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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 Panel on Plant Health (PLH)

Abstract

Following a request from the European Commission, the EFSA Panel on Plant Health (PLH) was asked to assess two publications, authored by Magarey et al. and Martínez-Minaya et al. from 2015, with regard to a need to update the EFSA Scientific Opinion from 2014 on the risk of *Phyllosticta citricarpa* (*Guignardia citricarpa*) for the EU territory. The EFSA PLH Panel was also requested to assess any other relevant scientific information published after the finalisation of the EFSA Scientific Opinion. The fungus *P. citricarpa* (McAlpine) Van der Aa causes the citrus disease citrus black spot (CBS), and is regulated as quarantine organism in Council Directive 2000/29/EC. The Panel assessed the two publications in detail as well as all relevant publications published until 31 March 2016. A comparison with the EFSA PLH Panel (2014) was made, survey data on CBS from South Africa used in Magarey et al. (2015) were evaluated, and the citrus production areas in the EU were characterised and compared with results from Magarey et al. (2015). Uncertainty and model sensitivity were discussed. It was concluded that the evidence presented in Magarey et al. from 2015 does not require an updating of EFSA PLH Panel (2014). The conclusion in the Opinion that probability of CBS establishment in the EU is moderately likely is not affected by the paper by Magarey et al. (2015) predicting establishment in some of the EU locations they selected. The high level of uncertainty regarding the probability of establishment is also unchanged by Magarey et al. (2015). The Panel concluded that Martínez-Minaya et al. (2015) does not provide new evidence requiring an update to EFSA PLH Panel (2014), principally because it had already been concluded that global climate zones are based on factors and thresholds that are broad and not necessarily representative of those that are critical for the pathogen and its host.

Keywords: Citrus black spot, risk of establishment, European Union, *Phyllosticta citricarpa*, *Guignardia citricarpa*, climate variability and modelling, uncertainty

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Summary

The Opinion is made at the request of the European Commission (Question number**:** EFSA-Q-2015-006010).

Request: The purpose of this request was to ask, pursuant to Article 29(1) of Regulation (EC) No 178/2002, an update of a scientific opinion in the field of plant health. In February 2014, the European Food Safety Authority (EFSA) published a 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. doi:10.2903/j.efsa.2014.3557 http://www.efsa.europa.eu/en/efsaioumal/doc/3557.pdf).

Since then, at least two scientific articles regarding *Phyllosticta citricarpa* have been published (see Annexes [of the mandate]). Therefore, EFSA was requested to assess the two mentioned scientific publications and any other relevant scientific information that may have been published after the finalisation of the EFSA Scientific Opinion of *P. citricarpa*. If in the light of the newly available scientific evidence, it becomes apparent that the Scientific Opinion needs to be updated, EFSA was mandated to do so by end of April 2016 based on the previous mandate, and asked to keep the European Union (EU) Commission informed.

[Encl: Magarey RD, et al., 2015. Prediction of *Phyllosticta citricarpa* using an hourly infection model and validation with prevalence data from South Africa and Australia. Crop Protection, 75, 104–114.

Martínez-Minaya J, et al., 2015. Climatic distribution of citrus black spot caused by *Phyllosticta citricarpa*. A historical analysis of disease spread in South Africa. Eur J Plant Pathol, 143, 69–83.]

Data and methodology: Literature searches were conducted on publications on *P. citricarpa* (citrus black spot (CBS)) that had appeared during the period from 1 January 2014 until 31 March 2016, using the pathogen's binomial and disease names as search terms in Web of Science. All publications retrieved during the search were evaluated for their relevance for the EFSA PLH 2014 Opinion; i.e. whether any new information provided necessitated an update of the Opinion. A literature search was also conducted in September 2015 of all papers citing: Magarey RD, Sutton TB and Thayer CL, 2005. A simple generic infection model for foliar fungal plant pathogens. Phytopathology, 95(1), 92–100, a key methodological source for both the Magarey et al. (2015) paper and the EFSA PLH Panel (2014) Opinion. Searches were based on Google Scholar, Scopus and Web of Science. Archived weather station data and grid cell data used in the previous EFSA Opinions of 2008 and 2014 were available for further analysis. In addition, the following data sources were used for the assessment:

- Simulated infection events from the output of the ascospore infection model presented in EFSA PLH Panel (2014)
- Infection scores obtained from the electronic supplementary material of Magarey et al. (2015)
- Citrus production data from National Authorities of the European Union (EU) Member States were requested and made available.
- Climate zone raster files by personal communication (A. Vicent, Instituto Valenciano de Investigaciones Agrarias (IVIA), Spain, personal communication, January 2016) with the corresponding author of Martínez-Minaya et al. (2015b).

Assessment: The assessment was conducted in line with the principles described in the EFSA Guidance on transparency in the scientific aspects of risk assessment (EFSA, 2009). The present document is structured according to the Guidance on the structure and content of EFSA's scientific opinions and statements (EFSA Scientific Committee, 2014). Uncertainties associated with weather variability, spatial-scale dependencies and model parameters were identified and analysed with regard to their impact on the final assessment outcome in line with the Draft Guidance on Uncertainty (EFSA Scientific Committee, 2016). The assessment was made according to the following structure:

1) Assessment and comparison of results and conclusions of recent papers on CBS with the EFSA opinion on CBS (EFSA PLH Panel, 2014).
2) Assessment of papers applying the generic infection model and the ascospore maturation model.
3) Evaluation of the survey data on CBS from South Africa used for validation in Magarey et al. (2015).
4) Characterising the citrus production areas in the potential area of establishment in the EU and comparing with results from Magarey et al. (2015).
5) Uncertainty and model sensitivity.

Conclusions: As a result of the assessment, the following conclusions were drawn:

Comparison of EFSA PLH Panel (2014) and Magarey et al. (2015)

The two studies show several similarities. Both basically used the same equations and parameter values to simulate infection by ascospores and by conidia. Both studies also used the Gompertz equation of Fourie et al. (2013) to describe the dynamics of ascospore production. However, the two studies show several differences concerning:

- the type of weather data used as model inputs and how it was used in the model (such as calculation of vapour pressure deficit);
- the temporal and spatial resolution of the simulations (lower numbers of years and locations were considered in Magarey et al. (2015));
- the biofix (i.e. when temperature summation begins) for the start of ascospore season;
- the method for estimating ascospore release;
- the type of output variables considered, and their post-processing, to assess the risk of establishment.

The number of sites showing high infection scores was lower in Magarey et al. (2015) than in EFSA PLH Panel (2014), but this result does not necessarily indicate a lower risk at the European scale because of the aforementioned assumptions in the simulation by Magarey et al. (2015). In addition, there were a smaller number of locations and years in that study compared to the gridded climatic data used by EFSA PLH Panel (2014), and no rationale was given for the choice of locations.

Although Magarey et al. (2015) and EFSA PLH Panel (2014) used very similar model equations and parameter values, it was found that the two series of model outputs were not significantly correlated. This opinion also shows that small differences in the model assumptions can have a strong impact on the model outputs. In order to improve the comparability of different modelling studies performed on CBS, it will be useful to share standard sets of weather data and to analyse the consequences of different assumptions made by the models using an ensemble approach.

Comparison of EFSA PLH Panel (2014) and Martínez-Minaya et al. (2015b)

The approach applied by Martínez-Minaya et al. (2015b) was not based on the use of an infection model. Martínez-Minaya et al. (2015b) analysed the suitability of the climates present in the Mediterranean Basin for CBS using the Köppen–Geiger zones and the Aschmann's classification criteria. The authors found that the climates of several areas located in southern Europe were suitable for CBS. There appears to be a strong overlap between the area reported as suitable by Martínez-Minaya et al. (2015b) and the area potentially suitable for CBS infection according to EFSA PLH Panel (2014), especially when the citrus production area is taken into account. However, EFSA PLH Panel (2014) had already concluded that global climate zones are based on factors and thresholds that are very broad and not necessarily representative of the climatic factors that are critical for the pathogen and its host, and hence for establishment.

Implications of issues raised by Martínez-Minaya et al. (2015b) as they affect Magarey et al. (2015)

Martínez-Minaya et al. (2015b) pointed out that CBS has spread in South Africa from the initial points of introduction. The current information about the distribution may not represent the full extent of spread in the country. This may not only be due to climatic restrictions, but also could be due to legislation restricting movement of infected citrus and citrus planting material. They were not able to quantify, however, how much climatic conditions and quarantine regulations have contributed to the current distribution. If CBS has not reached its maximum distribution in that country it would imply that some locations that are reported as free from the disease may still be suitable for the establishment of *P. citricarpa*. This, in turn would have implications about the conclusions reached by Magarey et al. (2015), as this study used the current presence and absence of CBS as a factor in the validation of their model.

The need to update EFSA PLH Panel (2014)

The Panel concludes that the evidence presented by Magarey et al. (2015) does not require an updating of EFSA PLH Panel (2014). The two approaches share a number of similarities but make some different biological assumptions that are identified above. The paper by Magarey et al. (2015) concerns the risk of establishment of CBS in different citrus producing regions of the world, including the EU. The EFSA PLH Panel (2014) conclusion of moderately likely probability of establishment was based on the

existence of favourable conditions in the risk assessment area for inoculum production and infection which is not affected by Magarey et al. (2015) who show that establishment is possible in some of the locations that they selected in the EU. The high level of uncertainty surrounding the probability of establishment is also unchanged by Magarey et al. (2015) (see Section 3.5 on uncertainty).

The Panel concludes that the information in the Martínez-Minaya et al. (2015b) paper does not provide new evidence that requires an update to EFSA PLH Panel (2014). This is principally because EFSA PLH Panel (2014) had already concluded that global climate zones are based on factors and thresholds that are very broad and not necessarily representative of the climatic factors that are critical for the pathogen and its host.

In conclusion, despite the number of scientific papers on CBS published since January 2014, the new biological information provided is insufficient to warrant an update of the EFSA 2014 Scientific Opinion.

Table of Contents

1. Introduction

1.1. Background and Terms of Reference as provided by the European Commission

The purpose of this letter was to request, pursuant to Article 29(1) of Regulation (EC) No 178/2002, an update of a scientific opinion in the field of plant health. In February 2014, the European Food Safety Authority (EFSA) published a 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; http://www.efsa.europa.eu/en/efsaioumal/doc/3557.pdf).

Since then, at least two scientific articles regarding *P. citricarpa* have been published (see Annexes). Therefore, the European Commission requested EFSA to assess these two scientific publications and any other relevant scientific information that may have been published after the finalisation of the EFSA Scientific Opinion of *P. citricarpa*. If in the light of the newly available scientific evidence, it becomes apparent that the Scientific Opinion needs to be updated, EFSA was mandated to do so by end of April 2016 based on the previous mandate, and asked to keep the Commission informed.

[Encl: Magarey RD, et al. 2015. Prediction of *Phyllosticta citricarpa* using an hourly infection model and validation with prevalence data from South Africa and Australia. Crop Protection 75, 104–114.

Martínez-Minaya J, et al., 2015. Climatic distribution of citrus black spot caused by *Phyllosticta citricarpa*. A historical analysis of disease spread in South Africa. Eur J Plant Pathol 143, 69–83.]

1.2. Interpretation of the Terms of Reference

The term 'Relevant scientific information that may have been published' in the Terms of Reference is taken to mean published in the peer-reviewed scientific literature. Papers in Conference Proceedings and chapters in edited books were also evaluated. The time bound set for publication of new papers is set at 31 March 2016. Where such new scientific information is available, it will be used to determine whether the relevant portion of the 2014 EFSA Opinion should be updated, specifically the risk assessment and uncertainty ratings to which the new information relates.

2. Data and methodologies

2.1. Data

Literature searches were conducted on publications on *P. citricarpa* and its previous name *G. citricarpa*, and the disease citrus black spot (CBS) that had appeared during the period from 1 January 2014 until 31 March 2016 in Google Scholar, Scopus and Web of Science. All publications retrieved during the search were evaluated for their relevance for the EFSA PLH 2014 Opinion, i.e. whether any new information provided necessitated an update of the Opinion. A literature search was also conducted in September 2015 of all papers citing: Magarey RD, Sutton TB and Thayer CL, 2005. A simple generic infection model for foliar fungal plant pathogens. Phytopathology, 95(1), 92–100, a key methodological source for both the Magarey et al. (2015) paper and the EFSA PLH 2014 Opinion. Searches were based on Google Scholar, Scopus and Web of Science.

Archived weather station data and grid cell data used in the previous EFSA Opinions of 2008 and 2014 were available for further analysis. In addition, the following data sources were used for the preparation of the tables and maps.

2.1.1. Citrus production data

Citrus production data from National Authorities of the European Union (EU) Member States were requested and made available.

2.1.2. Infection events from EFSA PLH Panel (2014)

The simulated infection events were obtained from the outputs of ascospores infection model presented in the EFSA PLH Panel (2014) opinion on CBS. The data were obtained by the number of daily infection events and information about daily rainfalls. As in EFSA PLH Panel (2014), the data from the model run with D_{50} = 3 h and T_{min} = 10°C were used.

2.1.3. Infection scores from Magarey et al. (2015)

Scores for infection by ascospores and pycnidiospores from Magarey et al. (2015) were taken from the web page of the article at Science Direct: http://www.sciencedirect.com/science/article/pii/S0261219415300387 (Table 2. Predictions of Proportion of Ascospores Trapped (PAT), ascosporic and pycnidiosporic infection period scores during the period of fruit susceptibility for study sites).

2.1.4. Climate zones from Martínez-Minaya et al. (2015b)

The climate zone raster files were obtained by personal communication (A. Vicent, Instituto Valenciano de Investigaciones Agrarias (IVIA), Spain, personal communication, January 2016) with the corresponding author of Martínez-Minaya et al. (2015b).

2.2. Methodologies

The assessment was conducted in line with the principles described in the EFSA Guidance on transparency in the scientific aspects of risk assessment (EFSA, 2009). The present document is structured according to the Guidance on the structure and content of EFSA's scientific opinions and statements (EFSA Scientific Committee, 2014). Uncertainties associated with weather variability, spatial-scale dependencies and model parameters were identified and analysed with regard to their impact on the final assessment outcome in line with the Draft Guidance on Uncertainty (EFSA Scientific Committee, 2016).

2.2.1. Assessment and comparison of results and conclusions of recent papers on CBS with the EFSA opinion on CBS (2014)

The assessment and the information provided in Magarey et al. (2015) and Martínez-Minaya et al. (2015b) were scrutinised and it was assessed whether these put aspects or conclusions of the opinion into question, supported the opinion or were neutral. All other scientific publications on CBS published since January 2014 were assessed to a lesser extent by a more narrative approach, applying a tiered method to analyse available information following the PROMETHEUS approach (EFSA (2015), where applicable to risk assessments. In all cases, the criterion for evaluation was set as whether or not new information was provided which would necessitate an update of EFSA PLH Panel (2014).

2.2.1.1. Magarey et al. (2015)

For the current EFSA evaluation, assumptions made and parameter values used by EFSA PLH Panel (2014) and Magarey et al. (2015) were compared to determine the differences between them. In particular, the differences were noted between model assumptions, model structure and parameter settings, and the approach taken to address uncertainty and model sensitivity. It was then evaluated if these differences and the conclusions reached would necessitate an updating of EFSA PLH Panel (2014).

2.2.1.2. Martínez-Minaya et al. (2015b)

The information in Martínez-Minaya et al. (2015b) was reviewed to determine whether an update of EFSA PLH Panel (2014) is required.

2.2.1.3. Other published papers

All papers published or accepted in peer-reviewed scientific journals on *Phyllosticta* (*Guignardia*) *cirtricarpa* and/or citrus black spot after EFSA PLH Panel (2014) to March 2016 were evaluated. Papers in Conference Proceedings and chapters in edited books were also evaluated. The criterion used for the evaluation was whether the publication added new information that would necessitate an update of the EFSA PLH Panel (2014) Opinion.

2.2.2. Assessment of papers applying the leaf wetness model and the ascospore maturation model

The Magarey et al. (2015) paper and EFSA PLH Panel (2014) both used the generic infection model of Magarey et al. (2005) as extended by the ascospore maturation model of Fourie et al. (2013). An evaluation of other papers which cite Magarey et al. (2005) and Fourie et al. (2013), but in relation to other plant pathogens, was made with regard to problems encountered, approaches found to solve these problems, or alternative approaches that have been proposed.

2.2.3. Evaluation of the survey data on CBS from South Africa used for validation in Magarey et al. (2015)

An evaluation of the paper by Carstens et al. (2012), cited in Magarey et al. (2015) describing surveys conducted between 1995 and 2010 in three citrus producing provinces of South Africa was made. This paper claims to identify areas in South Africa where CBS is present or absent. This categorisation carries implications for parameterisation and validation of subsequent CBS distribution models which use data on the presence or the absence of CBS, including the papers under evaluation in this Opinion.

2.2.4. Characterising the citrus production areas in the potential area of establishment in the EU and compare with Magarey et al. (2015)

Citrus production data from the National Authorities of the EU Member States were made available and used to prepare maps overlaid with establishment areas predicted by Magarey et al. (2015) and EFSA PLH Panel (2014).

2.2.5. Uncertainty and model sensitivity

An analysis was made of uncertainties, including model sensitivities, associated with the model predictions in EFSA PLH Panel (2014) and Magarey et al. (2015).

3. Assessment

3.1. Assessment and comparison of results and conclusions of recent papers on CBS with the EFSA opinion on CBS (2014)

Recent papers, including those by Magarey et al. (2015) and Martínez-Minaya et al. (2015b), were assessed and compared with the EFSA opinion on CBS (EFSA PLH Panel, 2014). The main differences in model assumptions, input data and results are compared and contrasted. Results of a technical hearing with authors of each paper are provided. Finally, further papers on CBS published between the publication of EFSA PLH Panel (2014) and the date of 31 March 2016 are summarised.

3.1.1. Magarey et al. (2015)

Magarey et al. (2015) developed an infection model of *P. citricarpa* using hourly weather data inputs and ascospore dispersal models. The model was validated against data from 18 locations where CBS is known to occur in parts of South Africa and Australia. The model was applied to regions in Europe and the US where CBS is not currently known to occur to provide CBS risk ratings for 67 specified locations in Europe and the US. The rationale for the choice of the locations was not given. In this section, differences in the biological parameters and assumptions made by Magarey et al. (2015) and EFSA PLH Panel (2014) are identified. Differences in approaches to model uncertainty and presentation of results are presented in sections 3.5.

3.1.1.1. General observations with regard to Magarey et al. (2015) relating to EFSA PLH Panel (2014)

Magarey et al. (2015) state that 'A pest risk assessment by the European Food Safety Authority concluded that quarantine measures should be maintained given the potential for establishment and spread', with reference to EFSA PLH Panel (2014). This statement does not appear in EFSA PLH Panel (2014). Risk management decisions are the remit of the regulatory authority, which is the European Commission in the case of the EU.

Magarey et al. (2015) repeat a model simulation exercise originally conducted by EFSA PLH Panel (2014), with a slightly modified modelling approach, with access to a database of presence/absence data for CBS that can be used for validation, but covering a shorter time period of 9 years (2003–2011) than the EFSA PLH Panel (2014) study covering 21 years (1989–2009). The Magarey et al. (2015) study applies a spatial resolution of 38 × 38 km while EFSA PLH Panel (2014) applied a 25 × 25 km resolution making like for like comparisons difficult. In order to examine the potential for persistence of the pathogen, the model output was examined in EFSA PLH Panel (2014) as a time series looking up the length of periods with the absence of environmental conditions conducive for disease development.

