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Short Notes

Temperature and incubation period affect *Septoria pistaciarum* conidium germination: disease forecasting and validation

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Summary. Septoria leaf spot is an important disease of pistachio trees in Greece. This study aimed to determine effects of temperature and the incubation period on germination of conidia of *Septoria pistaciarum*, and to evaluate a generic model to forecast pistachio leaf spot under the field conditions of Aegina Island, Greece. The optimum temperature for conidium germination was 23°C, and germination was inhibited at 35 and 4°C. At constant temperature of 23°C, conidia commenced germination after 9 h. The predictive model indexed disease risk close to 100 at 10 May at two locations (Rachi Moschona and Vigla) in 2017, and first leaf spot symptoms were observed on 17 May. Moderate to high disease severity (>25% leaves infected) were observed in unsprayed trees at the end of May. In 2018, the model indexed risk close to 100 on 9 May at Rachi Moschona, and first symptoms were observed on 18 May. Moderate to high disease severity (>25% leaves infected) were observed in unsprayed trees on 25th of May. This study has shown that the forecasting model can be used in Aegina Island, Greece, to predict the severity of Septoria leaf spot of pistachio.

Keywords. Generic model, temperature.

INTRODUCTION

Septoria leaf spot is an important disease of pistachio trees in Greece. Three species of *Septoria* have been reported on pistachio worldwide; *Septoria pistaciarum, S. pistaciae* and *S. pistacina* (Teviotdale *et al*., 2001). In Greece, the fungus was identified as *Mycosphaerella pistaciarum* by Chitzanidis (1956), and the pycnidial stage of *S. pistaciarum*. Eskalen *et al.* (2001) reported *S. pistaciarum* as the causal agent of leaf spot of pistachio in the Eastern Mediterranean and Southeast Anatolian regions. The main symptoms of this disease are development of round to irregular lesions (each of 1–2 mm diam. and containing one to many (~20) pycnidia per lesion (Young and Michailides, 1989; Aghajani *et al*., 2009). The lesions form between small veins on both sides of affected leaves. These spots may increase slightly in size with time, but generally remain small and isolated from one another. Hundreds of spots may develop on each infected leaf. Over time, large sections of the leaf turn tan in colour. In severe cases, trees defoliate prematurely which reduces the amount of carbohydrates produced and stored, ultimately decreasing tree vigour.

Meteorological factors play key roles in infection caused by Septoria leaf spot fungi. The onset and severity of the disease was affected by wet weather and temperatures from 15–25°C in Arizona, United States of America (Young and Michailides, 1989; Matheron and Call, 1998). In South Greece, these climate conditions occur mainly in the period from May to June each year.

Many models have been developed for forecasting the probability of infections for particular plant pathogens (Newlands, 2018). Modelling approaches have strengths and weakness, and model selection depends upon several factors. Magarey *et al.* (2005) developed a generic infection model based on temperature and wetness duration. This model is generic in the sense that it was developed to describe any pathosystem when appropriate parameters are supplied. The successful development of a plant disease forecasting system also requires the proper validation of a developed model to reduce the risk of two false predictions a) false positive predictions, in which a forecast was made for a disease when in fact no disease was found in a location, and b) false negative predictions, in which a forecast was made for a disease not to occur when in fact the disease was found (Esker *et al*., 2008).

The main aims of this study were a) to investigate the minimum, maximum and optimum temperatures for the conidia germination of *S. pistaciarum*, b) to examine the minimum duration of incubation period (in hours) for the conidia germination of *S. pistaciarum* and c) the evaluation of the generic model developed by Magarey *et al.* (2005) to forecast the pistachio leaf spot disease under the field conditions of Aegina Island, Greece.

