56063	39.90671	Other	14311	3	0	54132	6	1

(a): Site where *P. paracitricarpa* was found by Guarnaccia et al. (2017).

(b): sites where *P. citricarpa* was found by Guarnaccia et al. (2017); all other sites were negatives.

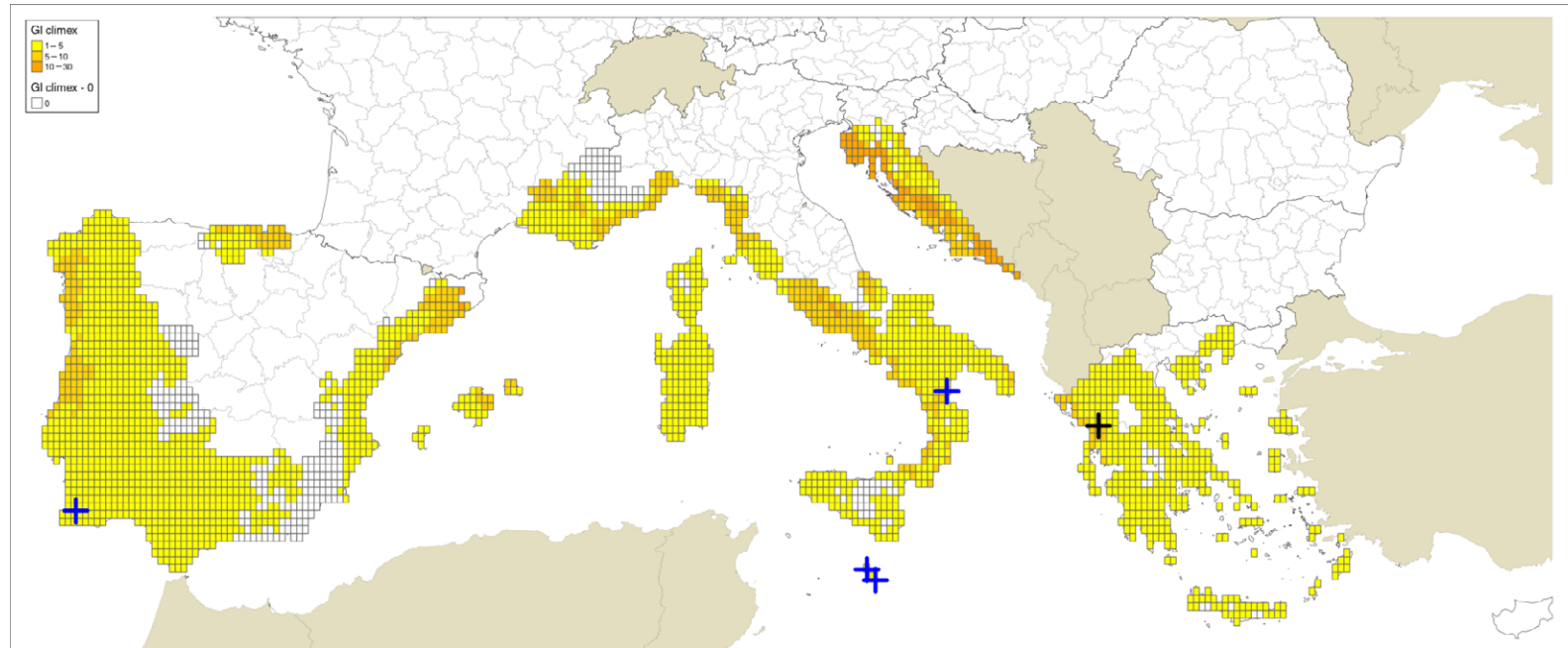


Figure A.1: Locations indicated by Guarnaccia et al. (2017) for their samplings: blue crosses indicate sites where *P. citricarpa* was reported in the leaf litter; a black cross indicates the site where *P. paracitricarpa* was reported in leaf litter in Greece. The map provides the Growth Indexes (GI) calculated based on CLIMEX model by Yonow et al. (2013), run with 0.1° latitude 0.1° longitude 1961–1990 climatology (New et al., 2002), which is expressing the climatic growth potential of the organism inferred from the temperature and humidity conditions

