

RESEARCH PAPERS

Validation of a mechanistic model for predicting fruit scab infection on different loquat cultivars

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Summary. Scab, caused by *Fusicladium eriobotryae*, is the main disease affecting loquat (*Eriobotrya japonica*) in the Mediterranean basin. A mechanistic epidemiological model developed in Spain to predict infection of loquat fruit by conidia was assessed in the main loquat cultivated area of Italy (Sicily). A 3-year study (2014–2016) was carried out in an experimental orchard on three loquat cultivars: Algerie, Peluche and San Filipparo. For each cultivar, output of the model was compared with observed scab development on fruits. The scab epidemics observed were different in different years and cultivars, representing a suitable data set for model validation. The model correctly predicted loquat scab, as demonstrated by the goodness of fit between model predictions and observed disease incidence on fruits ($R^2 > 0.85$), confirming the accuracy and robustness of the model for predicting scab development in loquat orchards. The use of the model for fungicide scheduling against *F. eriobotryae* may improve the management of loquat scab by reducing the number of required fungicide applications.

Key words: *Fusicladium eriobotryae*, *Eriobotrya japonica*, integrated pest management, disease forecast.

Introduction

Scab, caused by *Fusicladium eriobotryae* (Cavara) Sacc., is the main disease affecting loquat (*Eriobotrya japonica* Lindl.) in the Mediterranean basin (Sánchez-Torres *et al.*, 2009). This fungus can infect young twigs, leaves and fruits of loquat, causing circular olive-coloured spots (Sánchez-Torres *et al.*, 2009; González-Domínguez *et al.*, 2014c). Scabby fruit are unsuitable for sale, resulting in significant economic losses (Soler *et al.*, 2007).

In recent years, several studies have focused on the biology and epidemiology of *F. eriobotryae* (Sánchez-Torres *et al.*, 2009; González-Domínguez *et al.*, 2013; González-Domínguez *et al.*, 2014c), and its

life cycle has been described (González-Domínguez *et al.*, 2014b). The fungus overwinters in lesions that remain in trees after harvest; in the autumn, under favourable conditions, these lesions produce conidia, which are the primary inoculum. Conidia are dispersed by splashing rain to nearby fruits and leaves, where, under suitable temperature and moisture conditions, they germinate and penetrate host tissues (González-Domínguez *et al.*, 2013; González-Domínguez *et al.*, 2014c). Once infection has occurred, the fungus grows in the host tissues, and, after a latent period, produces conidiophores and new conidia, which form secondary inoculum. These secondary conidia cause infections throughout the growing season, when environmental conditions are suitable (González-Domínguez *et al.*, 2014c).

This information was used to develop a dynamic, weather-driven model to predict infection of fruit

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by *F. erobotryae* conidia (González-Domínguez *et al.*, 2014b). The model uses a mechanistic approach to describe the infection process (Rossi *et al.*, 2010), and was validated with data from the main loquat cultivation area of Spain (Callosa d'En Sarrià, Alicante province). For this purpose, epidemic information from 3 years (2011–2013) and two loquat orchards (cv. Algerie) was used. The model correctly predicted the occurrence of infection periods, and the severity of any infection period (González-Domínguez *et al.*, 2014b). The model therefore potentially provides improved loquat scab management; i.e., it should optimize scab management by helping loquat growers in the timing, and probably reduction, of fungicide applications. The model is currently used in the main cooperative of loquat farmers in Spain to schedule fungicide applications (González-Domínguez *et al.*, 2014a).

This model could be used in other regions of the Mediterranean basin where loquat is cultivated, such as Italy, Turkey or Morocco (Caballero and Fernández, 2002). However, before recommending its use in new loquat-growing areas, the model needs to be validated to assess accuracy (i.e., closeness of predicted to observed values) and robustness (i.e., capacity to perform well across different environmental conditions) (Rossi *et al.*, 2010). Moreover, model validation should include the main cultivars growing in the new area, which may differ in susceptibility to the pathogen.