METHODS

Assessment of effects of temperature and time on conidium germination

Leaves with typical symptoms of Septoria leaf spot were collected from a commercial pistachio orchard on Aegina Island, Greece in 2016. Identification of the pathogen as *S. pistaciarum* was based on the description of Crous *et al.* (2013). To enhance sporulation, infected leaves were placed in damp chambers for 2 d. Conidium suspensions were made by flooding the surface of leaf spots with sterile distilled water and filtering the resulting suspension through four layers of cheesecloth. Conidium concentration was adjusted to 10^5 conidia mL^{-}with a haemocytometer. One mL of suspension was spread into each Petri dish containing 2% malt extract agar (MEA), and the dishes were placed in a growth chamber (Emmanuel E. Chryssagis, Growth Plant Chambers - GRW 500/CMP2) (98 ± 3% relative humidity) under continuous wetness for 24 h at 4, 8, 10, 15, 20, 23, 25, 30, 32 or 35°C. The proportions of germinated conidia in the Petri dishes incubated at 23°C were recorded at 3, 6, 9, 12, 24, 36 and 48 h after placing in the growth chamber. Conidium germination was determined for 100 conidia, using a microscope at 400× magnification. A conidium was considered germinated when the germ tube length was at least equal to the greatest diameter of the swollen conidium. There were four Petri dishes for each treatment (Dhingra and Sinclair, 1985; Dantigny *et al*., 2006). This experiment was conducted twice.

General linear regression analysis was performed (SPSS Grad Pack 23, SPSS Inc.) to determine relationships between temperature, incubation period and conidium germination.

Disease prediction model

The following parameters were used to run a Septoria leaf spot predictive model based on the results produced in the above experiments: Minimum Temperature (Tmin) = 8°C, Maximum Temperature (Tmax) = 32°C, Optimum Temperature (Topt) = 23°C, Minimum Leaf Wetness (Wmin) = 9 h, Maximum Leaf Wetness (Wmax) = 24 h (based on the above results). This model was evaluated under the field conditions of Aegina Island. Leaf wetness was estimated from the hourly data: if the leaf wetness sensor indexed the hour as wet, it was designated as 1, or when the sensor indexed the hour dry, it is designated 0 (so the dry hours were not counted and were not considered). Continuous wet hours were summed to determine leaf wetness. However, if there was an interruption of fewer than or equal to 24 dry hours (based on the preliminary results produced in the laboratory) the summation of hours was continued. If the interruption of dry hours was longer than 24 dry hours, a new summation of hours was immediately started. Temperature was the average temperature during each wet period.

Cultivar susceptibility or inoculum level were not considered because insufficient information was available about their effects on the occurrence of infection.

Model accuracy in predicting the day of infection was evaluated by comparing actual and predicted times of symptom appearance. In the Aegina Island, which is one of the most important pistachio production areas in Greece, a telemetric meteorological station (Neuropublic S.A., Information Technologies & Smart Farming Services, Piraeus, 18545, Attica, Greece) was established to record weather data, and these data were used to run the model. The model was operated hourly, starting from 00.01 h on 1 May [aiming to include the periods favourable for development of the disease (May to June), and unfavourable for the development of the disease (July-August)], and ending at 31 August, using hourly leaf wetness and temperatures as driving variables for calculations The date of the first observation of leaf spot symptoms (in young leaves) was used to verify the indexing of the models, and the final intensity of the symptoms was recorded 15 d later by calculating the percentage of infected leaves in a sample of 100 leaves randomly selected from each of ten trees. The period of possible appearance of the disease was calculated on each day when Risk (LW, T) > 30, considering an incubation period indicated from our preliminary studies (incubation period estimated at 5 to 15 d in leaves). The model predictions were from 0 (when Disease Risk = 0) to 100 (Disease Risk = the greatest possible value). All the other values were distributed between 0 and 100. Previous preliminary study showed that no or very light symptoms were observed when the model predictions were from 0 to 29.

Two commercial pistachio fields (trees of cv. Aegina, 10–12 years old) were chosen to record the appearance of Septoria leaf spot symptoms in the locations Rachi Moschona and Vigla in 2017, and the experiment was repeated in the same field at Rachi Moschona in 2018. No results could be collected in 2018 from the Vilga location because of technical problems with the meteorological station). Selected trees did not show any symptoms of the disease before starting the trials. The trees (kept unsprayed) were inspected twice each week to determine the time of symptoms onset. When symptoms were unclear, the trees were carefully inspected for the appearance of the first symptoms. Leaves with leaf spot symptoms were marked and observed during the following surveys. Inspections ceased after the appearance of the first disease symptoms.

The predicted period of disease onset was then compared with the actual onset of disease. The model was judged to have provided accurate prediction of disease when the observed symptom onset coincided with the time interval predicted by the model (Giosuè *et al.*, 2000).

