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Prediction of *Phyllosticta citricarpa* using an hourly infection model and validation with prevalence data from South Africa and Australia

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A B S T R A C T

An hourly infection model was used for a risk assessment of citrus black spot (CBS) caused by *Phyllosticta citricarpa*. The infection model contained a temperature-moisture response function and also included functions to simulate ascospore release and dispersal of pycnidiospores. A validation data set of 18 locations from South Africa and Australia was developed based on locations with known citrus black spot prevalence. An additional 67 sites from Europe and the United States with unknown prevalence were also identified. The model was run for each location with 9 years of hourly weather data from the National Centers for Environmental Prediction (NCEP) Climate Forecast System Reanalysis (CFSR) database. The infection scores for the sites with known prevalence where ranked and a threshold for suitability in a given year was derived from the average score of the lowest ranked moderate prevalence site. The results of the simulation confirm that locations in Florida were high risk while most locations in California and Europe were not at risk. The European location with the highest risk score was Andravida, Greece which had 67% of years suitable for ascosporic infection but only 11% of years were suitable for pycnidiosporic infection. There were six other sites in Europe that had frequency of years suitable for ascosporic infection greater than 22% including Pontecagnano, Italy; Kekrya, Greece; Reggio Calabria, Italy; Cozzo Spadaro, Italy; Messina, Italy; and Siracusa, Italy. Of these six sites only Reggio Calabria had a frequency of years suitable for pycnidiosporic infection greater than 0%. These six sites are predicted to have prevalence similar or less than Messina, South Africa, *i.e.* low and occasional. Other sites in Europe would best be described as likely to have no prevalence based on very low simulated scores for both spore types. Although Andravida had a similar risk of infection to moderate locations in South Africa there was a difference in the seasonality of infection periods. The ascosporic infection period score was similar between the two sites, but Andravida had a much lower pycnidiosporic infection score in the middle of the period of fruit susceptibility than Addo, South Africa. In Europe favorable climatic conditions are discontinuous, i.e., there is a low frequency of suitable seasons. This raises doubts about the ability of the pathogen to persist at a location and cause disease loss when favorable seasons reoccur. These results suggest that Europe is less suitable for CBS than suggested by an earlier study produced by the European Food Safety Authority using a similar model. The findings from our model simulations suggest that only a few isolated locations in the extreme south of Europe are likely to have a low to marginal risk of *P. citricarpa* establishment.

1. Introduction

Citrus black spot (CBS) is caused by the fungus *Phyllosticta citricarpa* (McAlpine) Van der Aa (synonym *Guignardia citricarpa*) (Ascomycetes, Dothideales). CBS is found in parts of Asia, Africa,

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South America, and Australia (CABI, 2014) with a summer rainfall climate. In March, 2010, CBS disease was detected in a small area in Florida (Schubert et al., 2010). The disease has sparked considerable interest amongst scientists and regulators given the disputed European Union (EU) regulation of zero tolerance for CBS in fresh citrus fruit, despite technical arguments that EU climate is not suitable for CBS establishment and that infected fruit is not a pathway for CBS introduction (USDA APHIS, 2010; CBS Expert Panel, 2013; EFSA, 2014). A pest risk assessment by the European Food Safety Authority concluded that quarantine measures should be maintained given the potential for establishment and spread (EFSA, 2014). There have been efforts to develop quantitative pathway models to determine the risk of introduction of CBS on imported fruit from infested locations. One of the important data needs for such a model is the climatic suitability of citrus productions areas in the EU.

The causal organism of CBS disease has two stages: a sexual stage with airborne ascospores as inoculum and an asexual stage with water-dispersed pycnidiospores as inoculum. The peak inoculum production of these two stages occurs at different times, under contrasting conditions, and/or at different locations on the plant and therefore results in different epidemiological dynamics.

Ascospores are produced in infected leaf debris from fungal structures called pseudothecia that develop 40–180 days after infection (CABI, 2014). Alternate wet and dry periods and mild/warm temperatures aid pseudothecium maturation and ascospore release, where after ascospores may infect susceptible host plants (Kiely, 1948; Kotzé, 1981). Rainfall (or overhead irrigation) triggers the release of mature ascospores (Kotzé, 1963), but too much rainfall will disrupt ascospore discharge (Kotzé, 1981) and lead to the decomposition of the dead leaves destroying the substrate for *P. citricarpa* (Lee and Huang, 1973).

Fourie et al. (2013) modeled the effects of temperature and wetness on *Phyllosticta* ascospore dispersal based on ascospore trapping and concomitant weather data that were obtained for three localities over three seasons in the Limpopo province of South Africa. Temperature had a major influence on pseudothecium maturation and onset of ascospore dispersal (5th percentile) occurred after 907.1 degree days >10 °C accumulated from mid-winter (DDtemp). A Gompertz model described pseudothecium maturation, while a Gompertz equation was also used to predict the proportion of ascospores trapped per season from DDtemp data accumulated on wet or moist days from the first seasonal ascospore discharge.