The objective of the study reported here was to validate the accuracy and robustness of the model for scab infection in Sicily, the main loquat production area of Italy. Experiments were conducted during a 3-year period on three different loquat cultivars.

Materials and methods

Orchard characteristics and disease assessment

The 3-year study (2014–2016) was carried out in an experimental loquat orchard of the Dipartimento di Scienze Agrarie, Alimentari e Forestali, Università degli Studi di Palermo, located inside the 'Lo Giudice Farm' (38°04'N; 13°25'E; Sicily, Italy). Experiments were carried out on 20–25 year-old loquat trees of cultivars Algerie, Peluche and San Filipparo (also known as Sanfilippara), all grafted onto loquat seedlings. During the experiment, the orchard received no fungicides for scab control, but standard

cultural practices otherwise were performed during the growing season.

Air temperature (T, °C), relative humidity (% RH), and total rainfall (R, mm), were registered every 30 min using a weather station (HMP-45, Campbell Scientific) located close the experimental orchard. The weather station sensors were located at 2.00 m above ground level. Wetness duration (WD, in hours) (i.e., free water on the surfaces of the loquat fruit caused by either rain or dew), was assumed for any hour when $R > 0$ mm, or $RH > 89\%$, or vapour pressure deficit (VPD) > 1 hPa. VPD was calculated using T and RH, as indicated by Buck (1981).

For each year and loquat variety, scab incidence on fruit of six randomly selected trees was assessed every 2–3 weeks, from growth stage BBCH-701 (fruit size c. 10% of final size) to BBCH-805 (increased cultivar-specific fruit colouring) (Martinez-Calvo *et al.*, 1999). In total, seven disease assessments were performed in 2014, eight in 2015 and six in 2016, from mid-January to mid-May. In each tree, five shoots were randomly tagged, and the numbers of fruit per shoot were counted. Fruit on the tagged shoots were classified as healthy or diseased. A fruit was considered diseased if at least one visible scab lesion was present on its surface. Disease incidence was expressed as a percentage (diseased fruit/total fruit \times 100). Disease incidence data were used to calculate the area under the disease progress curve (AUDPC) for each year and each loquat variety.

Model structure

The weather data were used to operate the model, as described in González-Domínguez *et al.* (2014b). The model splits the disease cycle of *F. erobotryae* into different state variables, which change from one state to the following one based on rate variables or switches, that depend on the environmental conditions by means of mathematical equations (Figure 1 and Table 1). The model starts at fruit set (growth stage BBCH-701) and ends at harvest (BBCH-807). The model assumes that conidia of *F. erobotryae* are always present in the loquat-growing season and then in the orchard. The model considers that any measurable rain (i.e., $R > 0.2$ mm in 1 h) causes dispersal and deposition of conidia on loquat fruit, and triggers infection processes. Each site on the fruit that is occupied by a conidium is considered a potential infection site and is referred to as a lesion unit

Table 1. List of variables used in a loquat scab model, adapted from González-Domínguez *et al.* (2014b).

Acronym	Description
<i>T</i>	Air temperature
<i>R</i>	Rainfall
<i>WD</i>	Wetness duration
<i>DP</i>	Dry period
<i>LUH</i>	Unit of loquat fruit surface without conidia of <i>Fusicladium eriobotryae</i>
<i>LUUC</i>	Unit of loquat fruit surface with ungerminated conidia of <i>F. eriobotryae</i>
<i>LUGC</i>	Unit of loquat fruit surface with germinated conidia of <i>F. eriobotryae</i>
<i>LULI</i>	Unit of loquat fruit surface with latent infection by <i>F. eriobotryae</i>
<i>LUVI</i>	Unit of loquat fruit surface with visible scab lesions
<i>GER'</i>	Conidium germination rate
<i>INF'</i>	Pathogen infection rate
<i>SUR'</i>	Pathogen survival rate