A main criticism that has been raised by Magarey et al. (2015) against EFSA PLH Panel (2014) is the lack of validation of the EFSA modelling approach in an area with the known presence of CBS. Such a validation would need access to precise data on disease presence/absence/incidence/severity from areas where CBS occurs, along with weather data from locations in those areas. Magarey et al. (2015) claimed to have applied a form of validation which is evaluated in Section 3.5.3.

3.1.1.2. Comparison of biological parameters and assumptions

Magarey et al. (2015) adopted a similar modelling approach to EFSA PLH Panel (2014). Both studies are based on a combination of spore maturation and release model by Fourie et al. (2013) and an infection model by Magarey et al. (2005). However, there are some differences in terms of the model assumptions, model structure and parameter settings. Key differences can be found in the nature of the input weather data and biological assumptions made regarding ascospore infection events. First, the main differences between the approaches were identified. This was then further elaborated by a detailed comparison structured by the stages of the *P. citricarpa* disease cycle (see Figure 1 in EFSA PLH Panel (2014)). Some of the differences between the modelling approaches represent different but equally justifiable assumptions regarding the CBS pathosystem and usefully indicate areas for further research which could help to reduce uncertainties.

Magarey et al. (2015) used a grid with a spatial resolution of 38 × 38 km based on the weather data from the US National Centers for Environmental Prediction (NCEP) Climate System Forecast Reanalysis (CFSR) database. EFSA PLH Panel (2014) however adopted a higher resolution, interpolating station weather data from Monitoring Agricultural Resources (MARS) Crop Yield Forecasting System (MCYFS; Joint Research Centre (JRC) Monitoring Agricultural Resources Unit http://marswiki.jrc.ec.europa.eu/agri4castwiki/index.php/Interpolation_of_observed_weather) to a grid of 25 × 25 km cell size (note: this is four times higher than the spatial resolution of 50 km erroneously attributed to EFSA PLH Panel (2014) by Magarey et al. (2015)). Although the CFSR database has a high temporal resolution (hourly data are available), this is offset by the coarse spatial resolution. CFSR data used in Magarey et al. (2015) have been corrected by considering the altitude of citrus orchards. This was done by applying a fixed temperature coefficient based on the ratio between temperature and elevation to take into account the difference between the mean altitude of the grid cells and that of the orchards. The choice of spatial resolution can have profound consequences for the performance of disease distribution models and depends on many interacting epidemiological factors which occur at a range of spatial scales (Meentemeyer et al., 2012). These differences in spatial and temporal resolution between the two studies make direct comparisons difficult.

Magarey et al. (2015) restrict the number of ascospore release events using precipitation data. A default value of 0.2 mm/h rain is required in Magarey et al. (2015) to trigger ascospore release. In EFSA PLH Panel (2014), an assumption is also made that ascospore release is triggered by rain. In both cases, it is assumed that ascospore release cannot be triggered by moisture provided by dew or irrigation, rather than rainfall. Previous literature data also show high uncertainty about the role of rainfall, dew and irrigation water. For these studies, as noted in EFSA PLH Panel (2014), there was no robust method to distinguish between *P. citricarpa* and *Phyllosticta capitalensis*. Kiely (1949) trapped ascospores consistently throughout spring, summer and autumn, and noted that their numbers did not appear to correlate with periods of rainfall and concluded that dew provided sufficient wetting to initiate ascospore release. Under South African conditions, McOnie (1964a,b) trapped negligible levels of ascospores during spring, and observed ascospore release peaking from November to January and lower levels in autumn. However, release typically followed rain events and was heaviest during high-rainfall months (1964a). Ascospore release could not be correlated with flood irrigation or dew, although McOnie (1964a) conceded that irrigation was seldom necessary during November to January due to summer rainfall. Kotzé noted that ascospores are released when it rains and occasionally during irrigation (Kotzé, 2000 in Dummel et al., 2015).

Further differences between the biological model assumptions and parameters from EFSA PLH Panel (2014) and Magarey et al. (2015) are summarised in Table 1 and discussed below. For clarity, the comparisons are structured as they relate to the natural disease cycle of the pathogen. *P. citricarpa* has two infection cycles, a primary cycle driven by ascospores in the leaf litter and a secondary cycle involving pycnidiospores produced on lesions in fruit, twigs and leaves (see Figure 1 in EFSA PLH Panel (2014)). The disease cycle stages include: the start of the ascospore season, the subsequent dynamics of ascospore production, ascospore release, infection by ascospores, production of pycnidia, dispersal of conidia (pycnidiospores) and finally infection by conidia.

Table 1: Summary of the comparison of biological model parameters and assumptions from EFSA PLH Panel (2014) and Magarey et al. (2015) following the *P. citricarpa* disease cycle

Stages in the disease cycle	EFSA PLH Panel (2014)	Magarey et al. (2015)
Primary ascospore cycle		
Stage 1: Start of the ascospore season	Gompertz equation predicting the onset of ascospore release as a function of degree day accumulation of daily weather data from mid-winter (1 January) as the biofix and 10°C as the base temperature. Time of onset is defined as the moment at which the probability of spore discharge on days that are suitable for such discharge (3-day cumulative rainfall > 0.2 mm or vapour pressure deficit < 5 hPa) pass a predefined threshold	527.3 degree days above 10°C accumulated from mid-winter (1 January) were used as a proxy of date of the first trapped ascospore
Stage 2: Dynamic of ascospore production	Gompertz equation predicting the cumulative proportion of ascospores released per season as a function of degree-day accumulation only on days with measurable rainfall (> 0.1 mm) or vapour pressure deficit < 5 hPa)	The PAT equation was based on the degree days (> 10°C) accumulated from the first seasonal ascospore discharge (see step 1) on days with rainfall > 0.1 mm or vapour pressure deficit (VPD) < 5 hPa. PAT smoothed by a 7-day moving average and accumulated on days of infection when the daily infection risk was greater than zero
Stage 3: Ascospore release	Not considered in the model	Precipitation for the hour exceeds the dispersal threshold. Default value 0.2 mm rain h^{-1}. Precipitation requirement is allowed to accumulate over 2 h
Stage 4: Infection by ascospores	Parameter settings from EFSA (2008): T_{min} = 15°C; T_{opt} = 27°C; T_{max} = 35°C; D_{50} = 3 h; W_{min} = 15 h; W_{max} = 38 h. Equation published in Magarey et al. (2005) Ascosporic infection periods were those that occurred in a period with > 1% of the ascospores released according to model 2 in Section 3.3.2.4 of EFSA PLH Panel (2014). Infections predicted on days without ascospore inoculum available according to model 2 in Section 3.3.2.4 of EFSA PLH Panel (2014) were disregarded	Parameter settings as in EFSA (2008). Equation published in Magarey et al. (2005). Ascosporic infection periods were those that occurred on days with predicted ascospore release: the differential PAT was the PAT on a given day minus the PAT of the previous day
Secondary pycnidiospore cycle		
Stage 5: Production of pycnidia	Not considered in the model	Not considered. Time for starting conidial infections not specified
Stage 6: Dispersal of conidia (pycnidiospores)	On days with rainfall > 0	As for ascospore release
Stage 7: Infection by conidia	Model by Magarey et al. (2005); parameter settings from EFSA (2008): T_{min} = 10°C, T_{opt} = 25°C, T_{max} = 35°C, D_{50} = 3 h, W_{min} = 12 h, W_{max} = 35 h	Model by Magarey et al. (2005); parameter settings as in EFSA (2008)

D_{50}: duration of a dry period at relative humidities < 95% that will result in a 50% reduction in disease compared with a continuous wetness period; PAT: proportion of ascospores trapped; T_{max}: maximum temperature; T_{min}: minimum temperature; T_{opt}: optimum temperature; VPD: vapour pressure deficit; W_{max}: maximum value of the wetness duration requirement; W_{min}: minimum value of the wetness duration requirement.

Stage 1: Start of the ascospore season

In EFSA PLH Panel (2014), the Gompertz equation is used to predict the onset of ascospore release as a function of degree-day accumulation of daily weather data from mid-winter (1 January) as the

biofix and 10°C as the base temperature. The time of onset is defined as the moment at which the probability of spore discharge on days that are suitable for such discharge (3-day cumulative rainfall > 0.2 mm or VPD < 5 hPa) pass a predefined threshold. Fourie et al. (2013) recommend probability thresholds of 0.5 and 0.7, and EFSA PLH Panel (2014) evaluated both 0.5 and 0.7 thresholds.

However, in Magarey et al. (2015), the model is initiated when 'a value of 527.3 degree days above 10°C accumulated from mid-winter', where mid-winter is defined as 1 January by Fourie et al. (2013). Magarey et al. (2015) attribute the 527.3 degree days figure to Fourie et al. (2013); however, during the hearing (Appendix A), Magarey clarified that the value of 527.3 was incorrectly attributed to Fourie et al. (2013). Instead, this value was used in Magarey et al. (2015) as this represented the lowest decimal degree value when ascospores were observed in the field. A source for the specific value of 527.3 was not made available.

Stage 2: Dynamic of ascospore production

In EFSA PLH Panel (2014), the Gompertz equation was used to predict the cumulative proportion of ascospores released per season as a function of degree-day accumulation only on days with measurable rainfall (> 0.1 mm) or VPD < 5 hPa. When running the ascospore maturation and release model, it was observed that a minor proportion of the spores would not mature within one growing season, and would not be released until the following season. Although semiarid conditions are not particularly detrimental for leaf litter survival, the data available on citrus leaf litter decomposition indicate that it is unlikely that fallen leaves will maintain their integrity as a substrate for inoculum production for such a long period (Lee and Huang, 1973; Mondal and Timmer, 2002; Mondal et al., 2003; Upadhyaya et al., 2012; Bassimba et al., 2014). Therefore, only predictions for the first year have been considered (EFSA PLH Panel, 2014).

In Magarey et al. (2015), the PAT is a component of the ascosporic infection risk, i.e. the 'differential PAT was the PAT on a given day minus the PAT of the previous day. Ascosporic infection periods were those that occurred on days with predicted ascospore release'. PAT is calculated based on Fourie et al. (2013). However, differently from the original PAT equation which was based on daily data (temperature > 10°C, rainfall > 0.1 mm and VPD < 5 hPa) hourly data were used. Obviously, using hourly data and averaging them would provide a different result, and there is no mention on whether this difference was preliminarily evaluated or not.

Stage 3: Ascospore release

Ascospore release is not explicitly incorporated into EFSA PLH Panel (2014). Magarey et al. (2015) restrict ascospore release to periods where precipitation for the hour exceeds 0.2 mm, which is allowed to accumulate over a 2 h period. It was clarified in the hearing notes (Appendix A) that this meant 0.2 mm in 2 h. This is a small amount of rain that may be below the amount that is measurable. At the same time, ascospore release requires only moisture in fallen leaves which may be provided by dew, soil moisture or irrigation. Hence, by including the precipitation mechanism in the model, Magarey et al. (2015) is potentially susceptible to misclassification error on ascospore release events, i.e. ascospore release events occurring in days with no rain are not considered.

Moreover, Fourie et al. (2013) showed no close association between rain (both in a day and in 3-h intervals) and ascospore trapping noting that 'Rainfall was recorded on 243 of the 635 ascospore days. The 50th and 75th percentiles for daily rainfall were given as 0 and 2.4 mm, respectively. When rainfall during the 3 days leading up to an ascospore event was considered, these values were 2.1 and 13 mm, respectively; 396 days conformed to this criterion. In 81.6% of the cases (518 days), rainfall was recorded in the 7 days leading up to the event'.

As noted above, previous literature data also show high uncertainty about the role of rainfall, dew and irrigation water.

No significant relationship was observed by Dummel et al. (2015) between the ascospore release and the amount of rainfall. The variable most influencing the release of ascospores was days with more than 10 h of leaf wetness (D_{Mojt}), which counts the days of total wetting caused either by rain or by dew. The variable D_{Mojt} had the strongest effect on the correlation because it calculates the total days with wetness duration longer than 10 h (Kendall correlation coefficient = 0.68).

Irrespective of that, Magarey et al. (2015) remark that: 'The first step in the infection period module is to check if precipitation for the hour exceeds the dispersal threshold. For *P. citricarpa* this was set to the default value of 0.2 mm/h (Paul et al., 2004) for pycnidiosporic and ascosporic infection. While ascospores were trapped in the absence of measured rainfall (Fourie et al., 2013), other studies found that rainfall was a requirement for ascospore release (Kotzé, 1963; McOnie,

1964a). The precipitation requirement is allowed to accumulate over 2 h. Precipitation is required only to initiate an infection not to continue it'. Note that the 0.2 mm per value used was based on data from *Fusarium graminearum*; there is no evidence that the same rain threshold may apply to *P. citricarpa* (see hearing notes, Appendix A).

Stage 4: Infection by ascospores

In EFSA PLH Panel (2014), the model by Magarey et al. (2005) was applied; parameter settings are (see Table 1, Stage 4, under EFSA PLH 2014): T_{min} = 15°C; T_{opt} = 27°C; T_{max} = 35°C; D_{50} = 3 h; W_{min} = 15 h; W_{max} = 38 h.

Ascosporic infection periods were those that occurred in a period with ≥ 1% of the ascospores released. Infections predicted on days without ascospore inoculum available were disregarded. Furthermore, these infection periods occurred on days with predicted ascospore release: the differential PAT was the PAT on a given day minus the PAT of the previous day.

In Magarey et al. (2015), also the model by Magarey et al. (2005) was applied; parameter settings (see Table 1, Stage 4, under Magarey et al., 2015) set as in EFSA PLH Panel (2014).

Stage 5: Production of pycnidia

This stage was not considered in either EFSA PLH Panel (2014) or Magarey et al. (2015).

Stage 6: Dispersal of conidia

In both EFSA PLH Panel (2014) and Magarey et al. (2015), an infection period by conidia is initiated by rain. Studies by Perryman et al. (2014) confirm that conidia are splash dispersed.

Stage 7: Infection by conidia

The same infection model as used for ascospores was applied, but with different parameter settings (T_{min} = 10°C, T_{opt} = 25°C, T_{max} = 35°C, D_{50} = 3 h, W_{min} = 12 h, W_{max} = 35 h).

3.1.1.3. Conclusion on the need to update EFSA PLH Panel (2014) based on Magarey et al. (2015)

The Panel concludes that the evidence presented by Magarey et al. (2015) does not require an updating of EFSA PLH Panel (2014). The two approaches share a number of similarities but make some different biological assumptions that are identified above. Magarey et al. (2015) concerns the risk of establishment of CBS in different citrus-producing regions of the world, including the EU. EFSA PLH Panel (2014) concluded that the probability of establishment in the EU was moderately likely with high uncertainty. The EFSA PLH Panel (2014) conclusion of moderately likely probability of establishment was based on the existence of favourable conditions in the risk assessment area for inoculum production and infection which is not affected by Magarey et al. (2015) who show that establishment is possible in some of the study sites that they selected in the EU.

The high level of uncertainty surrounding the probability of establishment is also unchanged by Magarey et al. (2015) (see Section 3.5 on uncertainty). Both the approaches of Magarey et al. (2015) and EFSA PLH Panel (2014) involve exact thresholds on environmental factors at different stages in the organism life cycle for which there is insufficient scientific evidence to determine (as stated in EFSA PLH Panel (2014)). Magarey et al. (2015) do not conduct a full sensitivity analysis on their model which introduces uncertainty around their results, and the authors themselves indicate that some of their parameter choices could justifiably differ to those that were used (see hearing notes, Appendix A). One reason for the moderately likely rating for establishment by EFSA PLH Panel (2014) was the use of sprinkle and microsprinkle irrigation in parts of the EU citrus-growing area. Magarey et al. (2015) do not address the potential role of irrigation in ascospore release.

3.1.2. Martínez-Minaya et al. (2015b)

Martínez-Minaya et al. (2015b) conducted a historical analysis of CBS spread across South Africa in an attempt to identify climatic associations with CBS disease occurrence. The study assembled data on the CBS presence from the beginning of the CBS epidemic (1940–1950) until 2014. Information on air temperature and precipitation was sourced from the WorldClim database (Hijmans et al., 2005) and areas where CBS is absent in South Africa were compared with areas where CBS has been recorded as present between 1950 and 2014 in relation to both the average and the variation in temperature and rainfall characteristics. In addition, the climate classifications based on both the Köppen–Geiger system

(Peel et al., 2007) and the Aschmann (1973) criteria for the Mediterranean climate were compared with the CBS presence and absence in South Africa.

The Panel finds that Martínez-Minaya et al. (2015b) has compiled interesting data on the historical spread dynamics of CBS in South Africa. The Panel agrees with the conclusion of Martínez-Minaya et al. (2015b) that CBS expanded in South Africa from its initial geographical range in summer rainfall areas to neighbouring regions with markedly drier conditions and that these results contradict overall statements indicating that CBS occurs exclusively in climates with summer rainfall (Kotzé, 2000; Graham et al., 2014).

The analysis of the historical spread dynamics of CBS in South Africa by Martínez-Minaya et al. (2015b) is based on the hypothesis that not only climate, but also South African regulations prohibiting the movement of citrus and/or related plant propagation material, have played a role in restricting the current distribution of the disease in South African citrus production areas. However, Martínez-Minaya et al. (2015b) do not provide conclusive evidence of the relative contribution of regulatory prohibition and climatic barriers to CBS disease spread.

The Panel considers that the information in the Martínez-Minaya et al. (2015b) paper does not provide new evidence that requires an update to EFSA PLH Panel (2014). This is because:

- EFSA PLH Panel (2014) also summarised the use of climate classification approaches for assessing the suitability of the environment for plant pathogens such as *P. citricarpa* finding that the climate classification systems are based on broad environmental characteristics that do not necessarily represent the thresholds, durations and timings of the key environmental factors that are critical for disease development by the pathogen and its host. Thus, EFSA PLH Panel (2014) states that: 'climate zones (e.g. Köppen–Geiger) may not necessarily represent the environmental factors that are critical for the pathogen and its host for disease to develop, especially when considering the influence of microclimate (Vicent and García-Jiménez, 2008)'. This explains why EFSA PLH Panel (2014) decided not to apply climate classification systems to estimate the suitability of the climate in the EU citrus production areas for *P. citricarpa*. In relation to the suitability of the Mediterranean climate for *P. citricarpa*, EFSA PLH Panel (2014) summarised the Köppen–Geiger climates where *P. citricarpa* is found and noted that: 'It has been stated that *P. citricarpa* has failed to establish in Mediterranean climates (Paul et al., 2005; Yonow et al., 2013), but the extent to which the pathogen has or has not become established under Mediterranean climatic conditions depends on the definition of the Mediterranean climate'. EFSA PLH Panel (2014) quoted five definitions based on climate, geography, vegetation and other factors.
- The importance of irrigation in influencing pathogen distribution makes some climatic parameters that are key to the definitions of climatic zones much less relevant. This topic was explored in detail by EFSA PLH Panel (2014) noting that: 'practically all the commercial citrus orchards existing in the EU are irrigated nowadays (Carr, 2012)'. Martínez-Minaya et al. (2015b) included irrigation as an example of a cultural practice for CBS management but did not mention its role in, e.g. extending the duration of leaf wetness.
- Martínez-Minaya et al. (2015b) stated that 'the minimum temperature of the coldest month in grid cells with CBS present ranged from 2.3–11.3°C in 1950 to 0.4–12.9°C at present (Figure 5a)' but they also used the 50-year average to calculate climate zones. Thus, any change in climate during that period will not be apparent.
- While average climate based on 30-year climate normals may be appropriate for determining the distribution of Köppen–Geiger zones, the establishment of a particular pathogen in any area will depend on the variation in the key climatic parameters that are critical for its persistence during the 30-year period. Even if pathogen persistence can be related directly to the climatic variables defining Köppen–Geiger zones, the zone maps do not distinguish those areas where the climatic variables consistently lie within the parameters defining the zones during the 30-year period and those that do not.
- WorldClim (Hijmans et al., 2005) is a 50-year average and interpolates climate using partial thin plate smoothing splines based on the latitude, longitude and elevation of the meteorological station to four different spatial resolutions including the 5-min grid cells used by Martínez-Minaya et al. (2015b). Climate variables are provided for the median elevation in each grid cell based on the Shuttle Radar Topography Mission (SRTM). As noted in EFSA PLH Panel (2014), such elevations will be relevant to areas of citrus production in grid cells with

relatively uniform topography but in areas, like those along many Mediterranean coasts which have only a narrow coastal plain, they may be unrepresentative.