During wet periods, mature ascospores are forcibly ejected from the asci within the pseudothecia up to a centimeter high (Kiely, 1948; Kotzé, 1963). Ascospores are subsequently spread by wind and water (Kiely, 1948; Kotzé, 1963; Whiteside, 1965). Adequate hours of wetness, temperatures, and inoculum must be present simultaneously for infection to occur (Huang and Chang, 1972; Kotzé, 1981; Lee and Huang, 1973). For ascospores, a minimum of 15–38 h of wetness is required for successful germination and penetration (Kotzé, 1963), while pycnidiospore germination and appressoria formation require a minimum of 12 h of wetness at optimal temperatures (Noronha, 2002). Leaves are susceptible to infection up to 10 months of age (Truter et al., 2007), while fruit are susceptible for four to five months after petal fall independent of rainfall, temperature, or inoculum levels (Kotzé, 1963, 2000). Younger trees, less than 10 years old, appear to be less affected by CBS than older trees (Kiely, 1948); in trees up to 10 years old the susceptible period of fruit is limited to 3 months, and CBS is more easily controlled (Kiely, 1969).

The sexual ascospores are the primary source of tree to tree spread (Kotzé, 2000; McOnie, 1964, 1965), although act to increase disease intensity in individual trees (Kotzé, 1963, 2000; Spósito et al., 2008, 2011). The most important factors promoting epidemics are summer rains and proximity of lemon orchards which are highly susceptible to disease (Kotzé, 1981). Citrus black spot typically occurs in sub-tropical regions with summer rainfall.

Countries or regions with Mediterranean climates such as Spain and Portugal are thought to be unfavorable for the pathogen but this has been the subject of scientific debate. Despite many of years of trade between Europe and Asia and the importation of citrus propagative material, CBS has not established in Europe (Ramón-Laca, 2003). Likewise, CBS remains absent in citrus growing regions with Mediterranean type climates in Australia and South Africa, despite centuries of unregulated movement of citrus fruit and plant material from CBS-endemic areas within these countries (CBS Expert Panel, 2013).

A study by Paul et al. (2005) using CLIMEX concluded that in all European regions *P. citricarpa* had an ecoclimatic index (EI) of <8 compared to >20 in areas where the disease is prevalent (Paul et al., 2005). CLIMEX is a mechanistic model that uses both literature and distribution records to fit parameters. Reports by the European Food Safety Authority (EFSA) concluded that there was risk of CBS establishment in Europe (EFSA, 2008, 2014). The EFSA report was based upon CLIMEX using the Paul parameters but also questioned the values of the stress parameters used by Paul et al. (2005). A follow-up CLIMEX study by Yonow et al. (2013) also concluded that the European citrus production areas would not be at risk. A third CLIMEX study (Er et al., 2013), suggested that CBS could cause risk in Mediterranean climates, but this study has been challenged because it predicted suitability in locations where the disease is known not to occur and unsuitability in areas where the disease is known to occur (Graham et al., 2014; Yonow and Kriticos, 2014).

There have also been efforts to predict the risk of CBS using infection models. One study generated risk maps showing the frequency of years suitable for CBS based on thresholds developed from locations with known prevalence (Magarey et al., 2011). There were several limitations of the study including the use of daily weather data, the absence of an ascospore dispersal model and the crude spatial resolution of the National Centers for Environmental Prediction (NCEP) R2 database. This database has since been superseded by the NCEP Climate System Forecast Reanalysis (CFSR) database which was used in the present study. The CFSR data sets has a resolution of 38 km and includes a number of major advancements over the NCEP R2 database (Saha et al., 2010). These include greater spatial resolution and a more advanced description of the atmosphere.

The European Food Safety Authority used a more advanced version of the Magarey infection model to compare the number of infection periods at locations in Europe with those from South Africa and Australia (EFSA, 2008). The results showed that there were a similar number of infection periods in some parts of Europe to those in Addo, South Africa, which is a site with moderate CBS prevalence. One surprising finding from the EFSA study was far fewer pycnidiosporic infection periods than those for ascospores. One weakness of the EFSA study was that it only used data from two sites in South Africa and three years of weather data per site. In addition to the validation study, the EFSA panel also created maps of the EU at a 50 km resolution showing the number of potential infection events for CBS (EFSA, 2008, 2014).

The first objective of this study was to develop an infection model that used hourly weather data inputs and also incorporated ascospore dispersal models (Fourie et al., 2013). The second objective was to validate the model in locations with known black spot prevalence using a global weather data set. The validation included 18 locations from South Africa and Australia with known citrus black spot prevalence. For each location, the model was run for 9 years of weather data. This validation will be used to define thresholds for the number of infections periods required for a site