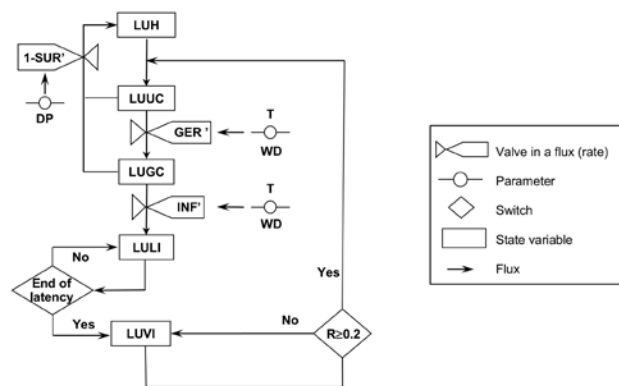


Figure 1. Relational diagram of the model simulating infection of loquat fruit by *Fusicladium eriobotryae*, adapted from González-Domínguez *et al.* (2014b).

(LU). During the infection process, an LU is initially healthy (LUH) and then becomes occupied by: an ungerminated conidium (LUUC) at the time of conidial dispersal; a germinated conidium (LUGC) at the time of conidial germination (following a ger-

mination rate: *GER'*); latent infections (LULI) after host penetration (following an infection rate: *INF'*); and visible and sporulating scab lesions at the end of latency (LUVI). Both LUUC and LUGC can fail to progress (following a survival rate: *SUR'*), and these LUs then return to being healthy, i.e., LUHs (Figure 1 and Table 1; González-Domínguez *et al.*, 2014b). *GER'* and *INF'* are influenced by temperature and wetness duration, and are calculated at hourly intervals; *SUR'* is influenced by dry periods (González-Domínguez *et al.*, 2013; 2014b). The model considers that any further rain event causes further dispersal and deposition of conidia if >5 h have passed after the previous dispersal event.

Data analysis

Model validation was performed by comparing model output (LULI in Figure 1) with observed data of disease incidence (DI). To take into account the lag between infection and onset of disease symptoms (i.e., the latent period), DI was shifted back 21 d (Sánchez-Torres *et al.*, 2009), as in the validation performed by González-Domínguez *et al.* (2014b). For each year and loquat variety, LULI values at the time of each disease assessment in the orchard were rescaled to the Σ LULI at harvest. Disease incidence was also rescaled to the final disease incidence (González-Domínguez *et al.*, 2014b). For each loquat variety, a linear regression was used to compare the predicted and observed data of the three years. A t-test was used to test the null hypotheses that “a” (the regression line intercept) was equal to 0 and that “b” (the regression line slope) was equal to 1 (Teng, 1980). The concordance correlation coefficient (CCC) and the coefficient of residual mass (CRM) were also calculated (Madden *et al.*, 2007; Rossi *et al.*, 2010). CCC is a measure of model accuracy (CCC = 1 indicates perfect agreement), and CRM is a measure of the tendency of the model to overestimate or underestimate the observed values (a negative CRM indicates a tendency of the model toward overestimation).

Results

In 2014, the model was operated between 1 January and 14 April, for cv. Algeria and Peluche. In this period, 401 mm of rain was recorded, triggering 57 infection periods (Figures 2a and b). For cv. San Filipparo, the model was operated between 1 Febru-