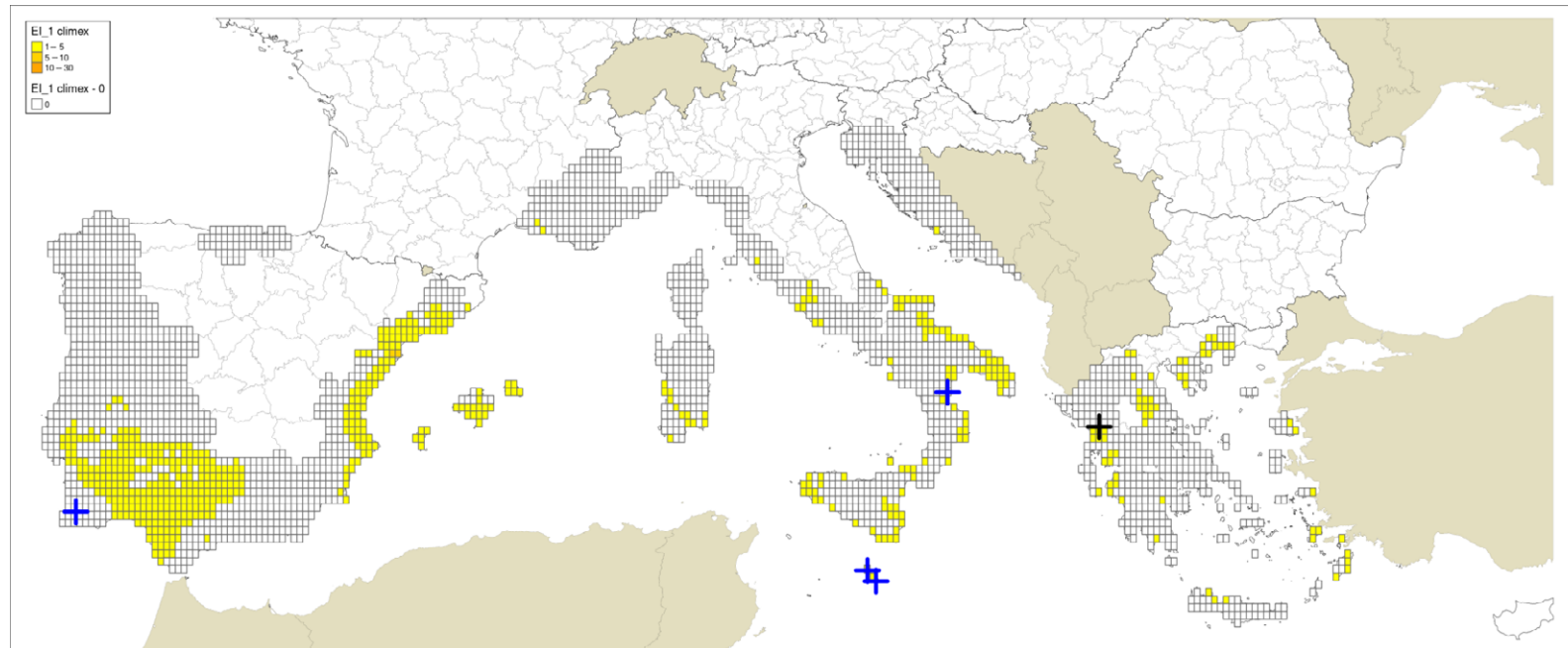


Figure A.2: Locations indicated by Guarnaccia et al. (2017) for their samplings: blue crosses indicate sites where *P. citricarpa* was reported in the leaf litter; a black cross indicates the site where *P. paracitricarpa* was reported in leaf litter in Greece. The map provides the Ecoclimatic Indexes (EI) calculated based on CLIMEX model by Yonow et al. (2013) run with 0.1° latitude 0.1° longitude 1961–1990 climatology (New et al., 2002). The EI is the growth potential (GI) with the predicted effect of stress factors subtracted