- A key additional challenge when attempting to relate the distribution and prevalence of CBS in South Africa with climate is not only the lack of maps showing the current situation at similar resolutions but also, as noted by Martínez-Minaya et al. (2015b), the extent to which CBS incidence is affected by fungicide spray programmes. Detailed information on disease prevalence, incidence, fungicide use and irrigation would be particularly helpful when comparing the situation in the Eastern Cape Province, the area in South Africa that has the lowest values of summer precipitation and moderate CBS prevalence, with southern Europe.
- In summarising the spread of CBS in South Africa, Martínez-Minaya et al. (2015b) did not take into account the potential adaptation of CBS to other climatic conditions although this might be an important factor with regard to potential future establishment in Europe. EFSA PLH Panel (2014) noted that: 'in the case of *P. citricarpa*, very little information is available regarding diversity in ecophysiological traits and its propensity for adaptation'.

3.1.2.1. Conclusion on the need to update EFSA PLH Panel (2014) with regard to Martínez-Minaya et al. (2015b)

The Panel concludes that the information in the Martínez-Minaya et al. (2015b) paper does not provide new evidence that requires an update to EFSA PLH Panel (2014). This is principally because EFSA PLH Panel (2014) had already concluded that global climate zones are based on factors and thresholds that are very broad and not necessarily representative of the climatic factors that are critical for the pathogen and its host, especially as these can be limited to only short time periods and occur only in the microclimate. The difficulties of defining the Mediterranean climate and thus also the suitability of this climate for *P. citricarpa* were also identified by EFSA PLH Panel (2014). Further issues were identified related to: the influence of irrigation practices and fungicide application on disease prevalence, the long time period covered by WorldClim and the lack of current South African host and pathogen maps at the same resolution as the WorldClim outputs.

3.1.3. Technical hearing with authors of the two papers

The full record of the hearing is given in Appendix A.

3.1.4. Papers published on *Phyllosticta citricarpa*/citrus black spot after EFSA PLH Panel (2014) to 31 March 2016

Since publication of the EFSA PLH Panel (2014) Opinion, a number of papers have been published in refereed journals, conference proceedings and as chapters in edited books. Other material has been placed on web sites, either as unpublished reports or as papers submitted for publication in journals for which no decision has yet been made as to acceptance. The terms of reference for the mandate request that in addition to the two main papers (Magarey et al., 2015; Martínez-Minaya et al., 2015b) for evaluation, other information that has been published should also be considered in relation to the EFSA PLH Panel (2014) Opinion. A literature search identified the publications shown in Appendix B.

Two publications were not evaluated further because their content had already been included in the 2014 EFSA PLH Panel Opinion (Makowski et al., 2014; Perryman et al., 2014).

Other papers covered a wide range of topics relating to CBS and the causal agent *P. citricarpa*. Of these, many had no direct relevance to the conclusions of EFSA PLH Panel (2014): relating to fungicide use or integrated pest management in citrus in South Africa (Carvalho et al., 2015; Grout, 2015; van Zyl et al., 2015; Junior et al., 2016); pathogen identification and/or taxonomy (Wickert et al., 2014; Steffen et al., 2015; Zhang et al., 2015); and one paper on biological control (Fialho et al., 2016). One book chapter (Barkley et al., 2014) addressed concerns of general biosecurity and criticised the EFSA (2008) Opinion; issues which were addressed in EFSA PLH Panel (2014). The PhD thesis from Brazil in Portuguese (Souza, 2015) is concerned with fungicide sensitivity along with the population structure and was not considered relevant to the EFSA PLH Panel (2014) Opinion.

Other publications had some relevance, but not directly to the present mandate: relating to misuse of CLIMEX mapping in the USA (Graham et al., 2014; Yonow and Kriticos, 2014); diagnostic and detection techniques (ISPM 27 (2014; now revoked); Kim et al., 2014; West and Kimber, 2015; Mariduena Zavala et al., 2014); or the implications for the European trade regulations (Laurenza and Montanari, 2014).

As well as Magarey et al. (2015) and Martínez-Minaya et al. (2015b), three other papers have direct relevance for the EFSA PLH Panel (2014) Opinion. Er et al. (2014) reported that the minimum

range (5–11.4°C) was lower than that used by Magarey et al. (2015) (15°C for ascospores and 10°C for pycnidiospores). In EFSA (2008), it was shown that T_{min} was one of the more influential parameters in the infection process (see Table 2, Section 3.5.1).

Dummel et al. (2015) in a Conference Proceedings proposed an ascospore release model that did not use or cite the Fourie model used in both EFSA PLH Panel (2014) and Magarey et al. (2015). Martínez-Minaya et al. (2015a), also in a Conference Proceedings, present a complementary Bayesian analysis of disease spread in South Africa.

Both Dummel et al. (2015) and Martínez-Minaya et al. (2015a) are published as proceedings of different meetings, and while some scrutiny may have occurred, it does not always correspond to a full peer-reviewing process. The Dummel et al. (2015) paper describes a study in Argentina, where the relationship between ascospore release (measured with spore traps) is correlated with environmental data over a 2-year period. Some striking differences in these data (compared to Fourie et al., 2013) include a lack of ascospores trapped at night. The approach by Dummel et al. (2015) was fitted to ordered logistic models to predict ascospore release (grouped as three levels: high, moderate and low) to weather data. Composite variables related to temperature (and its fluctuation) and precipitation recorded during the previous 7 days were the best explanatory variables. A direct comparison with the results of Fourie et al. (2013) is not possible due to differences in the data analyses and these results cannot be substituted in the simulation models presented by EFSA (2008) and EFSA PLH Panel (2014) nor by Magarey et al. (2015). Martínez-Minaya et al. (2015a) analyse the factors associated with spread of *P. citricarpa* with a Bayesian method called integrated nested Laplace approximation (INLA) that is an alternative to Markov chain Monte Carlo methods. Details in this paper were rather sparse and the authors reported that there were different environmental factors that affected spread in the years 1945, 1950 and 2014, and that inclusion of a dispersal kernel gave a better fit for each time period.

Despite the number of scientific papers on citrus black spot published since January 2014, the new biological information provided is insufficient to warrant an update of the EFSA 2014 Scientific Opinion.

3.2. Assessment of papers applying the leaf wetness model and the ascospore maturation model

EFSA PLH Panel (2014) and Magarey et al. (2015) both make extensive use of the generic infection model of Magarey et al. (2005) and the ascospore maturation model of Fourie et al. (2013). Other studies which have cited Magarey et al. (2005) (Appendix C) or develop ascospore maturation models, such as those in Fourie et al. (2013) (Appendix D), have been evaluated for the usefulness, any modifications which have been made and reported limitations in their use. The intention was not to determine whether Magarey et al. (2015) or EFSA PLH Panel (2014) made better use of these modelling approaches, but rather to point out the limitations of both approaches and ways in which these limitations have been addressed in the studies reported.

The generic infection model proposed by Magarey et al. (2005) has been used in a wide range of epidemiological studies at different spatial scales ranging from individual fields to regions, countries and continents; and temporal scales ranging from weeks to months, years and decades. It is particularly applicable for foliar pathogens where cardinal temperature points and relative humidity/leaf wetness requirements largely determine pathogen response. As such, it has been used to assess the risk of pathogen infection in relation to weather, either in relation to disease forecasting for an endemic pathogen or to establishment of a non-endemic, possibly newly emerging pathogen. The Magarey et al. (2005) paper is the most widely used generic infection model that in principle can be adapted to the specific case of a pathogen, if the values and their associated errors are available for the model parameters and the spatial scale over which the model is applicable.

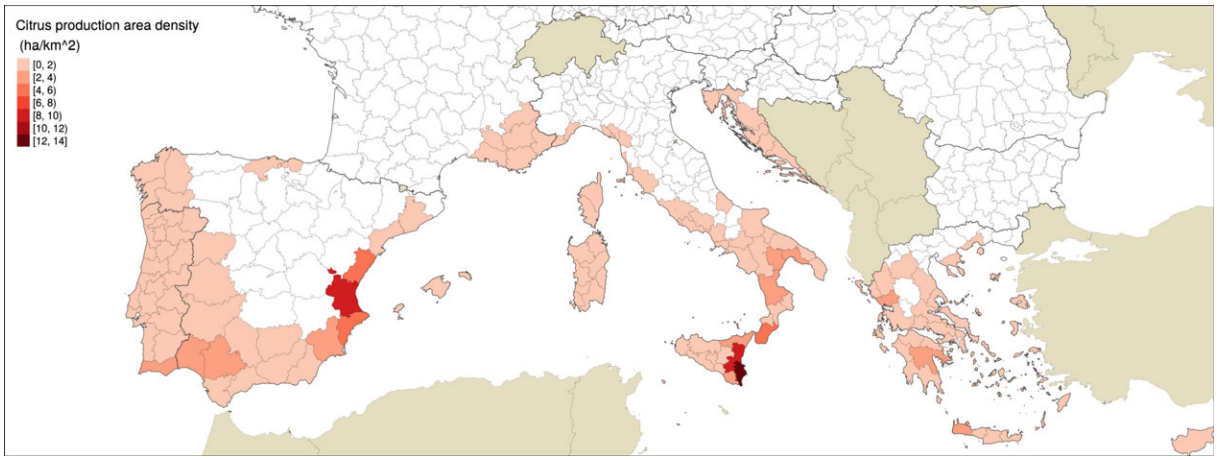
Fourie et al. (2013) developed ascospore maturation models for *P. citricarpa*. Specifically, the availability of ascospores for CBS has been simulated using and combining two equations: a first equation for defining the time when first seasonal ascospores are mature and the second equation for describing the dynamics of ascospore maturation during the season. These two equations have been developed following an empirical approach that has been widely used in the literature for fitting field observations to mathematical equations. The approach used by Fourie et al. (2013) illustrates the limitations of empirical modelling. In this approach, the model simply represents, as best as possible, the field dataset used for model development. Representativeness of this dataset is then critical for developing robust models, i.e. models able to accurately represent the reality in a range of different (and extreme) environmental conditions.

3.3. Comparison of the EFSA PLH Panel (2014) and Magarey et al. (2015) infection model outputs

In this section, the citrus production areas in Europe are shown and overlaid with the areas for which (a) Magarey et al. (2015) provided infections scores, both for ascospores and pycnidiospores and (b) EFSA PLH Panel (2014) identified potential areas of CBS establishment. To obtain sufficient information on probability of establishment of CBS, both ascospore and pycnidiospore infection scores have to be taken into account. A direct comparison of the number of infection periods from the simulations done by EFSA (2008) with the simulations in Magarey et al. (2015) is not possible, because the two sets of outputs represent different scales. Table 2 in Magarey et al. (2015) presents 'infection scores' for both ascospores and pycnidiospores. How the information from infection periods was translated into these scores was not stated even though this is central to their results and conclusions. One can assume that higher values represented higher risk. The maximum value for ascospores was 62.4 and for pycnidiospores it was 185.7. Minimum values were 0 for both spore types. The infection scores were benchmarked against values in South Africa, where there was a moderate level of the presence of CBS (Addo, Eastern Cape Province), and this was used as threshold for establishment. Different conclusions would have been reached if other sites with a lower level of CBS had been chosen as the benchmark. Furthermore, an analysis is provided on effects of rain and lack of rain on the release of ascospores, as there exists different theories on the need of rain events for ascospore release (see Section 3.1.1.2).

3.3.1. Citrus production areas in the EU at locations for which infection scores were predicted by Magarey et al. (2015)

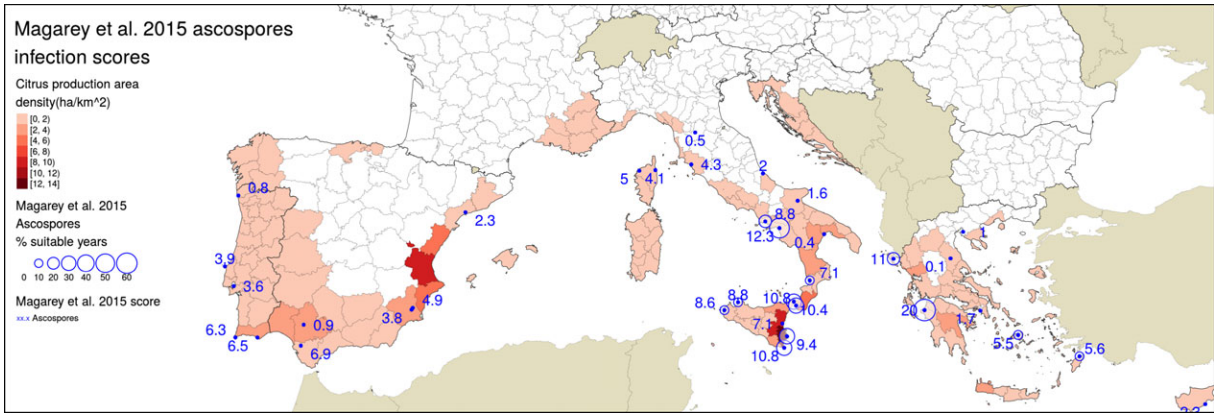
Figure 1 shows the citrus production areas in the EU listed in Appendix E based on Nomenclature of Territorial Units for Statistics 3 (NUTS3) regions colour coded according to the density of citrus production. The citrus production density of each NUTS3 region was calculated by dividing the area of citrus production in each NUTS3 region by the size of the NUTS3 region (density ha/km^2).



Color coding: red and reddish colours refer to different densities of citrus production in EU Member States. White refers to areas in the EU where citrus is not (commercially) produced. Beige represents the non-EU countries. For some NUTS3 regions, data transformations were applied, to ensure that all data followed the same NUTS3 classification: Croatia – the total area of citrus production in Adriatic Croatia was distributed to all NUTS3 bordering the coast; France – the total area (6 ha) of citrus production in Provence-Alpes-Côte d'Azur (FR82) and Corse (FR83) was distributed to NUTS3 regions (FR821-826) and (FR831-FR832) in relation to the area of the regions; Portugal – the data from the 2010 NUTS 3 regions were transferred approximately to the 2013 NUTS 3 regions following the description provided by the European Commission; Spain – the data for ES53 (Madrid) and ES62 (Murcia) (both NUTS2 regions) were distributed to NUTS3 regions according to the size of the area.

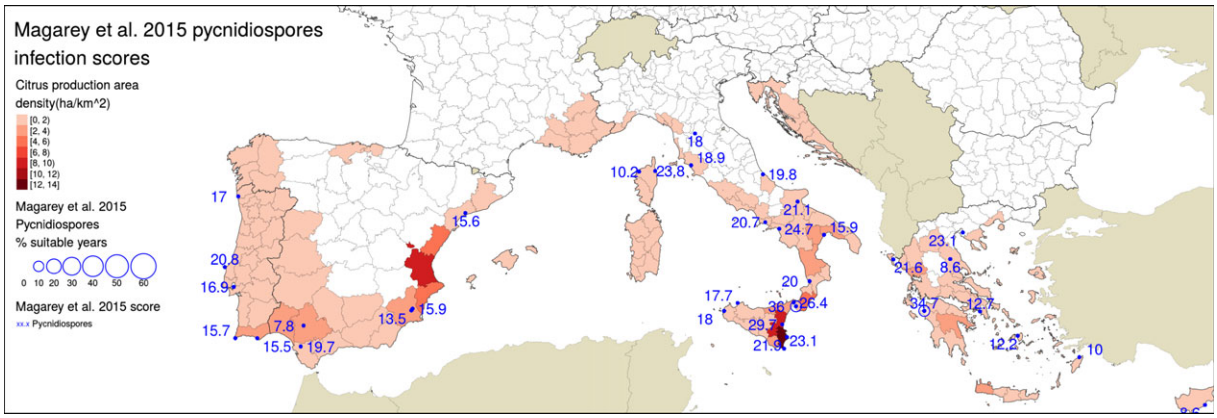
Figure 1: Map of NUTS3 citrus production density in the EU based on citrus production data extracted from national statistical databases of Portugal, Spain, France, Italy, Malta, Croatia, Greece and Cyprus

Figures 2 and 3 show the ascospore and pycnidiospore infection scores, respectively, reported by Magarey et al. (2015) for 33 locations in the EU. The maps indicate that several sites showing high infection scores are located in areas with citrus production, especially in Greece and in Italy, e.g. Andravida in Greece and Reggio Calabria in Italy had high infection scores both for ascospores and pycnidiospores.



The numbers associated with the blue dots indicate the ascospore infection scores reported by Magarey et al. (2015) for 33 (out of 36, since for 2 locations no matching grid cells from EFSA PLH Panel (2014) could be found and 2 locations are inside the same grid cell) locations in the EU, e.g. Andravida, Greece, has an infection score of 20 and was identified as a site of potential establishment. Scores were taken from Magarey et al. (2015), Table 2.

Figure 2: Ascospore infection scores and citrus production density in the EU. Locations are represented by blue dots, circles surrounding these dots present the percentage of suitable years

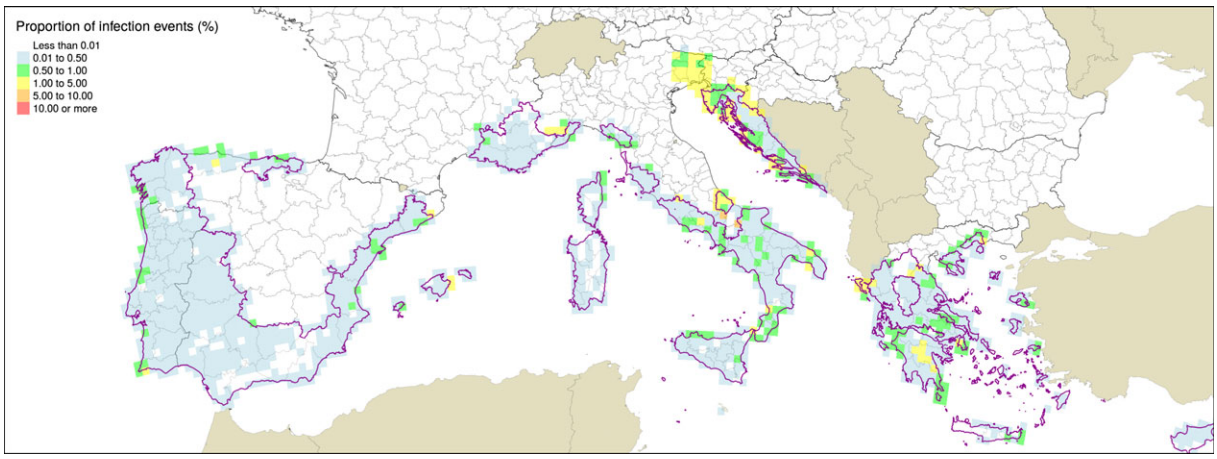


The numbers associated with the blue dots indicate the pycnidiospore infection scores reported by Magarey et al. (2015) for 33 (out of 36) locations in the EU, e.g. Reggio Calabria, Italy, has an infection score of 36. Scores were taken from Magarey et al. (2015), Table 2.