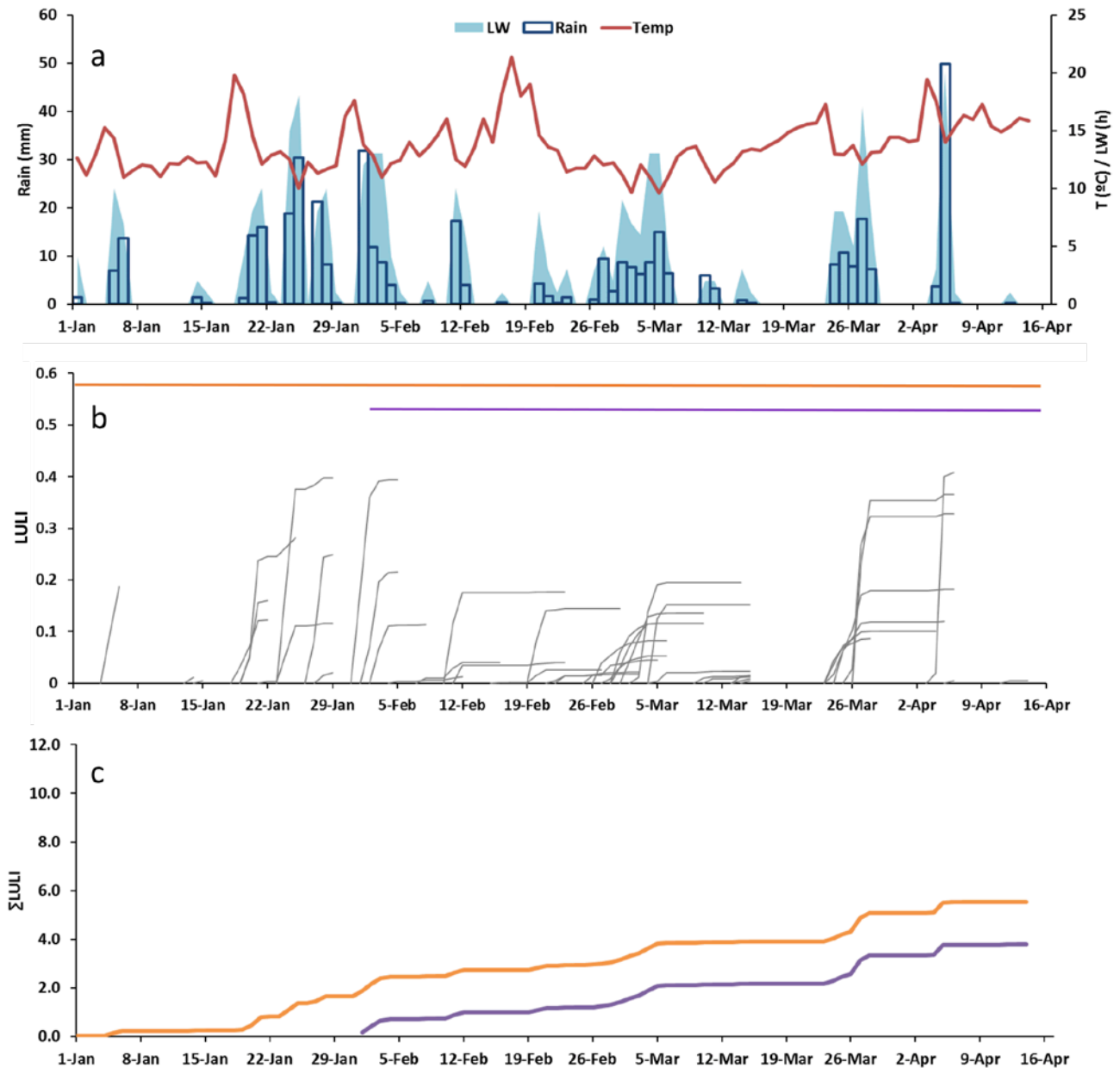


Figure 2. Weather data and model output for 2014. a: daily weather data; b: predicted increase of latent infections (LULI) for each infection period; c: predicted seasonal dynamics of the cumulative values of LULI (Σ LULI) for cv. Peluche and Algeria (orange line) and for cv. San Filipparo (purple line). Coloured lines at the top in b represent the periods in which the model was operated for cv. Peluche and Algeria (orange line) and for cv. San Filipparo (purple line).