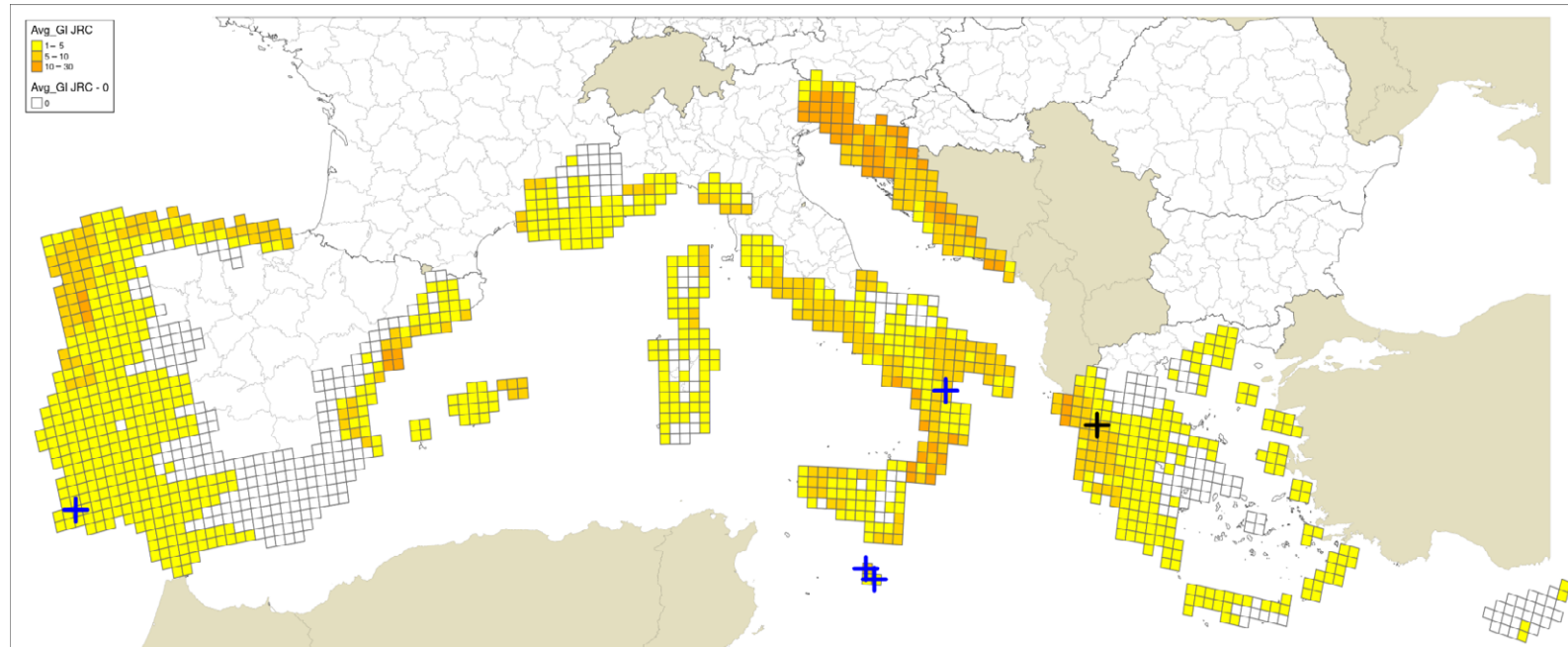


Figure A.3: Locations indicated by Guarnaccia et al. (2017) for their samplings: blue crosses indicate sites where *P. citricarpa* was reported in the leaf litter; a black cross indicates the site where *P. paracitricarpa* was reported in leaf litter in Greece. The map provides the Growth Indexes (GI) calculated based on CLIMEX model by Yonow et al. (2013), run with 25 × 25 km JRC Mars interpolated climate data, which is expressing the climatic growth potential of the organism inferred from the temperature and humidity conditions