Figure 3: Pycnidiospore infection scores and citrus production density in the EU. Locations are represented by blue dots, circles surrounding these dots present the percentage of suitable years

3.3.2. Citrus production areas in the EU compared with locations for which potential infection events were identified by EFSA PLH Panel (2014)

In Figure 4, the NUTS3 citrus production areas in the EU have been overlaid with the areas identified in EFSA PLH Panel (2014) as being at risk with regard to infection events by CBS. The map reveals that several citrus producing areas in Spain, Italy, and Greece have more than 1% of infection events.

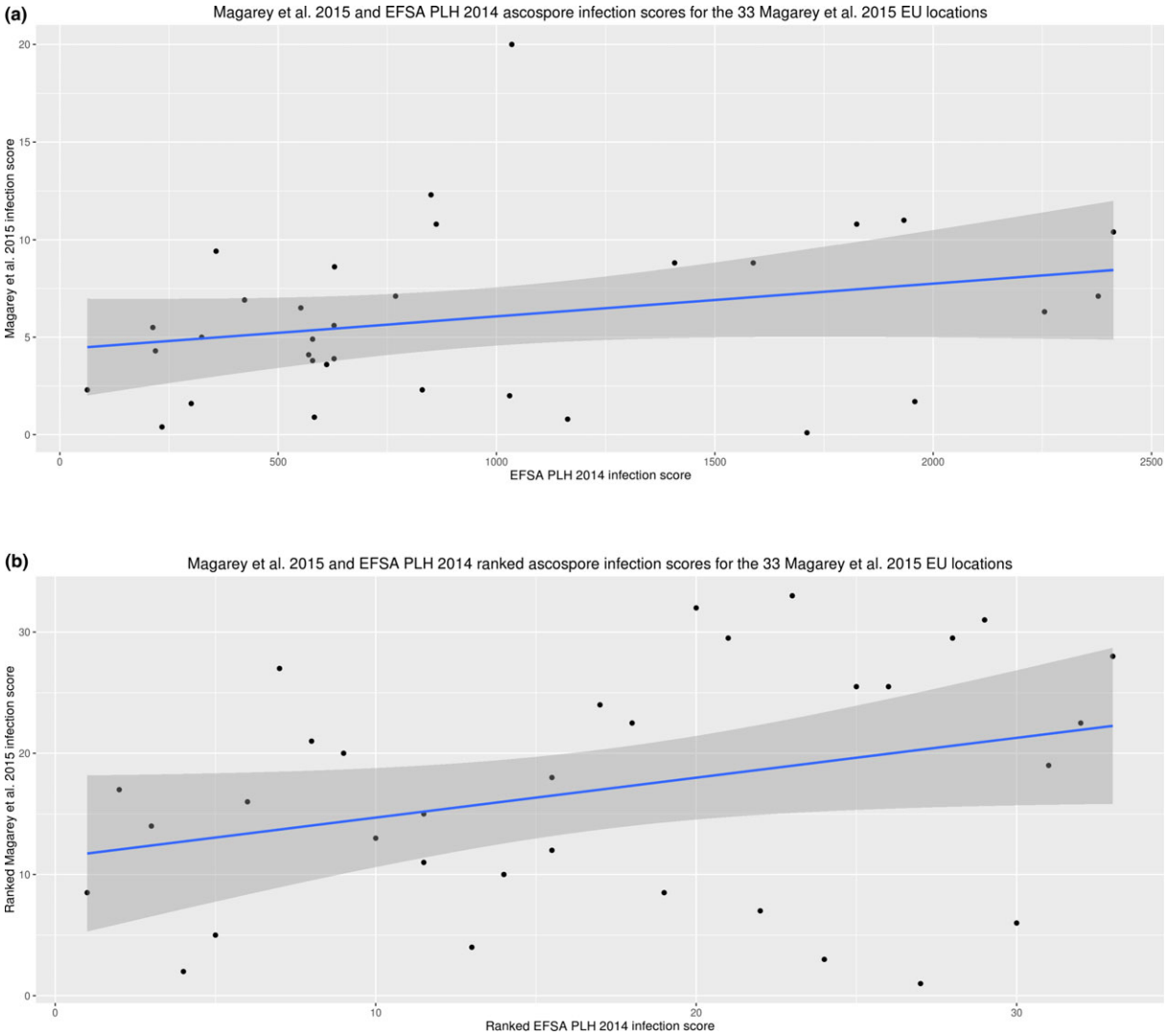


The areas demarcated by the purple lines include all the EU NUTS3 regions that provide citrus production data. The underlying 25 × 25 km grid cells show areas of the EU with greater than 1% citrus tree coverage. The derivation of this grid is described in Annex F of EFSA PLH (2014).

Figure 4: Overlap of EU citrus production regions with areas for which EFSA PLH (2014) predicted potential infections, differentiated into infection events (%), which go up to 5–10% in a few regions in Greece, Italy and Spain. Percentage of hours with weather conditions suitable for successful infection events by *Phyllosticta citricarpa* ascospores (generic infection model for foliar fungal pathogens by Magarey et al. (2005) with D_{50} = 3 h and T_{min} = 15°C), see also EFSA PLH (2014, figure 31)

3.3.3. Relationships between the outputs of the EFSA PLH Panel (2014) and Magarey et al. (2015) infection models

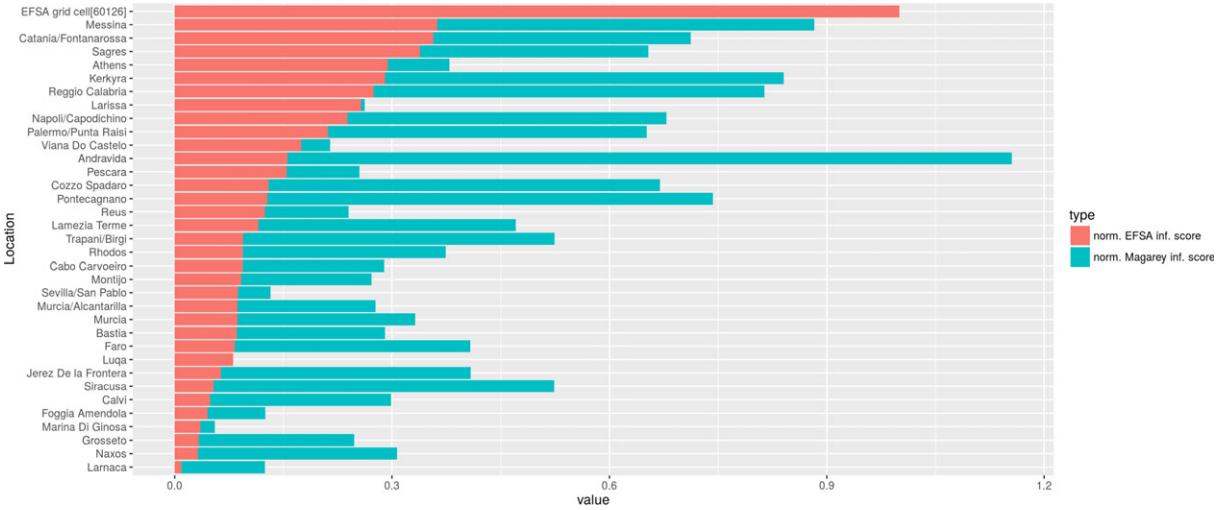
The outputs of the two ascospore infection models are shown in Figures 5 and 6 for the locations that feature in Magarey et al. (2015) to enable a direct comparison. Figure 5 shows the two series of outputs in absolute values (a) and in ranks (b). The absolute values of the infection scores are defined very differently in both papers (EFSA PLH Panel, 2014; Magarey et al., 2015) and are neither defined precisely in the underlying generic infection model (Magarey et al., 2005), so both papers took some freedom to get from the generic infection model to 'infection scores'. In the case of EFSA PLH (2014), information is given on the type of post-processing of the model outputs in the opinion, for Magarey et al. (2015), however, a clear definition of the 'infection scores' was not provided. Furthermore, the Magarey et al. (2015) data is based on different climate data, namely the NCEP CFSR database, while EFSA PLH Panel (2014) is based on the JRC-MARS database. Both use different years as well. Results thus do not reveal any clear relationship between the outputs of the two models. Correlations between absolute output values (Pearson correlation) and between ranks (Kendall and Spearman rank correlations) are not significant ($p > 0.05$). There is thus a low concordance between the two infection models. The plots support and illustrate clearly that the correlation between EFSA PLH Panel (2014) and Magarey et al. (2015) infection scores for the Magarey et al. (2015) locations is very low. This result indicates that the simulated infection scores are highly sensitive to the model assumptions, and that there is a high uncertainty about the true levels of risk of infection. It is striking however that both studies (EFSA PLH Panel, 2014; Magarey et al., 2015) use the same model, but come to uncorrelated infection scores. This shows that the model is not defined in enough detail to allow easy comparisons between different papers using the same model.



Absolute values and ranked scores are compared in a and b, respectively. Pearson, Kendall and Spearman correlation coefficients are not significantly different from zero ($p > 0.05$). Ascospore infection scores obtained with the EFSA PLH (2014) model and with the model of Magarey et al. (2015). In (a), the Magarey infection scores (see table 2 in Magarey et al., 2015), which range between 0 and 20, are plotted versus the EFSA PLH (2014) infection scores, which range between 0 and 2,000. If both values were well correlated, the graph would be monotonously increasing on both axes. In (b), the relative rank of each location is plotted in the two values per location (EFSA PLH Panel, 2014 scores, and Magarey et al., 2015 scores). The rank for both axes is therefore (0, 1, 2 … 33 as there are 33 locations). A perfect correlation would be a diagonal line in a 45° angle going through point 0, 0.

Figure 5: The two series of ascospore infection outputs of the EFSA PLH (2014) model and with the model of Magarey et al. (2015) in absolute values (a) and in ranks (b)

Figure 6 shows a direct comparison of the normalised ascospore infection scores for Magarey et al. (2015) with those obtained in EFSA PLH Panel (2014).



Outputs of the EFSA PLH (2014) model were divided by the maximum value obtained in Europe (the maximum is indicated by the upper red bar). Outputs of Magarey et al. (2015) were divided by the maximum value obtained with this model across the considered EU locations. Outputs of the EFSA PLH (2014) model were ranked in decreasing order.

Figure 6: Normalised values of the ascospore infection scores obtained with the EFSA PLH (2014) model and with the model of Magarey et al. (2015) for different locations in the EU (locations considered in Magarey et al., 2015)

3.4. Evaluation of the survey data on CBS from South-Africa used for validation in Magarey et al. (2015)

CBS was first recorded in South Africa in 1929 in areas around Pietermaritzburg (Doidge, 1929 in Paul et al., 2005) and is known to occur in a number of citrus-producing provinces namely KwaZulu-Natal, Mpumalanga, Limpopo, North West and the Eastern Cape (Carstens et al., 2012). Previously, attempts have been made to map the presence of CBS in South Africa using expert knowledge (Paul, 2006) and field surveys (Carstens et al., 2012). Carstens et al. (2012) provide details of surveys conducted in South Africa between 1995 and 2010 to ascertain the presence or absence of CBS in regions where the disease has not been previously reported, namely the Western Cape, the Northern Cape and the Free State (Carstens et al., 2012).

Initial surveys were conducted in 1995 in the Western Cape and included sampling of 860 commercial orchard trees across 11 magisterial districts. After this time, samples were drawn from both commercial orchard trees and residential trees from home gardens. In each year, one or more provinces were surveyed. Between 1998 and 2002 surveys were conducted in the Northern Cape, from 2002 to 2004 in the Western Cape and Free State, from 2005 to 2006 in the Northern Cape and Free State, and from 2007 to 2010 surveys were conducted in the Western Cape only. The authors detail the differing sample collection, processing and identification methods that occurred over the survey period (Carstens et al., 2012). Following the surveys, the authors concluded that 'the Western Cape, Northern Cape and Free State Provinces can be recognised as CBS pest free areas' (Carstens et al., 2012).

ISPM 4 (1995) provides guidelines on determination of a Pest Free Area and ISPM 6 (1997) provides guidelines on surveillance that can be included in a pest record and used to design specific surveys to ascertain the absence of a pest from an area. This includes guidance on the statistical basis of a survey which includes 'e.g. level of confidence, number of samples, selection and number of sites, frequency of sampling, assumptions'. Carstens et al. (2012) provide detailed information on the timing, location and numbers of sites and samples, although the authors do not use statistical methods to ascertain the level of confidence in their survey data. Due to imperfect sampling and detection methods and an inability to perform a complete census of an area, failure to detect a disease in an area can occur even if the disease is present. Generally, this can arise most often when disease occurs at low incidence. The level of confidence in an absence survey can be ascertained using a range of statistical methods which can use information on the number of samples and sensitivity of detection methods (e.g. Cannon, 2002; Madden et al., 2007). A common approach in plant pathology, based on

binomial sampling theory, is the 'rule of three' (Madden et al., 2007). This approximation from the binomial distribution determines the 95% confidence that, if no disease is found in a survey, the true incidence is less than a certain proportion.

Using the information available in Carstens et al. (2012) and from this statistical inference, levels of confidence can be given to a zero incidence of disease from surveys. For example, the 300 trees sampled in Citrusdal and Clanwilliam districts suggests, using the rule of three, that the true incidence of CBS would be less than 1%. In 2007–2008, larger sample sizes were collected from the Western Cape indicating true incidence would be less than 0.45% if both fruit and leaf samples are combined. However, in 2010, only 42 samples were taken in total and these were concentrated in two districts in the Western Cape only. There is a 95% confidence that the true incidence of CBS is less than 7.1% in these two districts sampled in 2010.

The results from Carstens et al. (2012) suggest that CBS is either absent or at some low level of incidence in the sampled provinces of South Africa in the years surveyed. However, the results should be interpreted in the light of the timings and locations of the surveys which vary throughout the survey period. For example, although the Western Cape included surveyed locations in 2010, the latest surveyed locations reported in the Northern Cape were in 2005. A cursory analysis reveals that in 2010 CBS incidence in the Western Cape is less than, but could be as high as 7.1% even though no disease was found. Lack of information on some aspects of the survey program, e.g. sensitivity and specificity of sampling methods and diagnostic protocols, prevents a fuller interpretation of the statistical basis of the surveys (e.g. level of confidence) as indicated in ISPM 6 (1997).

It should also be noted that assumptions of absence can have implications for model results informed or validated by the presence and absence data. Including information about possible misclassification of disease-present and disease-absent areas could increase the uncertainty of the conclusions of both Magarey et al. (2015) and Martínez-Minaya et al. (2015b). Thus, conclusions of models based on negative data that are not fully evaluated should be caveated when presenting model findings. Moreover, a range of methods have been developed to account of imperfect detection in models of species distribution (Lahoz-Monfort et al., 2014) that should be considered in models of CBS potential distribution.

Hence, it can be concluded that the prevalence of CBS in South Africa might be underestimated – better surveillance schemes could have impact with regard to identifying more areas being infested, with implications also on predictions with regard to Europe. There are implications also for attempts to validate an infection prediction model based on surveillance results, as discussed in 3.5.2.

3.5. Uncertainty and model sensitivity

3.5.1. Uncertainty and sensitivity analyses implemented in the reviewed papers

According to Makowski (2013), uncertainty and sensitivity analysis are two techniques for evaluating models. Although both techniques are often mixed together, they each have a different purpose. Uncertainty analysis (UA) comprises a quantitative evaluation of uncertainty in model components, such as the input variables and parameters for a given situation, to determine an uncertainty distribution for each output variable rather than a single value (Vose, 2000; De Rocquigny et al., 2008; Makowski, 2013). UA aims to answer the following question, 'what is the uncertainty associated with the output resulting from the uncertainty associated with the inputs?'. For example, a biological or environmental process can be inherently variable and this variability can be known and quantified. If this is included in the model, we can analyse the uncertainty this introduces to the model output. Analysis of uncertainty would identify how often and how closely the results of the model would predict what would happen in real life.

The main purpose of sensitivity analysis (SA) is to determine how sensitive the output of a model is with respect to elements of the model. The objective of a SA is to rank inputs according to their influence on the output. SA can be seen as an extension of uncertainty analysis. Its purpose is to answer the following question 'What are the most important uncertain inputs?'. SA seeks to identify which parameters are the most influential in affecting the results, allowing to analyse the consequences of being wrong about the values of the input parameters. The model output may be highly sensitive to the precise value of some parameters, but insensitive to others. Sometimes, SA is also used for a more general purpose, such as to understand how the model behaves when some input or parameter values are changed.

Methods of UA and SA implemented in EFSA (2008), EFSA PLH Panel (2014), Magarey et al. (2015), and Martínez-Minaya et al. (2015b) are briefly presented in Table 2.