RESULTS AND DISCUSSION

Effects of temperature and time on conidium germination

There were no significant differences between the two years for effects of temperatures (P = 0.175) or incubation period (P = 0.207), so the data from the two experiments were combined. Temperature influenced (P < 0.001, SE = 0.259) conidium germination. The optimum temperature for germination was 23°C, germination was strongly reduced at 32 and 8°C, and was inhibited at 35 and 4°C. The estimates of the parameters from the quadratic function are presented in Figure 1. Incubation period also influenced (P < 0.001, SE = 0.107) conidium germination. Under constant temperature at 23°C, germination was first observed after 9 h incubation. The greatest percentage of conidium germination was observed after 24 h incubation. No significant differences were detected in the proportions of germinated conidia after 24, 36 or 48 h incubation. Estimates of the parameters from the quadratic function are presented in Figure 2. According to Matheron and Call (1998), the onset and severity of Septoria leaf spot of pistachio in Arizona was affected by summer rainfall temperatures ranging from 15 to 25°C.

Control of the Septoria leaf spot has been based on disease prognosis. This method is adequate, but has disadvantages of inopportune and unnecessary spray applications. These increase the costs pistachio production and also possible environmental pollution with pes-

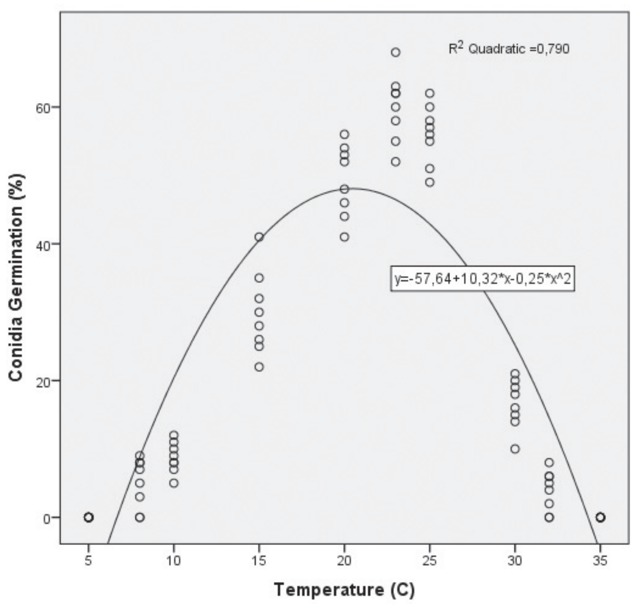


Figure 1. Proportions (%) of germinated *Septoria pistaciarum* conidia in Petri dishes held at different temperatures.

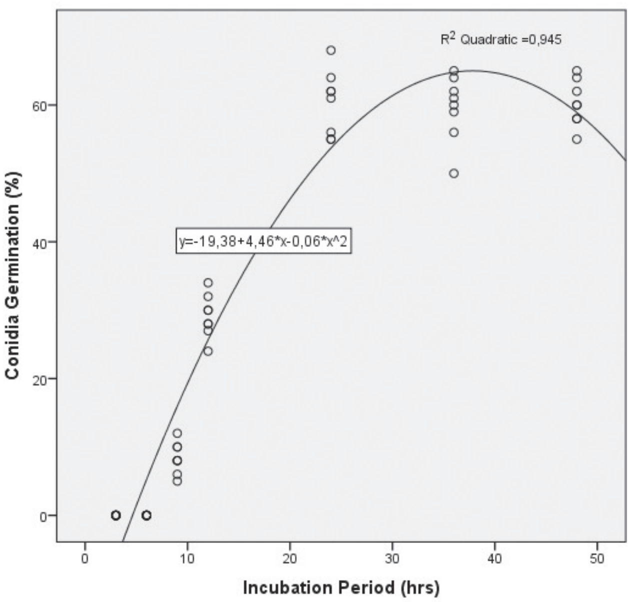
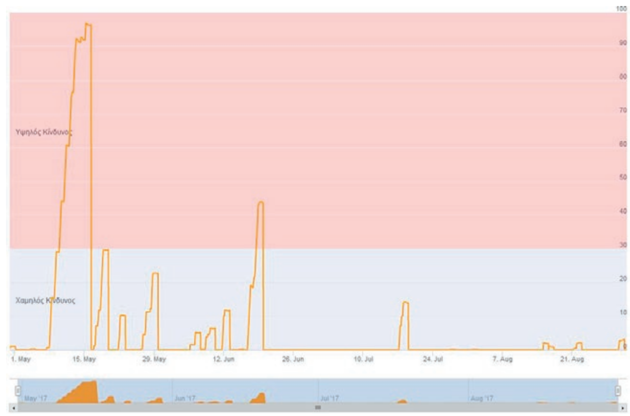
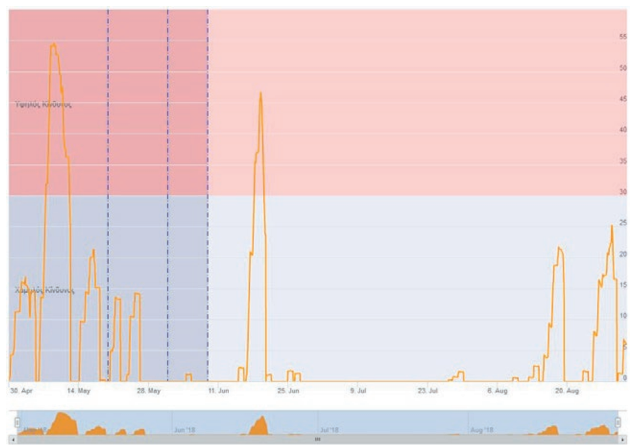