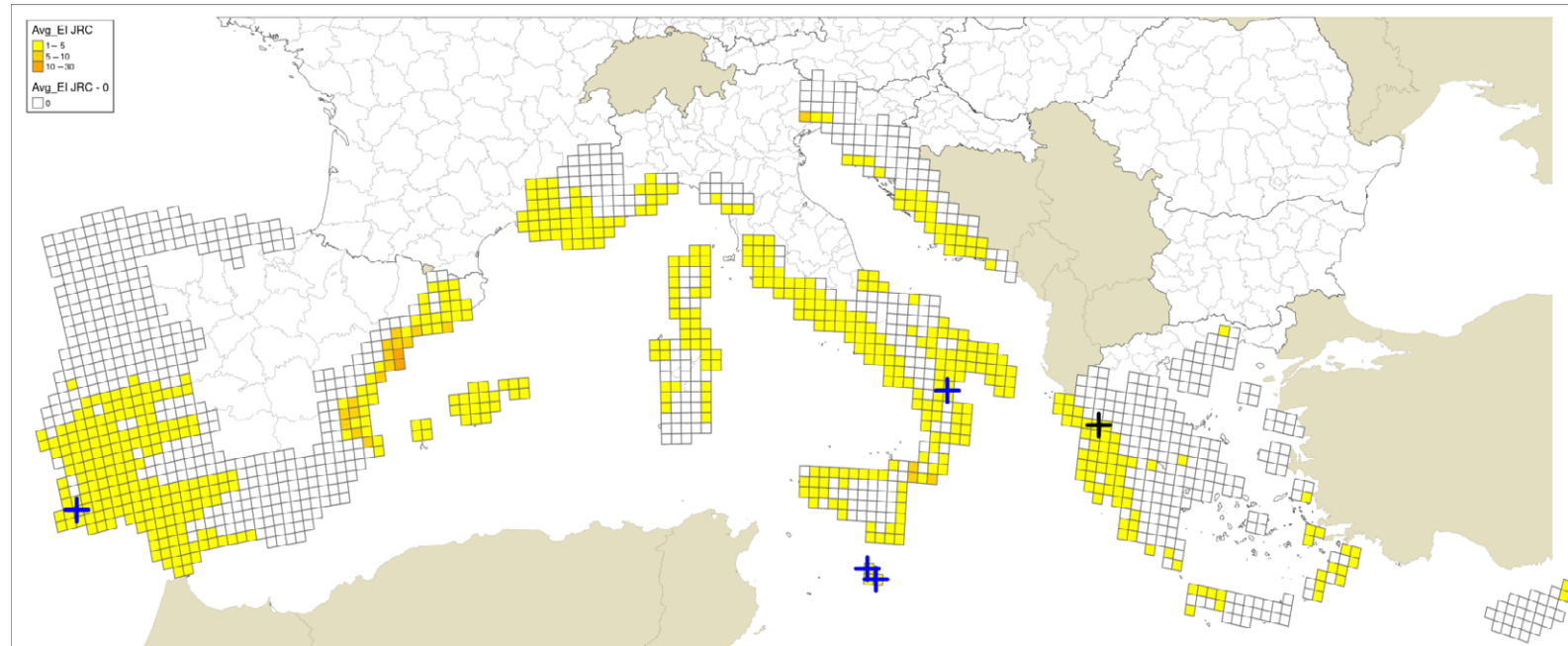


Figure A.4: Locations indicated by Guarnaccia et al. (2017) for their samplings: blue crosses indicate sites where *P. citricarpa* was reported in the leaf litter; a black cross indicates the site where *P. paracitricarpa* was reported in leaf litter in Greece. The map provides the Ecoclimatic Indexes (EI) calculated based on CLIMEX model by Yonow et al. (2013), run with 25 km × 25 km JRC Mars interpolated climate data. The EI is the growth potential (GI) with the predicted effect of stress factors subtracted

Appendix B – Ascospores and pycnidiospores infection

Table B.1: Locations sampled for investigation of presence of *Phyllosticta* spp. by Guarnaccia et al. (2017) (as indicated in Table 1 of Guarnaccia et al., 2017), compared with model results for ascospore and pycnidiospore infections according to the EFSA PLH Panel (2014)

City	Country	e	n	Grid_Code	Type of finding by Guarnaccia et al. (2017)	Pycnidiospores ($D_{50} = 3$ hours; $T_{min} = 10^{\circ}\text{C}$)			Ascospores ($D_{50} = 3$ hours; $T_{min} = 15^{\circ}\text{C}$; Fourie 0.7)		
						Mean number of infections in the period May–September (5 months fruit susceptibility)	Mean number of infections in the period May–November (7 months fruit susceptibility)	Mean number of yearly infection January–December (12 months leaf susceptibility)	Mean number of infections in the period May–September (5 months fruit susceptibility) in days with PAT	Mean number of infections in the period May–November (7 months fruit susceptibility) in days with PAT	Mean number of yearly infection January–December (12 months leaf susceptibility) in days with PAT
Arta-1	Greece	20.92959	39.16172	52147	<i>P. paracitricarpa</i>	36.09524	95.80952	111.9852	17.90476	24.52381	24.52381
Gozo-1	Malta	14.25991	36.05514	36125	<i>P. citricarpa</i>	25.85714	90.90476	145.3727	13.2381	36.42857	36.42857
Monchique-1	Portugal	-8.52623	37.3262	49044	<i>P. citricarpa</i>	54.57143	150.0476	241.1149	15.04762	30	30
Trebisacce-1	Italy	16.56482	39.91012	54132	<i>P. citricarpa</i>	34.42857	56.19048	60.90476	10	11.52381	11.52381
Zurrieq-1	Malta	14.5051	35.82385	35126	<i>P. citricarpa</i>	24.66667	91.90476	151.9951	10.47619	25.42857	25.42857
Acitrezza-1	Italy	15.16109	37.56108	43128	Other	16.71429	45.09524	57.61412	6.285714	8.095238	8.095238
Agia-1	Greece	23.92124	35.46598	38160	Other	15.80952	88.7619	130.2381	6.238095	18.42857	18.66667
Algemesi-1	Spain	-0.44977	39.20764	52073	Other	60.19048	105.2857	122.1264	19.52381	28.80952	28.80952
Algemesi-2	Spain	-0.47082	39.1969	52073	Other	60.19048	105.2857	122.1264	19.52381	28.80952	28.80952
Alginet-1	Spain	-0.45803	39.26007	52073	Other	60.19048	105.2857	122.1264	19.52381	28.80952	28.80952
Alginet-2	Spain	-0.41642	39.25141	52073	Other	60.19048	105.2857	122.1264	19.52381	28.80952	28.80952
Alhaurin_El_Grande-1	Spain	-4.67709	36.64537	43057	Other	39.33333	111.619	171.133	12.80952	25.61905	25.90476
Alhaurin_El_Grande-2	Spain	-4.69818	36.66469	43057	Other	39.33333	111.619	171.133	12.80952	25.61905	25.90476
Alikianos-1	Greece	23.90863	35.45666	38160	Other	15.80952	88.7619	130.2381	6.238095	18.42857	18.66667
Alikianos-2	Greece	23.90437	35.46238	38160	Other	15.80952	88.7619	130.2381	6.238095	18.42857	18.66667
Alikianos-3	Greece	23.9198	35.44644	37160	Other	16.33333	53.47619	63.04762	4.142857	6.809524	6.809524
Alikianos-4	Greece	23.94556	35.46622	38160	Other	15.80952	88.7619	130.2381	6.238095	18.42857	18.66667