Table 2: Methods of uncertainty and sensitivity analyses implemented in EFSA (2008), EFSA PLH Panel (2014), Magarey et al. (2015) and Martínez-Minaya et al. (2015b)

Reference	Model	Uncertainty analysis		Sensitivity analysis	
		Method	**Results**	**Method**	**Results**
EFSA (2008)	CLIMEX	None	None	Sensitivity analysis of the CLIMEX ecoclimatic index (EI) to the types of climatic input data (three different datasets). Sensitivity to parameter values was not analysed. See p. 28–34	The number of locations with EI > 5 (marginally suitable for CBS) depended on the climate datasets. With recent European data, some parts of the EU citrus production area were found 'marginally suitable for disease development'
EFSA (2008)	Simple infection model with interpolated climatic data in the EU	None	None	Sensitivity of the model outputs to the value of the parameter D_{50} (three different values were tested, 0, 3, and 14 h). See p. 43–44	Even using the most conservative value of D_{50} (0 h), potential infection events were still predicted to occur in the EU
EFSA (2008)	Infection model with data from agrometeorological stations	UA of the model outputs to the parameter values. The model was run using 48 combinations of parameter values and the model output distribution was summarised by its minimum, maximum and median values. See P50–60	Uncertainty was large. In all stations, but two, the maximum simulated percentage of time with potential successful infection by ascospores was higher than 20% for at least 1 month. The minimum percentage was always equal or close to zero	A global SA was performed in order to rank five uncertain model parameters according to their influences on the model outputs. See p. 52 and 54	The most influential parameters were D_{50} and T_{min}. The parameters T_{opt}, W_{max} and W_{min} had only a small effect on the model outputs
EFSA PLH Panel (2014)	1) Pseudothecium maturation and ascospore release simulations using the model of Fourie et al. (2013) 2) Infection model (model used in EFSA 2008)	None	None	The sensitivity analysis done in EFSA (2008) is mentioned. See p. 85 and 97	The results obtained in EFSA (2008) are summarised. See P97
EFSA PLH Panel (2014)	CLIMEX	None	None	SA of the ecoclimatic index to the spatial and temporal resolution of the climatic data inputs	For some of the EU citrus-growing areas, the climatic suitability classification varied from 'marginally suitable', through —'suitable' and even to 'highly suitable' based on the classification of the EI by Yonow et al. (2013) when changing either the spatial resolution or the temporal period of the climate data inputs

Reference	Model	Uncertainty analysis		Sensitivity analysis	
		Method	Results	Method	Results
Magarey et al. (2015)	Infection model	No formal uncertainty analysis was performed, but the model outputs were compared to observed prevalence. See comments below	None	None. Some results of the SA performed by EFSA (2008) are mentioned	None
Martínez-Minaya et al. (2015)	Comparison of climate conditions in different regions	None	None. The ranges of the means of the critical values in the Köppen–Geiger analyses were presented	None	None

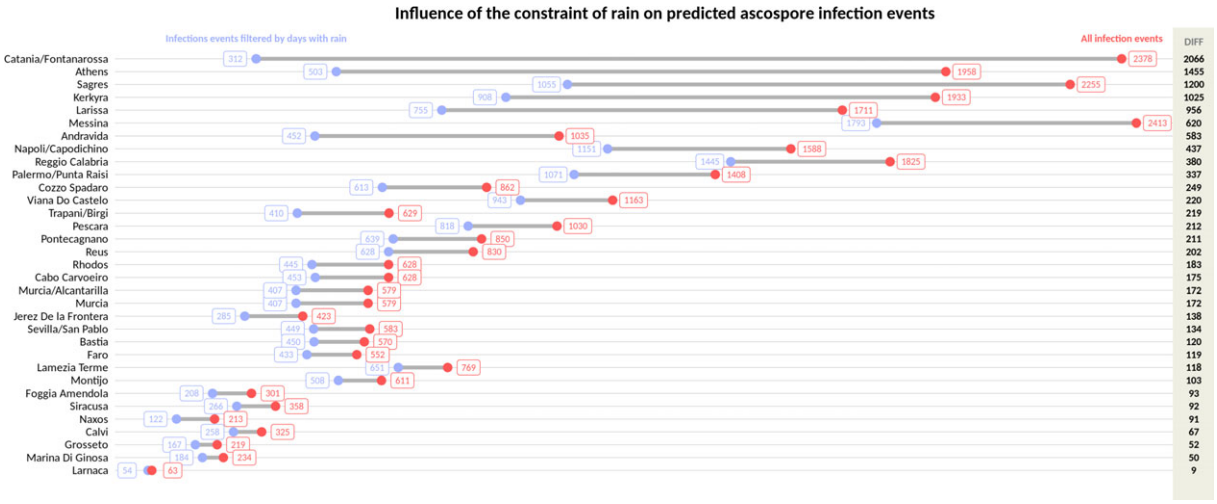
D_{50}: duration of a dry period at relative humidities < 95% that will result in a 50% reduction in disease compared with a continuous wetness period; EU: European Union; SA: Sensitivity analysis; T_{min}: minimum temperature; T_{opt}: optimum temperature; UA: Uncertainty analysis; W_{max}: maximum value of the wetness duration requirement; W_{min}: minimum value of the wetness duration requirement.

Table 2 indicates that the outputs of the infection model used by EFSA (2008) and by Magarey et al. (2015) were sensitive to the values of several parameters (especially, T_{min} and D_{50}). EFSA (2008) performed an UA with the infection model and found the uncertainty was large. Table 2 also indicates that the outputs of CLIMEX were influenced by the temporal and spatial resolution of the climate input data. In Magarey et al. (2015), no SA and no UA were performed. In this paper, the parameters were set equal to fixed values and their influences on the conclusions were not studied. Martínez-Minaya et al. (2015b) did not perform any UA and SA either. In particular, the authors did not consider the risk of misclassification of locations reported as disease free. False negatives (infected locations classified as 'disease free') could theoretically lead to an underestimation of the prevalence of CBS. This would decrease the sensitivity of the proposed classification (probability that the disease was considered absent in locations where it is actually present).

3.5.2. Sensitivity of the infection model output to effects of rain on the release of ascospores

The sensitivity of the infection model outputs was tested to the assumption that ascospore release is triggered by rain or not. To do that, (i) the numbers of infection events simulated on days with rain and (ii) the numbers of infection events simulated on days without rain (Appendix F) were calculated separately. Infection events are summed over the whole time period for each of the EU sites considered in Magarey et al. (2015).

Results indicate that the number of infection events occurring on days without rain is higher than 500 in several EU sites (Figure 7). The total number of infection events occurring on days without rain is equal to 241,928 when summed over all locations. If these events are added to the infection events occurring during rainy days (457,404), the total number of infection events will increase by more than 50%. The simulated number of infection events is thus highly sensitive to the assumption that ascospore release is triggered by rain. If wrong, this assumption leads to an underestimation of the number of infection events in the Magarey et al. (2015) model.



Color coding: blue ones are the infection events filtered by days with rain and the red ones are all infection events.

Figure 7: Total numbers of infection events filtered by events on days with rain versus non-rain. Infection events were obtained for each EU location considered in Magarey et al. (2015) by matching its location with the corresponding EFSA PLH 2014 model grid cell

3.5.3. Note on the validation procedure used by Magarey et al. (2015)

In Magarey et al. (2015, table 2), the authors compared the outputs of their models (ascospore and pycnidiospore infection scores) to the prevalence levels (Absent, Low, Moderate, High and Endemic) observed on 17 sites located in Australia and in South Africa. This analysis suffers from several limitations:

1) Magarey et al. (2015) did not precisely explain how the prevalence levels (Absent, Low, Moderate, High and Endemic) were defined.
2) The authors did not calculate any evaluation criterion to quantify the performance of the epidemiological model (the values of mean absolute error and of mean error reported in section 2.2 of Magarey et al. (2015) refer to the weather inputs, not to the epidemiological model outputs).
3) The number of observed prevalence levels used to assess the model outputs is very small (nine sites with moderate, high or endemic prevalence levels, and eight sites with low prevalence or absence of disease).

Concerning issues (2) and (3), several quantitative criteria could have been used to assess the epidemiological model from the categorical observed prevalence, especially the sensitivity, specificity, and area under the receiver operating characteristic (ROC) curve (AUC). Such criteria are frequently used to assess performances of pest models (e.g. Dupin et al., 2011 http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0020957). These criteria were estimated by the EFSA Panel on Plant Health (PLH Panel) in order to quantitatively assess the model outputs (ascospore and pycnidiospore scores) (Table 3). The observations of disease prevalence reported in Magarey et al. (2015) were split in two categories; the sites with moderate to endemic levels on one hand, and the sites with absent to low levels on the other hand. The capabilities of the ascospore and pycnidiospore infection scores to discriminate between these two categories were assessed using several standard criteria (sensitivity, specificity and AUC) computed with the R and winBUGS software (code available, see Appendix G).

Table 3: Results of the evaluation of the ascospore and pycnidiospore infection scores using the data reported in Table 2 of Magarey et al. (2015). Values of AUC, sensitivity and specificity estimated using both a frequentist method and a Bayesian method (in italics). The 95% confidence interval (for frequentist estimates) and credibility interval (for Bayesian estimates, in italic) are reported when available. Sensitivity and specificity were computed using thresholds equal to 13.4 and 48.4 for ascospore and pycnidiospore infection scores, respectively (as indicated in Magarey et al., 2015)

Criteria	Ascosporic score	Pycnidiosporic score
AUC	0.96 (0.89–1)	1 (–)
	0.95 (0.73–1)[(a)]	*0.96 (0.76–1)*[(a)]
Sensitivity	1 (0.66–1)[(b)]	0.89 (0.52–1)[(b)]
	0.93 (0.69–1)[(c)]	*0.84 (0.56–0.98)*[(c)]
Specificity	0.88 (0.47–1)[(b)]	1 (0.63–1)[(b)]
	0.82 (0.52–0.97)[(c)]	*0.93 (0.66–1)*[(c)]

AUC: area under the curve.
(a): Calculated using the method described in O'Malley et al. (2001).
(b): Calculated using the method of Clopper and Pearson (1934).
(c): Calculated using the Beta-Binomial model described in EFSA PLH Panel (2012).

If the values of sensitivity, specificity and AUC reported in Table 3 were perfectly estimated, they would indicate that the classifications derived from the epidemiological model were very good (because all criteria were close to one). However, the estimated values of the evaluation criteria are in fact very inaccurate. For example, the 95% confidence interval of the sensitivity calculated for the ascosporic score is [0.66, 1]. Its lower bound is thus much lower than 1. This result indicates that there is a high uncertainty about the actual performance of the model outputs. Because of this high uncertainty, we cannot exclude that 34% of the model classifications correspond to false negatives. In this case, 34% of the situations classified in the 'absent-low prevalence' category by the ascosporic score should in fact be classified in the 'moderate-endemic' category. These results do not necessarily mean that the model performs poorly, but they indicate that the uncertainty about the performance of the model of Magarey et al. (2015) is high due to the limited number of data used to evaluate its performance.

3.5.4. Towards ensemble modelling

In the EFSA (2008) analysis, an UA was done with 48 combinations of key parameters. If the key parameters for a dynamic simulation model via sensitivity analysis have been identified, and the actual value of the parameter in question is unsure, some idea as to the variability of the model output (its uncertainty) can be obtained by performing different model runs with a range of different values for these most important parameters. An 'ensemble' is where a single model is run a number of times using different initial conditions, parameter values, or model assumptions. The range of outcomes from these different ensembles allows a quantitative assessment of the range of possible behaviours. A 'multimodel ensemble' is a range of simulations from different models and different parameterisations and is termed 'ensemble modelling'. This range of possible outcomes is the explicit output of ensemble modelling (Araújo and New, 2007; Grenouillet et al., 2011).

Ensemble modelling is standard practice in many fields, including climate modelling (Thomson et al., 2006; Tebaldi and Knutti, 2007; Rodó et al., 2013), and in epidemiology the use of ensembles of epidemic models is now also being used to make improved predictions and to assess the potential outcome of control interventions (Cameron et al., 2015; Lindström et al., 2015; Villaverde et al., 2015). For example, Smith et al. (2012) used 14 different individual-based simulation models of the causal agent of malaria to assess vaccination programmes. The authors suggested this approach provided more secure conclusions than could have been obtained via the use of a single model. Lindström et al. (2015) use a weighted method for ensemble predictions of the 2001 UK Foot and Mouth epidemic.

In epidemiology, different models include different assumptions and different formulations of the processes that drive an epidemic and ultimately determine establishment and spread. As a result, these different models have different predictions which illustrate the inherent uncertainty arising from incomplete knowledge when assembling a model. To assess this uncertainty a multimodel ensemble can be run and the predictions combined. Ensemble modelling of epidemics is a compelling approach

where there is a lack of consensus between models due to structural uncertainty (i.e. where multiple assumptions can be justified) or differences in data calibration (Cameron et al., 2015). This approach allows for these type of uncertainties to be quantified and provides a better analysis of best- and worst-case scenarios.

4. Conclusions

4.1. Comparison of EFSA PLH Panel (2014) and Magarey et al. (2015)

The two studies show several similarities. Both basically used the same equations and parameter values to simulate infection by ascospores and by conidia. Both studies also used the Gompertz equation of Fourie et al. (2013) to describe the dynamic of ascospore production. However, the two studies show several differences concerning:

- the type of weather data used as model inputs and how it was used in the model (such as calculation of VPD);
- the temporal and spatial resolution of the simulations (lower numbers of years and locations were considered in Magarey et al. (2015));
- the biofix (i.e. when temperature summation begins) for the start of ascospore season;
- the method for estimating ascospore release;
- the type of output variables considered, and their post-processing, to assess the risk of establishment.

The number of sites showing high infection scores was lower in Magarey et al. (2015) than in EFSA PLH Panel (2014), but this result does not necessarily indicate a lower risk at the European scale because of the aforementioned assumptions in the simulation by Magarey et al. (2015). In addition, there were a smaller number of locations and years in that study compared to the gridded climatic data used by EFSA PLH Panel (2014), and no rationale was given for the choice of locations.

Although Magarey et al. (2015) and EFSA PLH Panel (2014) used very similar model equations and parameter values, it was found that the two series of model outputs were not significantly correlated. This opinion also shows that small differences in the model assumptions can have a strong impact on the model outputs. In order to improve the comparability of different modelling studies performed on CBS, it will be useful to share standard sets of weather data, and to analyse the consequences of different assumptions made by the models using an ensemble approach.

4.2. Comparison of EFSA PLH Panel (2014) and Martínez-Minaya et al. (2015b)

The approach applied by Martínez-Minaya et al. (2015b) was not based on the use of an infection model. Martínez-Minaya et al. (2015b) analysed the suitability of the climates present in the Mediterranean Basin for CBS using the Köppen–Geiger zones and the Aschmann's classification criteria. The authors found that the climates of several areas located in the southern Europe were suitable for CBS. There appears to be a strong overlap between the area reported as suitable by Martínez-Minaya et al. (2015b) and the area potentially suitable for CBS infection according to EFSA PLH Panel (2014), especially when the citrus production area is taken into account. However, EFSA PLH Panel (2014) had already concluded that global climate zones are based on factors and thresholds that are very broad and not necessarily representative of the climatic factors that are critical for the pathogen and its host, and hence for establishment.

4.3. Implications of issues raised by Martínez-Minaya et al. (2015b) as they affect Magarey et al. (2015)

Martínez-Minaya et al. (2015b) pointed out that CBS has spread in South Africa from the initial points of introduction. The current information about the distribution may not represent the full extent of spread in the country. This may not only be due to climatic restrictions, but also could be due to legislation restricting movement of infected citrus and citrus planting material. They were not able to quantify, however, how much climatic conditions and quarantine regulations have contributed to the current distribution. If CBS has not reached its maximum distribution in that country, it would imply that some locations that are reported as free from the disease may still be suitable for the establishment of *P. citricarpa*. This, in turn would have implications about the conclusions reached by

Magarey et al. (2015), as this study used the current presence and absence of CBS as a factor in the validation of their model.

4.4. Conclusion on the need to update EFSA PLH Panel (2014)

The Panel concludes that the evidence presented by Magarey et al. (2015) does not require an updating of EFSA PLH Panel (2014). The two approaches share a number of similarities but make some different biological assumptions that are identified above. The paper by Magarey et al. (2015) concerns the risk of establishment of CBS in different citrus producing regions of the world, including the EU. The EFSA PLH Panel (2014) conclusion of moderately likely probability of establishment was based on the existence of favourable conditions in the risk assessment area for inoculum production and infection which is not affected by Magarey et al. (2015) who show that establishment is possible in some of the locations that they selected in the EU. The high level of uncertainty surrounding the probability of establishment is also unchanged by Magarey et al. (2015) (see Section 3.5 on uncertainty).

The Panel concludes that the information in the Martínez-Minaya et al. (2015b) paper does not provide new evidence that requires an update to EFSA PLH Panel (2014). This is principally because EFSA PLH Panel (2014) had already concluded that global climate zones are based on factors and thresholds that are very broad and not necessarily representative of the climatic factors that are critical for the pathogen and its host.

Accordingly, despite the number of scientific papers on citrus black spot published since January 2014, the new biological information provided is insufficient to warrant an update of the EFSA 2014 Scientific Opinion.

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Abbreviations

AUC	area under the curve
CBS	citrus black spot
CFSR	Climate System Forecast Reanalysis
D_{50}	duration of a dry period at relative humidities < 95% that will result in a 50% reduction in disease compared with a continuous wetness period
D	hourly developmental rate, estimated from a temperature response function (originally developed for crop modeling) using three parameters: minimum temperature (T_{min}), optimum temperature (T_{opt}) and maximum temperature (T_{max})
D_{Mojt}	days with more than 10 h of leaf wetness, which counts the days of total wetting caused either by rain or by dew
EI	ecoclimatic index
INLA	integrated nested Laplace approximation
JRC	Joint Research Centre
MARS	Monitoring Agricultural Resources
MYCFS	MARS Crop Yield Forecasting System
NCEP	US National Centers for Environmental Prediction
NUTS	Nomenclature of Territorial Units for Statistics
PAT	proportion of ascospores trapped
PLH Panel	EFSA Panel on Plant Health
ROC	receiver operating characteristic
SA	sensitivity analysis
SRTM	Shuttle Radar Topography Mission
T_{max}	maximum temperature
T_{min}	minimum temperature
T_{opt}	optimum temperature
UA	uncertainty analysis
VPD	vapour pressure deficit
W_{max}	maximum value of the wetness duration requirement
W_{min}	minimum value of the wetness duration requirement (minimum hours of leaf wetness required to cause 5% severity or 20% incidence at optimum temperature)

Appendix A – Technical hearing with Antonio Vicent on the paper Martínez-Minaya et al. (2015b) and with Paul Fourie and Roger Magarey on the paper Magarey et al. (2015) (Annex to the Minutes of the 4th meeting of the Working Group on Citrus Black Spot on 26 January 2016)

The full minutes have been agreed by all participants to the technical hearing and can be found at: http://www.efsa.europa.eu/sites/default/files/wgcitrusblack.pdf

Antonio Vicent – Questions and Answers

1) Could you please justify the use in your paper of two systems for climatic classification: Köppen–Geiger and Aschmann? Explain differences and criticisms.

The scope was to check the statement that 'CBS does not occur in Mediterranean climates' (e.g. Kotzé, 2000; Yonow et al.*, 2013). How could Mediterranean climates be defined? Our study shows that in the Köppen–Geiger Mediterranean-type climates, CBS is absent in South Africa, as well as in the Aschmann's Mediterranean-type climate. However, in climates similar to those occurring in the Mediterranean Basin, CBS is present in South Africa. Therefore, these two different systems (Köppen–Geiger and Aschmann) were used and compared.*

Described parameters in the Köppen–Geiger system are e.g.:

- *mean temperature in hottest month and how that is defined exactly (as given in paper) (hottest month Thot ≥ 22; this is to differentiate between the two Mediterranean types, Csa and Csb. See Table 1 for Mediterranean-type (Cs) classification);*
- *parameters to be met specified for Mediterranean climates.*

Described parameters in the Aschmann system are e.g.:

- *used in other studies, e.g. Plos One paper (Klausmeyer and Shaw 2009);*
- *Parameters outlined.*

Köppen–Geiger maps were shown for 1950 and for 2014 (South Africa and the Mediterranean Basin; overlaid with CBS areas for South Africa), Aschmann maps were shown for South Africa and the Mediterranean Basin (again overlaid with CBS areas for South Africa).

A comparison of the maps shows that climatic areas where CBS occurs in South Africa nowadays are also found in the Mediterranean Basin.

Additional question by the WG: Based on your statement in the paper that 'The strong spatial autocorrelation detected in the current CBS distribution data (…) suggest that climate itself might not be the main factor limiting the spread of CBS in South Africa', do you think that CBS is absent from areas of South Africa because it did not arrive yet or because these areas are not suitable?

Maps of prohibited movement of citrus plants in South Africa suggest that it is the related dispersal constraints that have limited spread, not climatic suitability.

In fact in the paper, this issue is discussed in detail, stating the following:

'In general, the potential for natural spread of CBS by P. citricarpa ascospores and conidia is poorly understood'.

'Although the origin of CBS introductions remains generally unknown, human-assisted movement of infected plant material is considered the most important means of disease spread'.