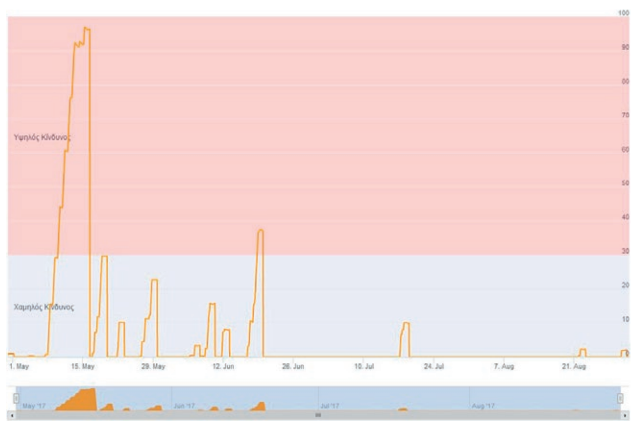
Figure 2. Proportions (%) of germinated *Septoria pistaciarum* conidia in Petri dishes held at 23°C for different incubation periods.



Location Rachi Moschona, 2017



Location Rachi Moschona, 2018



Location Vigla, 2017

Figure 3. Indexing of the generic predictive model (orange line) to forecast infections by *Septoria pistaciarum* on pistachio trees at two locations in 2017 and one location in 2018.

ticides. Disease forecasting has become an established component of quantitative epidemiology. Although it is difficult to exactly predict disease incidence, estimating possible ranges of disease intensity (risk) can be relatively easy. This improvement will provide disease management decision makers with valuable information. The introduction of predictive models to forecast disease appearance could reduce the number of spray applications required for disease control, and improve the effectiveness of spray applications conducted.

Predictive models for forecasting plant diseases are typically developed in specific climates and regions. Before using a model not field tested or validated for a specific location, the model must be tested for one or more seasons under local conditions to verify that it has good precision in that location. Predictive models may contain assumptions about site-specific conditions that may not apply for all areas. Input variables may need to be adjusted due to pathogen biology, and host phenology and variety in a specific area.

Magarey *et al.* (2005) developed a generic model appropriate for predicting the appearance of several plant diseases. This model requires climate parameters such as minimum, maximum and optimum temperatures, and minimum and maximum number of hours of leaf wetness. In the present study this approach was evaluated under field conditions for predict the appearance of pistachio leaf spot. The model indexed risk close to 100 on May 10 at two locations (Rachi Moschona and Vigla) in 2017 (Figure 3), and first symptoms of the disease were observed on May 17. Moderate to high intensity of the symptoms of the disease (>25% of leaves infected) were observed in the unsprayed trees at the end of May. In 2018, the model indexed risk close to 100 on May 9 at Rachi Moschona. The first symptoms of the disease were observed on May 18. Moderate to high intensity of the symptoms (>25% of leaves infected) were observed in the unsprayed trees on May 25. These results showed that the model correctly indexed infection periods.

Based on the results of this study, the Septoria leaf spot prediction model can be used on the Aegina Island, Greece. Greek pistachio producers now have the option to spray their trees only when the model predicts risks of infections. More investigations should be conducted to determine possible correlations between disease risk levels and the proportions of disease, and to determine the number of spray applications which are economically acceptable for pistachio production.

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