City	Country	e	n	Grid_Code	Type of finding by Guarnaccia et al. (2017)	Pycnidiospores ($D_{50} = 3$ hours; $T_{min} = 10^{\circ}\text{C}$)			Ascospores ($D_{50} = 3$ hours; $T_{min} = 15^{\circ}\text{C}$; Fourie 0.7)		
						Mean number of infections in the period May–September (5 months fruit susceptibility)	Mean number of infections in the period May–November (7 months fruit susceptibility)	Mean number of yearly infection January–December (12 months leaf susceptibility)	Mean number of infections in the period May–September (5 months fruit susceptibility) in days with PAT	Mean number of infections in the period May–November (7 months fruit susceptibility) in days with PAT	Mean number of yearly infection January–December (12 months leaf susceptibility) in days with PAT
Almeria-1	Spain	–2.40293	36.83464	43065	Other	11.2381	31.38095	42.56814	3.857143	8.333333	8.333333
Almeria-2	Spain	–2.40289	36.82883	43065	Other	11.2381	31.38095	42.56814	3.857143	8.333333	8.333333
Alzira-1	Spain	–0.49072	39.15696	52073	Other	60.19048	105.2857	122.1264	19.52381	28.80952	28.80952
Amfilochia-1	Greece	21.17164	38.96138	51148	Other	27.90476	82.57143	95.70936	13	17.61905	17.61905
Argo-1	Greece	22.74218	37.62865	46154	Other	15.47619	59	63.04762	4.238095	10.90476	10.90476
Argo-2	Greece	22.73931	37.65556	46154	Other	15.47619	59	63.04762	4.238095	10.90476	10.90476
Argos-1	Greece	22.66172	37.68659	46154	Other	15.47619	59	63.04762	4.238095	10.90476	10.90476
Arta-2	Greece	20.90379	39.15566	52147	Other	36.09524	95.80952	111.9852	17.90476	24.52381	24.52381
Arta-3	Greece	20.91826	39.16047	52147	Other	36.09524	95.80952	111.9852	17.90476	24.52381	24.52381
Barcellona P.G.-1	Italy	15.13679	38.11056	45127	Other	32.61905	68.09524	72.19048	7.952381	10	10
Brucoli-1	Italy	15.11052	37.29482	42128	Other	22.33333	60.47619	81.28407	8.857143	12.95238	12.95238
Canicattí-1	Italy	13.8409	37.35843	42123	Other	15.95238	43.90476	49.61248	6.380952	9.333333	9.333333
Carruba-1	Italy	15.19094	37.68463	43128	Other	16.71429	45.09524	57.61412	6.285714	8.095238	8.095238
Castello-1	Spain	–0.0862	39.90392	55075	Other	57.66667	100.5238	124.1429	23.71429	34.14286	34.38095
Castello-2	Spain	–0.08823	39.88386	55075	Other	57.66667	100.5238	124.1429	23.71429	34.14286	34.38095
Castello-3	Spain	–0.09095	39.88401	55075	Other	57.66667	100.5238	124.1429	23.71429	34.14286	34.38095
Cefalú-1	Italy	14.01227	38.02948	45124	Other	43.2381	93.33333	107.468	21.42857	33.14286	33.14286
Chania-1	Greece	24.05114	35.49315	38161	Other	13.09524	72.19048	105.1429	8.285714	19.90476	19.90476
Chania-2	Greece	23.94806	35.47789	38160	Other	15.80952	88.7619	130.2381	6.238095	18.42857	18.66667
citricarpa-1	NA	–8.51451	37.33241	49044	Other	54.57143	150.0476	241.1149	15.04762	30	30
Comiso-1	Italy	14.63716	36.94398	40126	Other	21.71429	56.14286	64.09524	12.09524	15.80952	15.80952
Conceicao-1	Portugal	–7.91693	37.04848	47046	Other	36.14286	99	177.4778	12.2381	25.42857	25.42857