'The introduction of citrus plants into the Western Cape, Eastern Cape and Northern Cape provinces was banned by this phytosanitary regulation (Anonymous 1984)'.

'The movement of citrus plants from KwaZulu-Natal, Mpumalanga, Gauteng, Limpopo, North West and Eastern Cape to the Western Cape, Northern Cape and Free State was banned due to CBS. Within the Western Cape, the movement of citrus plants was also banned from the easternmost to the westernmost magisterial districts due to CBS (Anonymous 2002, 2005a, 2005b; DAFF 2009)'.

'The strong spatial autocorrelation detected in the current CBS distribution data (…) suggest that climate itself might not be the main factor limiting the spread of CBS in South Africa. However, further modelling studies are necessary to weigh the relative contribution of environmental variables and spatial effects in disease distribution'.

'Among the ten climates present in citrus-growing areas in South Africa, the only ones where CBS was not detected were the Mediterranean-type Csa and Csb as well as the BWk arid cold dessert (Figures 2a and 4). However, these three climates together represented only about 13% of the citrus

area in the country and are restricted to locations in the Western Cape and Northern Cape furthest from CBS-affected areas (> 450 km)'.

'The introduction of citrus plants into the Western Cape, Eastern Cape and Northern Cape provinces was banned by this phytosanitary regulation (Anonymous 1984)'.

'The movement of citrus material in South Africa was not regulated until 1984, but quantitative trade data among provinces was not found. In any case, it seems conceivable that larger amounts of plant material were moved from CBS-affected areas to nearby regions than to distant provinces. Consequently, the potential for introduction might have been higher in regions adjacent to CBS-affected areas'.

2) Can the conclusion in your paper that 'CBS expanded in South Africa from its original geographic range in summer rainfall areas to arid regions in the nearby provinces of Limpopo and the Eastern Cape' be linked to the increase in citrus-growing areas and the use of irrigation?

Irrigation is required in South Africa (as well as worldwide) for commercial citrus production.

Pre-1970s/1980s = flood and furrow irrigation

Post-1970s/1980s = drip and sprinkle irrigation (mainly drip used worldwide)

In the 1990s, new export markets for South Africa citrus (e.g. EU Mediterranean countries) were opened, the citrus-growing area increased

From 1950 to 2014, there was an increase in South African citrus distribution and also in CBS prevalence (new improved data set of CBS distribution was assembled based on the Land Cover 2013–2014 GIS from the Ministry of Environmental Affairs of South Africa, with similar results to those published by Martínez-Minaya et al.*, 2015).*

CBS expanded into wet regions but also nearby dry regions from 1950 to 2014 (figure shown as in paper Martínez-Minaya et al. *2015, Figure 4), particularly into Arid Steppe areas (Limpopo and Eastern Cape provinces). A histogram of CBS and summer rainfall (BIO_{18}, precipitation of warmest quarter) indicates that from 1950 to 2014 CBS expanded into drier regions. As indicated before, dispersal constraints due to phytosanitary barriers may have also influenced distribution (via spread).*

See also the paper Martínez-Minaya et al. *2015, stating 'Furthermore, citrus areas in South Africa increased from 28,900 ha in 1961 to 73,900 ha in 2012 (FAO 2014) and regions in the Northern Cape province were not even cropped with citrus in 1950 (Reuther* et al. *1967)'.*

3) Did you verify the quality of the data from the WorldClim database by comparing with stations data?

WorldClim is widely used (4,754 articles available). It provides interpolated data from weather stations (high density of stations in South Africa).

Station data are available (Agricultural Research Council, South Africa) but for different timeframes and locations than used by WorldClim so it is difficult to compare. Some stations in areas of the Eastern Cape and Limpopo showing more outlying data were checked. Data were in line with those from WorldClim although no formal validation was conducted. Therefore, WorldClim was trusted.

4) The WorldClim data set is monthly data at 5 arc-min spatial resolution. Do you think that the selected resolution was suitable for describing the climatic variability of the growing areas? Is there sufficient spatial and temporal resolution to capture the dynamics of the disease?

Data used for climate were a 50-year monthly average from 1950 to 2000.

Previous studies have used a 30 arc-min resolution (Paul, 2006, Yonow 2013). WorldClim goes to 30 s (~1 × 1 km) but by interpolation. Considering the extent of the study, this high spatial resolution could be computationally problematic in further modelling studies.

A resolution of 5 arc-min (~10 × 10 km) is more computationally efficient and is in line with other biogeographical studies (Franklin 2010). It is adequate to describe climatic variability of the citrus areas in South Africa and the two temporal scenarios available (1950–2014), with a trade off with computational time and accuracy. Data from WorldClim can be downloaded at 30″, 2.5′, 5′ and 10′ (http://www.worldclim.org/current).

As stated in the paper 'This preparatory work was part of a larger modelling project where the potential geographical range of CBS will be estimated based on relevant environmental variables and spatial effects'.

5) How large were the relevant squares in the spatial autocorrelation analysis?

Spatial resolution 5 arc-min (~10 × 10 km). Only citrus areas were considered in the spatial autocorrelation analysis.

Moran's Index at different distances was analysed and is available for future work. The analysis showed that clustering was evident at a range of spatial scales.

Roger Magarey (RM) and Paul Fourie (PF) – Questions and Answers

1) What is the rationale for requiring 0.2 mm rain to trigger an ascospore infection period?

Answer RM: While ascospores were trapped in the absence of measured rainfall (Fourie et al.*, 2013), other studies found that rainfall was a requirement for ascospore release (Kotzé, 1963; McOnie, 1964; Lee and Huang, 1973). The 0.2 mm threshold was based on* Gibberella zeae *because no threshold was available for P. citricarpa. This is a small amount of rainfall. Some measurable rainfall makes infection more effective.*

Additional question by the WG: Some papers indicate that rainfall does not have an effect. What is your opinion on not considering ascospore in no rain situations?

Answer RM: Given that so much rain is needed for infection (in order to cause such long-lasting leaf wetness as required by the leaf wetness duration parameters in the infection model for P. citricarpa), requiring 0.2 mm rain is not consequential to the model. I cannot remember if we tried with and without rain but my opinion is that it would have a negligible effect.

PF informed the WG that the 0.2 mm rain was a trigger to the infection model, not ascospore dispersal. In Magarey et al.*, 2015, ascospore dispersal was predicted by the Fourie-ascospore dispersal model, which was driven by degree days accumulated on wet/moist conditions (DDwet accumulated when > 0.1 mm rain or < 5 hPa VPD conditions).*

Additional question by the WG: Please comment on the probability that infection could also happen with dew, no rain.

Answer RM: My opinion is that dew would not be sufficient to sustain enough infection/wetness period but this could be looked at in a model.

2) Furthermore, precipitation requirement is allowed to accumulate over 2 h. Does this mean 0.2 mm/h for 2 h or 0.2 mm total in 2 h?

A precipitation total of 0.2 mm in 2 h is needed to start an infection event. This is a very small amount of rain.

Additional question by the WG: This is such a small amount that it is below the threshold able to be measured.

Answer RM: From memory 0.2 was a minimum.

Answer PF: Simulated weather data was used; hence the mechanical measurement concern is inconsequential.

3) Why were 527.3 degree days (DDtemp) selected in Magarey et al. (2015) for initiating the calculation of infection periods? It seems there is no evidence in Fourie et al. (2013) for using that as a threshold. Can you provide/indicate the evidence for the choice of this value?

Answer RM: A value of 527.3 degree days was used because this represented the lowest DD value when ascospores were observed in field. This serves as a substitute for the observed first ascospore. I apologise this value does not appear to be in Fourie et al. *(2013). PF advised that this figure be used as it represents the lowest DD value when ascospores are observed.*

Answer PF: Yes, that was an absolute minimum, so that the model would not miss any ascospores dispersal events. The 1st percentile was 768.3°C (Fourie et al.*, 2013); this may have been more realistic.*

Additional comment by the WG: The distribution should be used rather than the minimum, or at least a min–max range (to avoid cutting off the maturation curve too early and to avoid anticipation of the end of the ascospore season).

Answer PF: But the ascospores dispersal model accurately predicted the initial lag phase. RM: It may have been a problem in warm sites (extreme sites such as Darwin Australia) but not a problem in less warm, i.e. Mediterranean climates. PF referred the WG to the delta-proportion of ascospores trapped (delta-PAT) results in the article, which at no site reached 1.0, indicating that the dispersal curve was not ended too early.

Additional question by the WG: What is the effect of irrigation? It should provide enough humidity? This is an uncertainty.

Answer PF: Yes, but in general citrus orchards are all irrigated so it should be a normalising factor.

Additional question by the WG: But it will be more of a factor in dry climates than wet climates.

Answer PF: Positive and negative control sites with dry/arid climates were included in our study and so this concern has been captured and thus it should be a normalising effect.

4) Why was the T-model applied in the Fourie et al., 2013 assessment not used in the model in Magarey et al., 2015?

Answer RM: The use of the T-model might have improved the Magarey-2015 model. However, the dispersal model fairly accurately predicted the lag phase following actual and predicted onset of dispersal biofix.

5) Why are there moving averages for proportion of ascospores trapped (PAT)? Does this reduce the opportunity to have days (or periods) with PAT = 0?

Answer RM: A moving average smoothes the data and increases the number of days when PAT > 0.

Additional question by the WG: Using a 7-day moving average should reduce the number of days with PAT = 0?

Answer RM: It increased the number of days with PAT > 0, i.e. we are in agreement. Moving average is used because a weekly PAT is more accurate than daily.

6) Can you clarify what 'smoothed PAT for days of infection was accumulated on days when the daily infection risk was greater than zero' means?

Answer RM: On days when infection is greater than zero, the PAT was accumulated. This provides a measure of the proportion of ascospores that contribute to infection as opposed to those released on days that are not suitable for infection and do not contribute to disease severity. I apologise that these values were not reported in the manuscript. This is a minor error in the paper.

7) Apart from a preliminary sensitivity test to a single model parameter, why was a sensitivity analysis not looked at?

Answer RM: A sensitivity analysis of the generic infection model was published elsewhere (Makowski et al.*, 2011). The sensitivity analysis was performed on D_{50} because there was no information as to its value, so it was useful to determine that its value was not critical (in this particular case). A sensitivity analysis was not needed for other parameters because their values were known with greater confidence. Information on all parameters was fairly solid except for D_{50}. We had greater confidence in the values of the other parameters and so no sensitivity analysis was done. An informal sensitivity analysis was performed as far as I remember.*

8) Can you clarify on equation No. 3 – subscripts instead of superscripts?

Answer RM: These should have been subscripts. Sorry. A minor error in the paper is noted.

9) Can you clarify on the use of D_{50}?

Answer RM: D_{50} is the dry interruption or the minimum dry hours that will stop an infection period. Its value was not known with confidence, so we used 3 h as was used by EFSA.

10) In the calculations of vapour pressure deficit (VPD, equations No. 5 and 6), there is a change in units from days to hours. Would this make a difference to the calculation of DDwet2? Please elaborate on temperature also for this question.

Answer RM: VPD was calculated on an hourly basis and the average value on a given day was used to calculate DDwet2. The base temperature was 10°C and degree days were calculated from hourly data. The change from hourly to daily should not be critical provided we did not underestimate the impact of VPD on spore release. If I was repeating this work, I would consider using the minimum hourly VPD per day. This would have been an improvement.

Additional question by the WG: Is this consistent with the work carried out with PF? How did you calculate daily from hourly? The two papers used two different approaches (daily vs hourly values for VPD) and this might cause a difference.

Answer RM: Looking at the paper, I did calculate it on an hourly basis. We are looking at extreme wetting events at high temperature, those events would be driven by heavy rainfall. VPD did not have much contribution. It may have made a larger contribution if calculated differently. As we had negative controls, it would just have had uniform effect across sites.

Roger Magarey – Questions and Answers

1) Could you please provide the data/readings for the values assessed in Table 1 of the Annex to the Magarey et al., 2015 paper?

*I do not have the CFSR and station weather data used to calculate these statistics, which were calculated by the vendor ZedX inc. You would need to contact them for these data (*Russo@zedxinc.com*).*

2) In the interpretation of the grid cell data, did you adjust for humidity and the other meteorological variables as well as for temperature in taking into account the elevation of the citrus grove? What source did you use for the elevation of the citrus groves?

The elevation correction only accounted for temperature. RH, leaf wetness and other variables were not corrected. Temperature typically decreases, while RH and leaf wetness likely increase with increasing elevation. Since citrus groves in the EU are often at lower elevation, we likely overestimated leaf wetness and predicted CBS infection levels. In future studies, it would be best to correct for both RH and leaf wetness. However, we believe failure to correct for elevation for RH and leaf wetness could only lead to an overestimation of infection. We used Google Earth to estimate elevation from grove latitude and longitude.

3) Is a grid size of 38 km adequate to minimise the variability inside the grid?

Ideally, the analysis should be conducted at the highest possible resolution and this was 38 km at the time of the study, as far as available global data. So, preferably we would go to finer resolution. But the problem with this is that there is no global availability of fine resolution data, hence no comparable data sets between countries at that resolution.

In my opinion, the use of a global grid data set is important to ensure that the variables are as consistent as possible between regions (see below). The study should be repeated if higher resolution data were available. If repeating this exercise, we would use a calibration dataset from Europe. Using a finer resolution might likely decrease the calculated risk, as the elevation of citrus groves is likely lower than the mean elevation of the grid (see comment above).

4) Can you expand on the advantage to use gridded data instead of station data?

The CFSR data sets provide a globally consistent source of weather data. This minimises issues with variables, such as RH and leaf wetness, where there may be differences between weather stations in terms of instrumentation used to measure these variables. Another issue with station data is missing values which need to be filled in before modelling can begin. There are also errors associated with the use of grid data as was described in the paper; most notably inaccuracies in mountainous areas, especially when using coarse resolution.

5) How is the general applicability of the infection model, and which thresholds are related to disease severity?

I think an infection model approach is very applicable to determine CBS risk, as regularity of infection is likely the limitation to establishment. I believe that in determining if a location is likely to be at risk, both ascosporic and pycnidiosporic infection levels should be considered. Thresholds should be determined through comparison with positive and negative control sites, including marginal sites. This does miss other factors like overwintering, but infection is likely to be the most limiting factor in establishing the disease. The need for positive and negative controls (including marginal sites) should be reiterated.

6) How do you get from the infection prediction derived from your model to a prediction of establishment?

This is determined by the infection threshold for both spore types. The threshold is based on positive and negative control sites, including marginal sites. In order for CBS to establish the infection score, it would need to be at least as high as the marginal sites. This is explained in detail in the paper. The unique thing about the paper by Magarey et al. *(2015) is the count of numbers of favourable years (high frequency of favourable years needed for successful establishment). RM noted the need for regular frequency of suitable years, otherwise climate would not be suitable.*

7) How might irrigation influence or change the probability of establishment of the pathogen?

Irrigation if applied overhead could extend the duration of an infection period and thus increase risk if applied at the right time. Under tree irrigation probably has negligible influence. Required leaf wetness duration needed for infection is not probable under conventional irrigation conditions, the CBS expert panel concluded that irrigation would not have an effect (see comments of CBS Expert Panel on EFSA 2013 and 2014).

Additional question by the WG: In Europe, many orchards have branches touching the soil, could this change the effect of irrigation on leaf wetness?

Yes, the soil surface could stay wet and remain wet longer than leaf wetness. This could be something for management in Europe, i.e. by pruning away branches in contact with the ground.

Paul Fourie – Questions and Answers

1) In the model, any ascospore release can potentially cause infection. Is this true?

This was assumed in the Magarey et al. *(2015) model when leaf wetness and temperature conditions were met and smoothed PAT was > 0. However, a minor ascospore release in nature will have a lower likelihood to cause infection given the low inoculum potential. Therefore, one needs negative and positive control sites for a realistic interpretation of the model output. Using the threshold from marginal sites, it is important for interpreting the model.*

2) Do you have information on the relationship between ascospore dose and disease incidence/severity?

No, specific information is not available for CBS. There is some ongoing research. The logical assumption, as in other pathosystems, is that the greater the inoculum dose, the greater the incidence/severity.

Additional question by the WG: But higher severities will be reached with higher doses. So how can we manage different inoculation doses with an infection model?

At the moment it is just a yes/no.

Comment RM: This is a great question, it relates to PAT on days with infection. For apple scab, the PAT had a big impact on final severity. In this case, as the disease needs long infection events, it was not as well correlated. There are limited numbers of events to cause infection, so for CBS the exact amount of inoculum is not the issue, more the issue is if it is present or not. For CBS, in my opinion, it is the number of wetness events that is important, not the inoculum load. P. citricarpa survives and persists in infected leaves. Citrus leaf has a lifespan of 2 years, so if there is not a string of favourable years then new leaf infection would not occur, and potential ascospore inoculum will be depleted.

3) 10°C are used as a basal temperature for DD accumulation – a justification for this is not given in the paper. Why was this temperature used?

Basal temperature of 10°C was used as equivalent of the lower cardinal temperature for the organism (Lovell, 2004 – A perspective on the measurement of time in plant disease epidemiology. Plant Pathol. 53:705–712; EFSA, 2008; Kotzé, 1963; Noronha, 2002). Also, 10°C was linked to the lower threshold of ascospore infection period. See review paper. In fact, this basal temperature could have been made higher as cold stress was identified as the biggest limiting factor for P. citricarpa.

4) Why was 1 January defined as mid-winter?

1 July was used for Southern Hemisphere, while 1 January was used for Northern Hemisphere (Rossi et al.*, 2009 – Predicting the dynamics of ascospore maturation of Venturia pirina based on environmental factors. Phytopathology 99:453–461).*

Additional questions/comments

Question by the WG: 0.6 was used as an adjustment for lapse rate (adjustment of temperature due to elevation), why?

Answer RM: 0.6 is the value that is used as a standard for a parcel of air that cools.

Comment by the WG: 0.6 is the rate used for humid air. But for dry air, it should be 1.0, i.e. 0.6 will underestimate conditions on ground.

Answer RM: Ok, but we are interested in the times when there is wet potential (wet and dry adiabatic rate).

Question by the WG: In his presentation, PF mentioned that both spore trap and weather station were located inside the orchard. This is a concern as weather data should be recorded under standard weather conditions (e.g. open field, etc.). How would this affect model?

Answer PF: This is not correct, the paper says that weather stations were not inside but were close (1 km). This was a mistake in a comment made in the presentation.

Question WG: In case, we need input or output data from these studies, what data would be available?

Answer PF: My question would be what do you want to do with data?

Answer RM: Some data may be available via the vendor (Zedx inc) and I would need to check with them if the data can be shared. Ideally this would be done as a cooperative exercise rather than a critique. It would need to be a negotiation and cooperative exercise.

Comment by the WG: RM raised the question in slide 24 that EFSA predicted fewer pycnidiospore infection events than ascospore infection events, why? We don't have a perfect answer but the main difference is that in EFSA simulations we only had this requirement for initial rain event for pycnidiospores, not for ascospores.

Comment PF: Also EFSA did not restrict to citrus susceptibility events/periods. This would be good to look at with an independent model validation exercise.

Question PF: The EFSA mandate was to consider the two papers as well as other relevant scientific information. What did you consider? Did you also consider the critiques of the different papers, specifically the Martínez-Minaya et al. *critique and the Perryman* et al. *critique? The Perryman* et al. *critique is particularly relevant as the Perryman paper was used by EFSA to argue that fruit is a probable pathway.*

Comment WG: Today the agenda is to just look at the two papers. The WG is working also on other publications, however, unpublished (non-peer-reviewed) manuscripts as those referred to above, were not considered as per the EU mandate.