City	Country	e	n	Grid_Code	Type of finding by Guarnaccia et al. (2017)	Pycnidiospores ($D_{50} = 3$ hours; $T_{min} = 10^{\circ}\text{C}$)			Ascospores ($D_{50} = 3$ hours; $T_{min} = 15^{\circ}\text{C}$; Fourie 0.7)		
						Mean number of infections in the period May–September (5 months fruit susceptibility)	Mean number of infections in the period May–November (7 months fruit susceptibility)	Mean number of yearly infection January–December (12 months leaf susceptibility)	Mean number of infections in the period May–September (5 months fruit susceptibility) in days with PAT	Mean number of infections in the period May–November (7 months fruit susceptibility) in days with PAT	Mean number of yearly infection January–December (12 months leaf susceptibility) in days with PAT
Curiglia-1	Italy	16.20376	38.76773	48131	Other	70.85714	140.1429	163.3563	19.04762	24.80952	24.80952
El_Ejido-1	Spain	-2.71999	36.79521	43064	Other	17.14286	32.66667	38.57143	2.142857	3.571429	3.571429
Estellencs-1	Spain	2.481876	39.6535	53083	Other	45.71429	95.04762	103.4762	10.66667	12.90476	12.90476
Faro-1	Portugal	-7.91681	37.10846	48046	Other	29.80952	84.47619	132.6929	14.80952	23.28571	23.28571
Faro-2	Portugal	-7.91743	37.06264	47046	Other	36.14286	99	177.4778	12.2381	25.42857	25.42857
Giarratana-1	Italy	14.97442	36.88344	40127	Other	26	69	74.47619	9.428571	14	14
Gouria-1	Greece	21.25765	38.45498	49149	Other	35.95238	107.4286	137.9179	18	30.90476	30.90476
Gozo-2	Malta	14.2593	36.04907	36125	Other	25.85714	90.90476	145.3727	13.2381	36.42857	36.42857
Gozo-3	Malta	14.26012	36.03753	36125	Other	25.85714	90.90476	145.3727	13.2381	36.42857	36.42857
Gozo-4	Malta	14.27936	36.04965	36125	Other	25.85714	90.90476	145.3727	13.2381	36.42857	36.42857
Gozo-5	Malta	14.28445	36.05817	36125	Other	25.85714	90.90476	145.3727	13.2381	36.42857	36.42857
Grotte-1	Italy	15.17701	37.67993	43128	Other	16.71429	45.09524	57.61412	6.285714	8.095238	8.095238
Guardia-1	Italy	15.17592	37.66271	43128	Other	16.71429	45.09524	57.61412	6.285714	8.095238	8.095238
Kirtomados-1	Greece	23.91666	35.47875	38160	Other	15.80952	88.7619	130.2381	6.238095	18.42857	18.66667
Leni-1	Italy	14.59752	38.04442	45126	Other	29.90476	68	75.09524	11.66667	14.42857	14.42857
Leni-2	Italy	14.82713	38.55289	47126	Other	NA	NA	NA	NA	NA	NA
Lentini-1	Italy	15.0209	37.32058	42127	Other	27.2381	64.33333	81.36617	39.04762	50.09524	50.09524
Malaga-1	Spain	-4.42706	36.76176	44058	Other	23.19048	48.2381	50.95238	3.714286	4.571429	4.571429
Mascali-1	Italy	15.1925	37.76768	44128	Other	20.14286	45.71429	54.33333	9.619048	10.66667	10.66667
Mascali-2	Italy	15.19464	37.76826	44128	Other	20.14286	45.71429	54.33333	9.619048	10.66667	10.66667
Massafra-1	Italy	17.14411	40.54476	57134	Other	39.04762	62.14286	62.57143	9.333333	10.95238	10.95238
Mastro-1	Greece	21.28054	38.43029	49149	Other	35.95238	107.4286	137.9179	18	30.90476	30.90476

City	Country	e	n	Grid_Code	Type of finding by Guarnaccia et al. (2017)	Pycnidiospores ($D_{50} = 3$ hours; $T_{min} = 10^{\circ}\text{C}$)			Ascospores ($D_{50} = 3$ hours; $T_{min} = 15^{\circ}\text{C}$; Fourie 0.7)		
						Mean number of infections in the period May–September (5 months fruit susceptibility)	Mean number of infections in the period May–November (7 months fruit susceptibility)	Mean number of yearly infection January–December (12 months leaf susceptibility)	Mean number of infections in the period May–September (5 months fruit susceptibility) in days with PAT	Mean number of infections in the period May–November (7 months fruit susceptibility) in days with PAT	Mean number of yearly infection January–December (12 months leaf susceptibility) in days with PAT
Mesquita-1	Portugal	−8.28949	37.21367	48045	Other	41.38095	109.5238	189.0903	12.90476	31.04762	31.04762
Mesquita-2	Portugal	−8.29781	37.20453	48045	Other	41.38095	109.5238	189.0903	12.90476	31.04762	31.04762
Mineo-1	Italy	14.69086	37.35072	42126	Other	17.71429	45	54.2381	11	14.47619	14.47619
Moncada-1	Spain	−0.39458	39.58855	54074	Other	56.61905	97.57143	115.0443	17	24.95238	24.95238
Monchique-2	Portugal	−8.50369	37.33623	49044	Other	54.57143	150.0476	241.1149	15.04762	30	30
Monchique-3	Portugal	−8.49223	37.33224	49044	Other	54.57143	150.0476	241.1149	15.04762	30	30
Motta_S._Anastasia-1	Italy	14.88602	37.4821	42127	Other	27.2381	64.33333	81.36617	39.04762	50.09524	50.09524
Motta_S._Anastasia-2	Italy	14.95416	37.46971	42127	Other	27.2381	64.33333	81.36617	39.04762	50.09524	50.09524
Nafplio-1	Greece	22.78527	37.58931	46155	Other	21.14286	52.28571	73.66174	10.71429	15.71429	15.71429
Nafplio-2	Greece	22.69559	37.5751	46154	Other	15.47619	59	63.04762	4.238095	10.90476	10.90476
Nafplio-3	Greece	22.6968	37.58229	46154	Other	15.47619	59	63.04762	4.238095	10.90476	10.90476
Nafplio-4	Greece	22.87484	37.5888	46155	Other	21.14286	52.28571	73.66174	10.71429	15.71429	15.71429
Nicolosi-1	Italy	15.02948	37.61127	43127	Other	18.33333	42	52.09524	8.047619	9.285714	9.285714
Niscemi-1	Italy	14.3934	37.13978	41125	Other	20.28571	53.2381	65.33333	10	17.14286	17.14286
Noto-1	Italy	15.09545	36.8465	40128	Other	25.47619	76.47619	100.7603	9	18.14286	18.14286
Pachino-1	Italy	15.08699	36.72003	39128	Other	24.66667	107.1905	165.33	14.90476	39.85714	39.85714
Pachino-2	Italy	15.08941	36.72233	39128	Other	24.66667	107.1905	165.33	14.90476	39.85714	39.85714
Pedara-1	Italy	15.06654	37.60871	43127	Other	18.33333	42	52.09524	8.047619	9.285714	9.285714
Pizzo Calabro-1	Italy	16.22601	38.76039	48131	Other	70.85714	140.1429	163.3563	19.04762	24.80952	24.80952
Ribera-1	Italy	13.24185	37.49711	42121	Other	42.47619	98.04762	124.7094	21.57143	30.95238	30.95238

City	Country	e	n	Grid_Code	Type of finding by Guarnaccia et al. (2017)	Pycnidiospores ($D_{50} = 3$ hours; $T_{min} = 10^{\circ}\text{C}$)			Ascospores ($D_{50} = 3$ hours; $T_{min} = 15^{\circ}\text{C}$; Fourie 0.7)		
						Mean number of infections in the period May–September (5 months fruit susceptibility)	Mean number of infections in the period May–November (7 months fruit susceptibility)	Mean number of yearly infection January–December (12 months leaf susceptibility)	Mean number of infections in the period May–September (5 months fruit susceptibility) in days with PAT	Mean number of infections in the period May–November (7 months fruit susceptibility) in days with PAT	Mean number of yearly infection January–December (12 months leaf susceptibility) in days with PAT
Ribera-2	Italy	13.25207	37.50442	42121	Other	42.47619	98.04762	124.7094	21.57143	30.95238	30.95238
Riposto-1	Italy	15.19935	37.69647	43128	Other	16.71429	45.09524	57.61412	6.285714	8.095238	8.095238
Rocca_Imperiale-1	Italy	16.61795	40.10839	54132	Other	34.42857	56.19048	60.90476	10	11.52381	11.52381
San_Gregorio-1	Italy	15.10097	37.5623	43127	Other	18.33333	42	52.09524	8.047619	9.285714	9.285714
Scordia-1	Italy	14.86915	37.28153	42127	Other	27.2381	64.33333	81.36617	39.04762	50.09524	50.09524
Seville-1	Spain	–5.96282	37.50854	48053	Other	22.7619	88.47619	144.9557	9.809524	27.14286	27.14286
Seville-2	Spain	–5.95491	37.48298	48053	Other	22.7619	88.47619	144.9557	9.809524	27.14286	27.14286
Sikoula-1	Greece	21.0834	39.08593	52148	Other	27.7619	65.57143	71.47619	14.33333	16.33333	16.33333
Silves-1	Portugal	–8.39084	37.16415	48044	Other	52.33333	128.0476	234.5599	16.33333	33.19048	33.19048
Soller-1	Spain	2.709609	39.76453	53084	Other	46.33333	91.28571	101.2742	17.2381	20.66667	20.66667
Soller-2	Spain	2.7266	39.77012	53084	Other	46.33333	91.28571	101.2742	17.2381	20.66667	20.66667
Terme_Vigliatore-1	Italy	15.16324	38.1458	45128	Other	27.19048	54.7619	62.8555	6.809524	8.095238	8.095238
Torremolinos-1	Spain	–4.50413	36.67272	43057	Other	39.33333	111.619	171.133	12.80952	25.61905	25.90476
Trebisacce-2	Italy	16.56063	39.90671	54132	Other	34.42857	56.19048	60.90476	10	11.52381	11.52381