RM: Would you consider making a recommendation that a blind, independent model comparison be considered to resolve this impasse?

Appendix B – Publications on citrus black spot, January 2014–March 2016 in scientific journals, conference proceedings or as chapters in edited books, considered in the current opinion based on the criterion as to whether new information was provided which necessitated an update of EFSA PLH Panel (2014)

Papers with results already described in EFSA PLH Panel (2014)		
Makowski et al. (2014)	Comparison of statistical models in a meta-analysis of fungicide treatments for the control of citrus black spot caused by *Phyllosticta citricarpa*. European Journal of Plant Pathology 139(1), 79–94	Meta-analysis of fungicide treatments/trials for control of CBS. Results already in EFSA PLH Panel (2014)
Perryman et al. (2014)	Splash dispersal of *Phyllosticta citricarpa* conidia from infected citrus fruit. Scientific Reports 4, 6568	Splash dispersal of conidia from infected fruit. Results already in EFSA PLH Panel (2014), but criticised in unpublished web report from Citrus Research International
Papers with no direct relevance for EFSA PLH Panel (2014)		
Barkley et al. (2014)	Invasive pathogens in plant biosecurity. Case study: Citrus biosecurity. In: The Handbook of Plant Biosecurity. Springer, Netherlands, pp. 547–592	General review on invasive plant pathogens and biosecurity. Cited EFSA (2008) which they claim conclusions were based on infection events rather than establishment. This issue was addressed in EFSA PLH Panel (2014) Opinion
Carvalho et al. (2015)	Control of Citrus Black Spot and juice quality after spraying fungicide in contrasting water volumes. Aspects of Applied Biology 122: 431–436	Technical paper on optimising efficiency in spraying for CBS control
Fialho et al. (2016)	Proteomic response of the phytopathogen *Phyllosticta citricarpa* to antimicrobial volatile organic compounds from *Saccharomyces cerevisiae*. Microbiological Research 183, 1–7. Needs adding to Table (no relevance)	Response of *P. citricarpa* to a biocontrol yeast
Grout (2015)	The status of Citrus IPM in South Africa. Acta Horticulturae 1065: 1091–1095	General review of citrus IPM in South Africa
Junior et al. (2016)	Spray volume and fungicide rates for citrus black spot control based on tree canopy volume. *Crop Protection*, *85*, 38–45	Relating to fungicide use in Brazil
Souza (2015)	*Phyllosticta citricarpa*: diversidade genética temporal em pomares de citrus sinensis e sensibilidade a fungicidas. PhD Thesis, Universidade Estadual Paulista 'Júlio de Mesquita Filho', Brazil	Brazilian PhD thesis in Portuguese
Steffen et al. (2015)	Identification of pests and pathogens recorded in Europe with relation to fruit imports. EPPO Bulletin 45(2), 223–239	EPPO report on pest identifications related to imports that cites EFSA PLH Panel (2014) Opinion, but does no more than quote the 'increased risk of transfer' for CBS
Wickert et al. (2014)	Molecular and pathogenic study of *Guignardia* spp. isolates associated to different hosts. Advances in Microbiology 4, 2: 42304	Molecular typing of *Guignardia* spp. from different hosts – mainly relevant for *G. mangiferae*
Zhang et al. (2015)	Synopsis of *Phyllosticta* in China. Mycology 6(1), 50–75	Up-to-date taxonomy of *Phyllosticta* (in China)
van Zyl et al. (2015)	Reduced volume spray application in South African citrus orchards: effects on deposition quantity, quality and uniformity. Julius-Kühn-Archiv 448, 51	Reduced volume spray applications for CBS in South Africa

Papers with some relevance for EFSA PLH Panel (2014)		
Graham et al. (2014)	Response to 'Potential distribution of citrus black spot in the United States based on climatic conditions', Er et al. 2013. European Journal of Plant Pathology 139 (2), 237–240	Similarly to Yonow and Kriticos comment on the misuse of CLIMEX in relation to the potential distribution of CBS in the USA
ISPM 27 (2014)	Diagnostic Protocols DP 5: *Phyllosticta citricarpa* (McAlpine) Aa on fruit. International Plant Protection Convention (now revoked)	Diagnostic protocols on fruit for CBS
Kim et al. (2014)	Citrus black spot detection using hyperspectral imaging. International Journal of Agricultural and Biological Engineering 7(6), 20–27	Detection methods for fruits infected with CBS. Hyperspectral methods for fruit in country of origin intended for export
Laurenza and Montanari (2014)	Pest risk analysis – recent trends in the EU and its trade implications: the Citrus Black Spot case. European Journal of Risk Regulation 2: 201–207	Pest risk analysis and trade implications for CBS
Mariduena Zavala et al. (2014)	Genetic variation among *Phyllosticta* strains isolated from citrus in Florida that are pathogenic or nonpathogenic to citrus. *Tropical Plant Pathology, 39*(2), 119–128	Genetic analysis of isolates of *P. citricarpa* and *P. capitalensis* isolates in Florida showing that *P. citricarpa* isolates are identical, indicating that the perfect stage may not be important there
West and Kimber (2015)	Innovations in air sampling to detect plant pathogens. Annals of Applied Biology 166(1), 4–17	General review of air sampling techniques to detect plant pathogens. Complements and cites the Perryman et al. (2014) paper for CBS
Yonow and Kriticos (2014)	Misconstrued risks from citrus black spot in colder climates: a response to Er et al. 2013. European Journal of Plant Pathology 139(2), 231–236	Letter concerning the misapplication of CLIMEX modelling to CBS – but mainly concerned with the Er et al. (2014) paper cited in EFSA PLH Panel (2014) Opinion
Papers with direct relevance for EFSA PLH Panel (2014)		
Dummel et al. (2015)	Predictive model for ascospore release of *Phyllosticta citricarpa* using climatological data. Acta Horticulturae 1065, 953–963	Predictive model for ascospore release using climatological data. A Conference Proceedings
Er et al. (2014)	Isolation and biological characterization of *Guignardia* species from citrus in Florida. J. Plant Pathol. 96: 43–55	Phylogenetic and phenotypic comparison of *P. citricarpa* and *P. mangiferae*. Optimal and maximum growth temperatures for *P. citricarpa* were similar, but the minimum range (5–11.4°C) that was lower than that used by Magarey et al. (2015) (15°C for ascospores and 10°C for pycnidiospores)
Magarey et al. (2015)	Prediction of *Phyllosticta citricarpa* using an hourly infection model and validation with prevalence data from South Africa and Australia. Crop Protection 75: 104–114	Prediction of CBS based on prevalence data from South Africa and Australia. Main paper to be evaluated according to the mandate
Martínez-Minaya et al. (2015a)	Climatic and spatial factors associated with citrus black spot. A Bayesian analysis of disease spread in South Africa. Proceedings CEB-EIB 2015, Bilbao, Spain 23–25 September, 2015, 4 pp	Bayesian analysis of disease spread. A Conference Proceedings
Martínez-Minaya et al. (2015b)	Climatic distribution of citrus black spot caused by *Phyllosticta citricarpa*. A historical analysis of disease spread in South Africa. European Journal of Plant Pathology 143(1), 69–83	Climatic distribution and disease spread of CBS in S Africa. Main paper to be evaluated according to the mandate

CBS: citrus black spot.

Appendix C – Publications citing Magarey et al. (2005) infection model

A literature search at the start of the mandate in September 2015 identified 35 papers which cited Magarey et al. (2005) and these were categorised according to the ways in which they used (or did not use) the generic infection model (Table C.1).

Many papers simply cite Magarey et al. (2005) as background material, without actually applying it to the study in question and thus these studies provide no information on its usefulness. Some papers cite Magarey et al. (2005), but use alternative approaches for characterising pathogen response functions in relation to weather and for assessing risk. Other papers do use Magarey et al. (2005), but adapt the model to other components of the disease cycle, or modify the approach due to perceived limitations in application, especially in relation to model sensitivity, issues of scale and weather variability, and interactions with crop-specific factors. The EFSA (2008) Opinions on citrus black spot made full use of the basic model, but introduced modifications, as did Magarey et al. (2015).

Table C.1: Publications citing Magarey et al. (2005) infection model

Authors	Title	Comments
Papers which cite the model by Magarey et al. (2005) but use alternatives		
Caubel et al. (2012)	Generic response functions to simulate climate-based processes in models for the development of airborne fungal crop pathogens	Develop generic repose functions for infection in relation to crop growth. Cite Magarey et al. (2005) respective to leaf wetness but thereafter make no mention. Use sigmoidal Weibull function with four cardinal points and test robustness. Leaf wetness calculated either from RH or dew-point temperature. Difficult to use for comparative purposes but can be mentioned as an alternative approach
Guyader et al. (2013)	Modelling the effects of temperature and leaf wetness on monocyclic infection in a tropical fungal pathosystem	Use a Weibull function for appressorial formation at constant leaf wetness. Use beta function for response to temperature and multiply the two together to get combined response. Only cite Magarey for the data used, not the infection model
Launay et al. (2014)	Climatic indicators for crop infection risk: application to climate change impacts on five major foliar fungal diseases in Northern France	Climatic indicators for infection risk used for climate change impacts. Cites Magarey et al. (2005) as a generic infection model that can be adapted and used to predict infection risk at a large spatial scale. However, this may not provide a continuous assessment of infection risk under changing climatic conditions. Develops a continuous response function to climatic variables (ClimInfeR) as opposed to threshold approaches (Magarey et al. (2005)). Increasing uncertainty of climate effects of wetness duration
Leca et al. (2011)	Comparison of Penman–Monteith and non-linear energy balance approaches for estimating leaf wetness duration and apple scab infection	Estimation of leaf wetness duration using Penman–Monteith/non-linear energy balance equations. Consider errors involved
Viruega et al. (2011)	Factors affecting infection and disease development on olive leaves inoculated with *Fusicladium oleagineum*	Standardised 'Analytics' model for disease (Olive fusicladium) severity. Cites Magarey et al. (2005) as the source for leaf wetness data
Papers which directly use the model by Magarey et al. (2005) but modify and point out limitations		
Bregaglio and Donatelli (2015), Bregaglio et al. (2010, 2011, 2012, 2013)		Use the Magarey et al. (2005) model to lesser or greater extent with many modifications in this suite of papers, and comment on its suitability in various contexts. Consider heterogeneity at various scales and explore the effect of variability in climatic variables. Takes a comprehensive approach to model performance and behaviour in terms of accuracy, correlation, anomalous behaviour and robustness under different conditions. Sensitivity analysis done by means of standard deviations and truncation of parameter values

Authors	Title	Comments
Eyre et al. (2012)	Rating and mapping the suitability of the climate for pest risk analysis	Comment that Magarey's model is highly dependent on accurate climate response data (see also Makowski et al., 2011). Consider location data to be largely irrelevant
Makowski et al. (2011)	Estimation of leaf wetness duration requirements of foliar fungal pathogens with uncertain data – an application to *Mycosphaerella nawae*	The authors estimate some parameters of the Magarey et al. (2005) model with a likelihood-free method of Bayesian estimation, often referred to as 'Approximate Bayesian Calculation' or ABC. They tested this with a data set for a pathogen of persimmon where only limited experimental data were available. It provides a distribution of the model parameters which could be used in an uncertainty analysis
Moral and Trapero (2012)	Mummified fruit as a source of inoculum and disease dynamics of olive anthracnose caused by *Colletotrichum* spp	Colletotrichum on mummified fruit. Adapts the infection model to sporulation
Moral et al. (2012)	Effect of temperature, wetness duration, and planting density on olive anthracnose caused by *Colletotrichum* spp	Use the Magarey et al. (2005) model with two critical disease thresholds
Richard et al. (2013)	Effect of pea canopy architecture on microclimate and consequences on ascochyta blight infection under field conditions	Cite Magarey et al. (2005) and development of generic infection model. Then uses it in relation to crop architecture/canopy features, including relationship with leaf wetness duration. Differences between canopy layers are shown
Rossi et al. (2015)	Use of systems analysis to develop plant disease models based on literature data: grape black-rot as a case-study	Grape black rot – estimated parameters for infection model for both ascospores and conidia, using the Magarey et al. (2005) paper
Skjøth et al. (2015)	Quality of the governing temperature variables in wrf in relation to simulation of primary biological aerosols	Biological aerosols – cite Magarey et al. (2005) as the basis for infection models but consider variation in the cardinal temperatures at different spatial resolutions
Vicent and García-Jiménez (2008)	Risk of establishment of non-indigenous diseases of citrus fruit and foliage in Spain: an approach using meteorological databases and tree canopy climate data	General paper on citrus diseases. Cites Magarey et al. (2005) only but discusses uncertainty

Appendix D – Assessment of papers applying ascospore maturation models in comparison with the Fourie approach (Fourie et al., 2013)

Fourie et al. (2013) take an empirical approach in developing the ascospore maturation model for *P. citricarpa.* In the empirical approach, there are three main steps: (i) collection of field data under different environmental conditions (locations × years); (ii) development of equations fitting the time–ascospore relationships and selection of the best equation by means of standard statistical analysis; and (iii) validation of the equation against independent data (i.e. in additional locations and years). A second approach exists for developing disease models, the fundamental or mechanistic approach. Both approaches have strengths and weaknesses, which can be analysed using a pathogen similar to *P. citricarpa*, i.e. *Venturia inaequalis*, the apple scab fungus, for which an extensive literature exists.

For apple scab, different field data have been used for predicting dynamics of ascospore maturation from: (i) development of the fruiting bodies into the leaf litter through microscopic observations (e.g. James and Sutton, 1982); (ii) the presence of mature ascospores in fruiting bodies (e.g. MacHardy, 1982); to (iii) airborne spores ejected from fruiting bodies, through different types of spore traps. Methods based on sampling and direct examination of the pseudothecia in the orchard over time may be considered more accurate than those based on spore traps, which integrate several biological processes over time: development of pseudothecia, ascospore maturation, release and dispersal. Ascospore release and dispersal are regulated by different mechanisms and influencing variables than the earlier stages of ascocarp development and ascospore maturation. Release depends upon absorption of water by the ascocarp and this water is mostly provided by intermittent rain, dew or irrigation; ascocarp development and ascospore maturation mostly depend on temperature and moisture of the leaf litter.

In addition to confounding different biological mechanisms, some types of errors bias spore trap data and the use of spore traps for developing models predicting the onset of ascospore release in a season should be used with caution. A first error source is the trap efficiency. Efficiency of a spore trap depends on the trap type, maintenance and setting. In Fourie et al. (2013), no information is provided about efficiency of the spore traps used. A second error source is the trap positioning in the orchard, in terms of height above the soil and distance from the inoculum source. In Fourie et al. (2013), spore traps were positioned between trees at least four to five rows into mature citrus orchards (> 13 years old) that were mostly surrounded by other citrus orchards, with the orifice at 0.5 m above soil level; no information is available on the disease severity level in the orchards. A third error source is the identification and enumeration of the spores on the tape, considering that only a small part of the tape is usually examined and that spore identification is based on its size and shape. In Fourie et al. (2013), *Phyllosticta* ascospores were counted on a compound microscope at ×400 magnification, and possibly overestimated because ascospores of *P. citricarpa* cannot be distinguished from those of *P. capitalensis.* In Fourie et al. (2013), degree days are the only driver for predicting the first seasonal ascospore trapping; the use of degree days calculated by considering moisture conditions did not improve the prediction accuracy of the equation based on temperature only. Fourie et al. (2013) provide information about the distribution of rainfall, relative humidity and vapour pressure deficit (VPD) during 2,000 *Phyllosticta* ascospore release events, but this information does not explain whether rainfall, relative humidity or VPD provided sufficient moisture to the leaf litter for fungal development. In addition, no information is provided on irrigation, which may have influenced the moisture of the leaf litter holding pseudothecia. The lack of effects of moisture in the Fourie equation raises the question of equation accuracy in different environments, in which moisture could be an important influencing factor. For apple scab, the role of moisture on ascospore maturation and how much it influences the dynamics of ascospores maturation has long been clarified (Gadoury and MacHardy, 1982a–c; MacHardy and Gadoury, 1985; Stensvand et al., 1997; Rossi et al., 1999; 2000).

In Fourie et al. (2013), there is no validation of the ascospore maturation equation against an independent data set. The many papers published on ascospore maturation models for apple scab (most recently Alves and Beresford, 2013; Jankowski and Masny, 2014; Roubal and Nicot, 2016) clearly show that accuracy of ascospore maturation models need careful validation – and often parameterisation – before being used in different environments. Lacking this validation, the model of Fourie et al. (2013) should be used with caution for extrapolating ascospore dynamics in environments different from those where the model has been developed.

Alternatively, a mechanistic model could be developed. Mechanistic models are process-based models, in which the biological process to be modelled is split in different stages, and processes

influencing the advancement of the pathogen from a stage to another are considered, as well as the influence of the external (environmental) variables on the rate of these processes. For example, in a mechanistic model for apple scab (Rossi et al., 2007), the ascospore dynamics were predicted starting from the ontogenesis of pseudothecia, through the stages of pseudothecial development and ascospore maturity over time, to ascospore release, with each stage simulated by specific model compartments. Mechanistic models, based on processes rather than field data, can provide accurate prediction when tested against field data over a range of different (and extreme) environments.

Appendix E – Citrus production data

The data were collected from the national statistical offices of the individual countries of the European Union. In most cases the data were available on Nomenclature of Territorial Units for Statistics 3 (NUTS3) level, in some cases extrapolated from NUTS2. These cases are listed in Table E.1. The data was then combined into a single EU data set, retaining the latest available data for each country. The table shows as well the latest year of which data was available.