Appendix C – Data provenance

The following table shows for each map on which data files it is based on. The data files are deposited on <http://zenodo.org>, as 10.5281/zenodo.1003121 (<https://doi.org/10.5281/zenodo.1003121>)

Table C.1: Locations of data files on which figures of this Scientific Opinion are based on

Figure	map file name	input data file
1	locationDensity.png	locations.csv euCitrusSurface.rds
3	maghareyAscLocations.png	euCitrusSurface.rds mag2015_table 1.csv locations.csv
4	asc3_15_5m.png	locations.csv mag2015_table 1.csv
5	asc3_15_7m.png	locations.csv asco_avg_WSPR.zip/Asco_3_15_R7_Model_AVG_WSPR.xlsx
6	asc3_15_12m.png	locations.csv asco_avg_WSPR.zip/Asco_3_15_R7_Model_AVG_WSPR.xlsx
7	maghareyPycLocations.png	euCitrusSurface.rds locations.csv mag2015_table 1.csv
8	pyc3_10_5m.png	locations.csv pycnidio_avg_WR.zip/Pycnidio_3_10_Model_AVG_WR.xlsx
9	pyc3_10_7m.png	pyc3_10_7m.png pycnidio_avg_WR.zip/Pycnidio_3_10_Model_AVG_WR.xlsx
10	pyc3_10_12m.png	locations.csv pycnidio_avg_WR.zip/Pycnidio_3_10_Model_AVG_WR.xlsx
12	climateGI_climex.png	Yonow_et_al_2013_CLIMEX_with_10minresolution_1961-1990.zip locations.csv
13	climateEI_climex.png	Yonow_et_al_2013_CLIMEX_with_10minresolution_1961-1990.zip locations.csv
14	climateGI_jrc.png	Yonow_et_al_2013_CLIMEX_with_10minresolution_1961-1990.zip locations.csv
15	climateEI_jrc.png	Yonow_et_al_2013_CLIMEX_with_10minresolution_1961-1990.zip

The data files are available as part of the upper mentioned deposit.

The identity of the data files can be verified with the following md5 checksums:

984cd97090f1d84cce8659f3cf9855c0	asco_avg_WSPR.zip
3181603536251722eb5046758941fe01	euCitrusSurface.rda
09bddeda76d4f428a66701658bd68f10	Guarnaccia_et_al_2017 - comparison with model predictions.xlsx
fc76546c6ad19fd7c43b0b292290cb47	locations.csv
34117b40e8ab9a456a1076b7aa5acf63	mag2015_table 1.csv
5fa0b207f62e2e2c3e1bd17aa1aa1f7f	mag2015_table 2.csv
41d56dec5592f8187982af679cc9cc4a	martinez2015.zip
bfd72bb018eb8b6d9e00220d58d83b4	pycnidio_avg_WR.zip
9fc7144478dee24db65f48a6f18c9bda	spores2.zip
74be3b7bb8b56ba29b7f52e0dcc47990	Yonow_et_al_2013_CLIMEX_with_10minresolution_1961-1990.zip
4dd6a44c815cd33db4adf5285eddd737	Yonow_et_al_2013_CLIMEX_with_JRC.zip

The complete computing environment, which allows easy reproduction of all maps, is deposited as well as a Docker container: Doi: 10.5281/zenodo.1044096 (<https://doi.org/10.5281/zenodo.1044096>)

The tables mag2015_table 1.csv and mag2015_table 2.csv are originated from Magarey et al. (2015), accessible here: <http://www.sciencedirect.com/science/article/pii/S0261219415300387>