Table E.1: Citrus product surface area per NUTS3 region

Country	Year	NUTS.Code	NUTS3.name	ha	Citrus_density
CY	2013	CY000	Κύπρος (Kypros)	2,629	0.3181
EL	2012	EL305	Ανατολική Αττική (Anatoliki Attiki)	75.1	0.0535
EL	2012	EL306	ΔυτικήΑττική (Dytiki Attiki)	5	0.005362
EL	2012	EL307	Πειραιάς, Νήσοι (Peiraias, Nisoi)	604.8	0.7025
EL	2012	EL411	Λέσβος, Λήμνος (Lesvos, Limnos)	115.8	0.05688
EL	2012	EL412	Ικαρία, Σάμος (Ikaria, Samos)	130.5	0.18
EL	2012	EL413	Χίος (Chios)	632.8	0.7459
EL	2012	EL421	Κάλυμνος, Κάρπαθος, Κως, Ρόδος (Kalymnos, Karpathos, Kos, Rodos)	919.6	0.3728
EL	2012	EL422	Άνδρος, Θήρα, Κέα, Μήλος, Μύκονος, Νάξος, Πάρος, Σύρος, Τήνος (Andros, Thira, Kea, Milos, Mykonos, Naxos, Paros, Syros, Tinos)	298.7	0.1245
EL	2012	EL431	Ηράκλειο (Irakleio)	610.5	0.2583
EL	2012	EL432	Λασίθι (Lasithi)	107	0.06531
EL	2012	EL433	Ρεθύμνη (Rethymni)	374.5	0.2793
EL	2012	EL434	Χανιά (Chania)	4,512	2.107
EL	2012	EL515	Θάσος, Καβάλα (Thasos, Kavala)	0.3	0.0001463
EL	2012	EL527	Χαλκιδική (Chalkidiki)	11.9	0.004242
EL	2011	EL531	Γρεβενά, Κοζάνη (Grevena, Kozani)	0.6	0.0001079
EL	2012	EL541	Άρτα, Πρέβεζα (Arta, Preveza)	6,468	2.599
EL	2012	EL542	Θεσπρωτία (Thesprotia)	1,343	0.9335
EL	2012	EL543	Ιωάννινα (Ioannina)	1.2	0.0002526
EL	2012	EL612	Λάρισα (Larisa)	0.5	$9.773e^{-05}$
EL	2012	EL613	Μαγνησία, Σποράδες (Magnisia, Sporades)	80	0.03207
EL	2012	EL621	Ζάκυνθος (Zakynthos)	157	0.4195
EL	2012	EL622	Κέρκυρα (Kerkyra)	559.4	0.924
EL	2012	EL623	Ιθάκη, Κεφαλληνία (Ithaki, Kefallinia)	146.2	0.1744
EL	2012	EL624	Λευκάδα (Lefkada)	155.8	0.4631
EL	2012	EL631	Αιτωλοακαρνανία (Aitoloakarnania)	3,505	0.6915
EL	2012	EL632	Αχαΐα (Achaia)	3,296	1.085
EL	2012	EL633	Ηλεία (Ileia)	4,519	1.862

Country	Year	NUTS.Code	NUTS3.name	ha	Citrus_density
EL	2012	EL641	Βοιωτία (Voiotia)	11.2	0.004067
EL	2012	EL642	Εύβοια (Evvoia)	309.4	0.07941
EL	2012	EL644	Φθιώτιδα (Fthiotida)	3.3	0.0007917
EL	2012	EL645	Φωκίδα (Fokida)	194.6	0.09787
EL	2012	EL651	Αργολίδα, Αρκαδία (Argolida, Arkadia)	12,694	2.094
EL	2012	EL652	Κορινθία (Korinthia)	3,377	1.587
EL	2012	EL653	Λακωνία, Μεσσηνία (Lakonia, Messinia)	9,763	1.607
ES	2013	ES111	A Coruña	201	0.02527
ES	2013	ES112	Lugo	7	0.0007114
ES	2013	ES113	Ourense	2	0.0002789
ES	2013	ES114	Pontevedra	105	0.02362
ES	2013	ES130	Cantabria	24	0.0045
ES	2013	ES213	Bizkaia	1	0.0004509
ES	2013	ES415	Salamanca	3	0.0002517
ES	2013	ES431	Badajoz	44	0.002159
ES	2013	ES432	Cáceres	2	0.000106
ES	2013	ES511	Barcelona	3	0.0003965
ES	2013	ES514	Tarragona	8,940	1.463
ES	2013	ES521	Alicante/Alacant	29,208	5.385
ES	2013	ES522	Castellón/Castelló	34,392	5.418
ES	2013	ES523	Valencia/València	87,216	8.528
ES	2013	ES531	Eivissa y Formentera	239.9	0.3901
ES	2013	ES532	Mallorca	1,347	0.3901
ES	2013	ES533	Menorca	258.5	0.3901
ES	2013	ES611	Almería	8,501	1.055
ES	2013	ES612	Cádiz	2,690	0.3973
ES	2013	ES613	Córdoba	11,121	0.8708
ES	2013	ES614	Granada	1,035	0.089
ES	2013	ES615	Huelva	19,479	2.085
ES	2013	ES616	Jaén	9	0.0007185
ES	2013	ES617	Málaga	10,752	1.61
ES	2013	ES618	Sevilla	29,022	2.245
ES	2013	ES620	Murcia	35,731	3.405
ES	2013	ES705	Gran Canaria	893	NA
ES	2013	ES709	Tenerife	520	NA
FR	2013	FR821	Alpes-de-Haute-Provence	1.671	0.000235
FR	2013	FR822	Hautes-Alpes	1.373	0.000235
FR	2013	FR823	Alpes-Maritimes	1.024	0.000235
FR	2013	FR824	Bouches-du-Rhône	1.243	0.000235
FR	2013	FR825	Var	1.426	0.000235
FR	2013	FR826	Vaucluse	0.8533	0.000235
FR	2013	FR831	Corse-du-Sud	730.5	0.1854
FR	2013	FR832	Haute-Corse	863.4	0.1854
HR	2014	HR031	Primorsko-goranska županija	264.5	0.07084
HR	2014	HR032	Ličko-senjska županija	389.7	0.07084
HR	2014	HR033	Zadarska županija	263	0.07084
HR	2014	HR034	Šibensko-kninska županija	213.7	0.07084
HR	2014	HR035	Splitsko-dalmatinska županija	324.9	0.07084

Country	Year	NUTS.Code	NUTS3.name	ha	Citrus_density
HR	2014	HR036	Istarska županija	206.5	0.07084
HR	2014	HR037	Dubrovačko-neretvanska županija	125.7	0.07084
IT	2014	ITC31	Imperia	19	0.01623
IT	2014	ITC32	Savona	30	0.01898
IT	2014	ITC34	La Spezia	6	0.006668
IT	2014	ITF14	Chieti	6	0.002351
IT	2014	ITF31	Caserta	451	0.1758
IT	2014	ITF33	Napoli	1,050	0.9296
IT	2014	ITF34	Avellino	16	0.005916
IT	2014	ITF35	Salerno	1,760	0.3721
IT	2014	ITF43	Taranto	7,660	3.263
IT	2014	ITF44	Brindisi	145	0.08206
IT	2014	ITF45	Lecce	585	0.2219
IT	2014	ITF46	Foggia	612	0.09003
IT	2014	ITF47	Bari	128	0.03457
IT	2014	ITF48	Barletta-Andria-Trani	15	0.01011
IT	2014	ITF51	Potenza	50	0.007949
IT	2014	ITF52	Matera	6,891	2.083
IT	2014	ITF61	Cosenza	15,365	2.436
IT	2014	ITF62	Crotone	1,555	0.961
IT	2014	ITF63	Catanzaro	3,644	1.622
IT	2014	ITF64	Vibo Valentia	2,045	1.915
IT	2014	ITF65	Reggio di Calabria	14,943	5.049
IT	2014	ITG11	Trapani	660	0.2894
IT	2014	ITG12	Palermo	6,460	1.397
IT	2014	ITG13	Messina	6,400	2.123
IT	2014	ITG14	Agrigento	5,208	1.856
IT	2014	ITG15	Caltanissetta	216	0.1102
IT	2014	ITG16	Enna	2984	1.263
IT	2014	ITG17	Catania	31,712	9.682
IT	2014	ITG18	Ragusa	3,050	2.067
IT	2014	ITG19	Siracusa	23,755	12.29
IT	2014	ITG25	Sassari	106	0.02569
IT	2014	ITG26	Nuoro	317	0.08402
IT	2014	ITG27	Cagliari	2,526	0.5844
IT	2014	ITG28	Oristano	440	0.152
IT	2014	ITG29	Olbia-Tempio	42	0.01276
IT	2014	ITG2A	Ogliastra	284	0.1605
IT	2014	ITG2B	Medio Campidano	112	0.07782
IT	2014	ITG2C	Carbonia-Iglesias	110	0.07756
IT	2014	ITI11	Massa-Carrara	10	0.008488
IT	2014	ITI12	Lucca	3	0.001665
IT	2014	ITI16	Livorno	10	0.008212
IT	2014	ITI1A	Grosseto	3	0.0006692
IT	2014	ITI43	Roma	10	0.0019
IT	2014	ITI44	Latina	605	0.2759
IT	2014	ITI45	Frosinone	12	0.003791
MT	2010	MT001	Malta	52.7	NA
MT	2010	MT002	Gozo and Comino/Ghawdex u Kemmuna	58.6	NA

Country	Year	NUTS.Code	NUTS3.name	ha	Citrus_density
PT	2009	PT111	Minho-Lima	64	0.0294
PT	2009	PT112	Cávado	161	0.1324
PT	2009	PT119	Ave	64	0.04518
PT	2009	PT11A	Grande Porto	35	0.01767
PT	2009	PT11B	Tâmega	120	0.04196
PT	2009	PT11C	Entre Douro e Vouga	6	0.003377
PT	2009	PT11D	Douro	443	0.1133
PT	2009	PT11E	Alto Trás-os-Montes	28	0.005175
PT	2014	PT150	Algarve	14,222	3.099
PT	2009	PT16B	Oeste	262	0.1249
PT	2009	PT16D	Baixo Mondego	137	0.08412
PT	2009	PT16E	Pinhal Litoral	26	0.006264
PT	2009	PT16F	Pinhal Interior Norte	22	0.009446
PT	2009	PT16G	Baixo Vouga	61	0.01955
PT	2009	PT16H	Beira Interior Sul	138	0.0314
PT	2009	PT16I	Dão-Lafões	365	0.1151
PT	2009	PT16J	Serra da Estrela	61	0.01007
PT	2014	PT170	Área Metropolitana de Lisboa	390	0.1459
PT	2009	PT181	Alentejo Litoral	599	0.1236
PT	2009	PT184	Baixo Alentejo	733	0.09264
PT	2009	PT185	Lezíria do Tejo	572	0.142
PT	2009	PT186	Alto Alentejo	295	0.05145
PT	2009	PT187	Alentejo Central	226	0.03267
PT	2014	PT200	Região Autónoma dos Açores	417	NA
PT	2014	PT300	Região Autónoma da Madeira	117	NA

Appendix F – Table for rain/no rain

In order to assess the impact of inclusion or exclusion of the condition of rain on the model, Table F.1 compares the total number of infection events from EFSA PLH 2014, according to presence/non-presence of rain on a given day. In total, there were 457,404 infection events in the presence of rain, with an additional 241,928 infect events in the absence of rain.

Table F.1: EFSA PLH Panel (2014) infections for Magarey et al. (2015) locations

Country	Location	EFSA infections[(a)]	EFSA infections filtered[(b)]	Magarey asco_days average[(c)]	Magarey asco suit yrs[(d)]	Magarey pyc average[(e)]	Magarey pyc suit_yrs[(f)]
Greece	Andravida	1,035	452	20.0	67	34.7	11
Italy	Pontecagnano	850	639	12.3	56	24.7	0
Greece	Kerkyra	1,933	908	11.0	22	21.6	0
Italy	Reggio Calabria	1,825	1,445	10.8	33	36.0	11
Italy	Cozzo Spadaro	862	613	10.8	33	23.1	0
Italy	Messina	2,413	1,793	10.4	33	26.4	0
Italy	Siracusa	358	266	9.4	33	21.9	0
Italy	Napoli/Capodichino	1,588	1,151	8.8	22	20.7	0
Italy	Palermo/Punta Raisi	1,408	1,071	8.8	11	17.7	0
Italy	Trapani/Birgi	629	410	8.6	11	18.0	0
Italy	Catania/Fontanarossa	2,378	312	7.1	0	29.7	0
Italy	Lamezia Terme	769	651	7.1	11	20.0	0
Spain	Jerez De la Frontera	423	285	6.9	0	19.7	0
Portugal	Faro	552	433	6.5	0	15.5	0
Portugal	Sagres	2,255	1,055	6.3	0	15.7	0
Greece	Rhodos	628	445	5.6	11	10.0	0
Greece	Naxos	213	122	5.5	11	12.2	0
France	Calvi	325	258	5.0	0	10.2	0
Spain	Murcia	579	407	4.9	0	15.9	0
Italy	Grosseto	219	167	4.3	0	18.9	0
France	Bastia	570	450	4.1	0	23.8	0
Portugal	Cabo Carvoeiro	628	453	3.9	0	20.8	0
Spain	Murcia/Alcantarilla	579	407	3.8	0	13.5	0
Portugal	Montijo	611	508	3.6	0	16.9	0
Spain	Reus	830	628	2.3	0	15.6	0
Cyprus	Larnaca	63	54	2.3	0	8.6	0
Italy	Pescara	1,030	818	2.0	0	19.8	0
Greece	Athens	1,958	503	1.7	0	12.7	0
Italy	Foggia Amendola	301	208	1.6	0	21.1	0
Greece	Thessaloniki/Mikra	NA	NA	1.0	0	23.1	0
Spain	Sevilla/San Pablo	583	449	0.9	0	7.8	0
Portugal	Viana Do Castelo	1,163	943	0.8	0	17.0	0
Italy	Firenze	NA	NA	0.5	0	18.0	0
Italy	Marina Di Ginosa	234	184	0.4	0	15.9	0
Greece	Larissa	1,711	755	0.1	0	8.6	0
Malta	Luqa	536	424	NA	NA	NA	NA

(a): EFSA infections: total number of infection events from EFSA PLH Panel (2014).
(b): EFSA infections filtered: number of infection events, excluding days without rain from EFSA PLH Panel (2014).
(c): asco_days_average: ascospores infection score according to Magarey et al. (2015).
(d): asco_suit_yrs: ascospores suitable years according to Magarey et al. (2015).
(e): pyc_average: pycnidiospores infection score according to Magarey et al. (2015).
(f): pyc_suit_years: pycnidiospores suitable years according to Magarey et al. (2015).

Appendix G – R Code used to calculate the area under the ROC curve for the Magarey et al. (2015) infection scores

```
library(pROC)

TAB<-read.table("MagareyData.txt",sep="\t",header=T)
head(TAB)

###############
##Ascospores##
###############

Prevalence<-TAB$ObsPrevalence[TAB$ObsPrevalence!="Unknown"]
Risk<-TAB$AvgDayPAT[TAB$ObsPrevalence!="Unknown"]

length(Prevalence)

#ROC analysis
PrevBin<-as.character(Prevalence)
PrevBin[Prevalence=="Endemic"|Prevalence=="High"|Prevalence=="Moderate"]<-1
PrevBin[PrevBin!=1]<-0
Risk[PrevBin==0]
Risk[PrevBin==1]

par(mfrow=c(1,2))
hist(Risk[PrevBin==0])
hist(Risk[PrevBin==1])
par(mfrow=c(1,2))
hist(log(Risk[PrevBin==0]))
hist(log(Risk[PrevBin==1]))

table(PrevBin)
Risk_roc<-roc(PrevBin,Risk)
Risk_roc$sensitivities
Risk_roc$specificities
Risk_roc$predictor
plot(Risk_roc)
Risk_roc$auc
ci.auc(Risk_roc)

#Exact conf. interval for the sensitivity (th=13.4)
length(Risk[Risk>=13.4 & PrevBin==1])
length(Risk[PrevBin==1])
binom.test(length(Risk[Risk>=13.4&PrevBin==1]),length(Risk[PrevBin==1]),
p=length(Risk[Risk>=13.4&PrevBin==1])/length(Risk[PrevBin==1]))

#Posterior for the sensitivity (th=13.4)
#beta(1+x,n-x+1)
qbeta(0.025,1+length(Risk[Risk>=13.4&PrevBin==1]),1+length(Risk
[PrevBin==1])-length(Risk[Risk>=13.4&PrevBin==1]))
qbeta(0.975,1+length(Risk[Risk>=13.4&PrevBin==1]),1+length(Risk
[PrevBin==1])-length(Risk[Risk>=13.4&PrevBin==1]))
qbeta(0.5,1+length(Risk[Risk>=13.4&PrevBin==1]),1+length(Risk[PrevBin==1])-
length(Risk[Risk>=13.4&PrevBin==1]))

#Exact conf. interval for the specificity (th=13.4)
length(Risk[Risk<13.4&PrevBin==0])
length(Risk[PrevBin==0])
binom.test(length(Risk[Risk<13.4&PrevBin==0]),length(Risk[PrevBin==0]),
p=length(Risk[Risk<13.4&PrevBin==0])/length(Risk[PrevBin==0]))

#Posterior for the specificity (th=13.4)
#beta(1+x,n-x+1)
qbeta(0.025,1+length(Risk[Risk<13.4&PrevBin==0]),1+length(Risk[PrevBin==0])-
length(Risk[Risk<13.4&PrevBin==0]))
qbeta(0.975,1+length(Risk[Risk<13.4&PrevBin==0]),1+length(Risk[PrevBin==0])-
length(Risk[Risk<13.4&PrevBin==0]))
qbeta(0.5,1+length(Risk[Risk<13.4&PrevBin==0]),1+length(Risk[PrevBin==0])-
length(Risk[Risk<13.4&PrevBin==0]))
```

```
##################
##Pycnidiospores##
##################

Prevalence<-TAB$ObsPrevalence[TAB$ObsPrevalence!="Unknown"]
Risk<-TAB$AvgInfPyc[TAB$ObsPrevalence!="Unknown"]
Risk[PrevBin==0]
Risk[PrevBin==1]

par(mfrow=c(1,2))
hist(Risk[PrevBin==0])
hist(Risk[PrevBin==1])
par(mfrow=c(1,2))
hist(log(Risk[PrevBin==0]))
hist(log(Risk[PrevBin==1]))

length(Prevalence)

#ROC analysis

PrevBin<-as.character(Prevalence)
PrevBin[Prevalence=="Endemic"|Prevalence=="High"|Prevalence=="Moderate"]<-1
PrevBin[PrevBin!=1]<-0

table(PrevBin)
Risk_roc<-roc(PrevBin,Risk)
Risk_roc$sensitivities
Risk_roc$specificities
Risk_roc$predictor
plot(Risk_roc)
Risk_roc$auc
ci.auc(Risk_roc)

#Exact conf. interval for the sensitivity (th=48.4)

length(Risk[Risk>=48.4&PrevBin==1])
length(Risk[PrevBin==1])
binom.test(length(Risk[Risk>=48.4&PrevBin==1]),length(Risk[PrevBin==1]),
p=length(Risk[Risk>=48.4&PrevBin==1])/length(Risk[PrevBin==1]))

#Posterior for the sensitivity (th=48.4)
#beta(1+x,n-x+1)
qbeta(0.025,1+length(Risk[Risk>=48.4&PrevBin==1]),1+length(Risk
[PrevBin==1])-length(Risk[Risk>=48.4&PrevBin==1]))
qbeta(0.975,1+length(Risk[Risk>=48.4&PrevBin==1]),1+length(Risk
[PrevBin==1])-length(Risk[Risk>=48.4&PrevBin==1]))
qbeta(0.5,1+length(Risk[Risk>=48.4&PrevBin==1]),1+length(Risk[PrevBin==1])-
length(Risk[Risk>=48.4&PrevBin==1]))

#Exact conf. interval for the specificity (th=48.4)

length(Risk[Risk<48.4&PrevBin==0])
length(Risk[PrevBin==0])
binom.test(length(Risk[Risk<48.4&PrevBin==0]),length(Risk[PrevBin==0]),
p=length(Risk[Risk<48.4&PrevBin==0])/length(Risk[PrevBin==0]))

#Posterior for the sensitivity (th=48.4)
#beta(1+x,n-x+1)
qbeta(0.025,1+length(Risk[Risk<48.4&PrevBin==0]),1+length(Risk[PrevBin==0])-
length(Risk[Risk<48.4&PrevBin==0]))
qbeta(0.975,1+length(Risk[Risk<48.4&PrevBin==0]),1+length(Risk[PrevBin==0])-
length(Risk[Risk<48.4&PrevBin==0]))
qbeta(0.5,1+length(Risk[Risk<48.4&PrevBin==0]),1+length(Risk[PrevBin==0])-
length(Risk[Risk<48.4&PrevBin==0]))
```