ary and 14 April, with less rain (267 mm recorded) and 45 infection periods. Although infections periods were distributed through the recording season, they were more frequent during three periods: the last 2 weeks of January, and the first and last week

of March (Figure 2b). In this year, high levels of scab incidence were observed in the loquat experimental orchard in all three cultivars (Figure 3). Symptoms appeared first on cv. Peluche and Algeria, and then on cv. San Filipparo. In mid-March, disease incidence

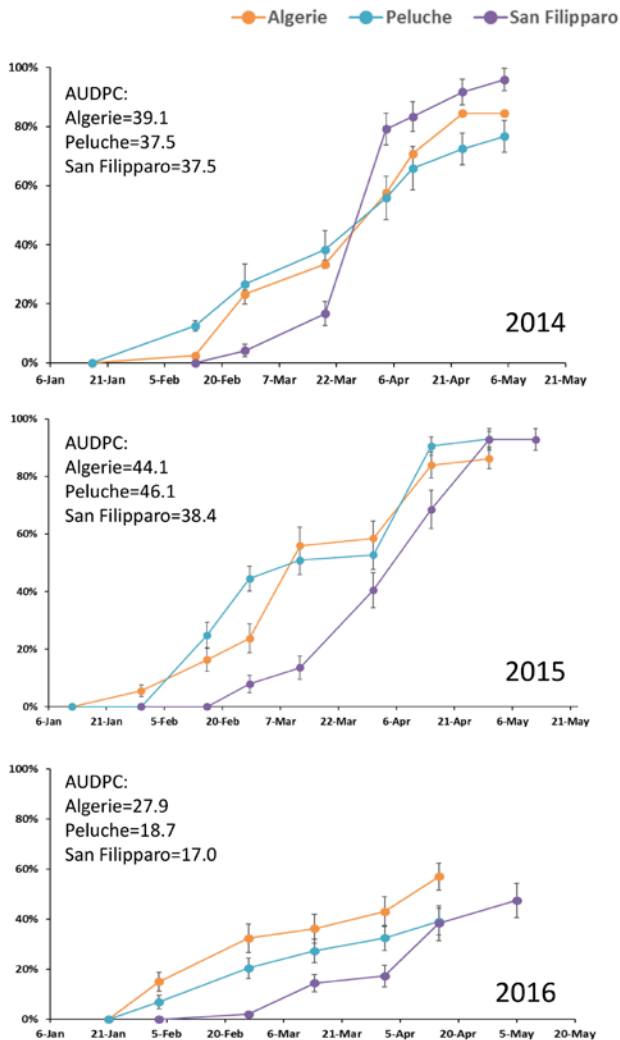


Figure 3. Mean percentages of loquat fruit affected by scab in three different cultivars and years. Whiskers indicate standard errors. Values of area under disease progress curve (AUDPC) are indicated for each cultivar and year.

was greater for cv. *Algerie* and *Peluche* (respectively, 33 and 38% of fruit affected) than for cv. *San Filipparo* (17%). However, major increase in disease incidence was observed in cv. *San Filipparo* in the next week, and final disease incidence was greater for cv. *San Filipparo* (96% of fruit affected), than for cv. *Algerie* (84%) and *Peluche* (76%) (Figure 3).

In 2015, the model was operated between 1 January and 9 April for cv. *Algerie* and *Peluche*, in which 479 mm of rain was recorded, triggering 87 infection periods (Figures 4a and b). For cv. *San Filipparo*, 62

infection periods were predicted by the model in the period between 1 February and 14 April, with 313 mm of rain recorded (Figures 4a and b). Distribution of these infection periods showed three peaks, the first at the end of January, then in mid-February and the beginning of March (Figure 4c). Scab symptoms on loquat trees appeared first on cv. *Algerie* and *Peluche*, with two periods of disease increase, in the last 2 weeks of February and the first 2 weeks of April (Figure 3). For cv. *San Filipparo*, disease incidence increased from the beginning of March until the end of April, when 93% of the fruits were affected (Figure 3).

In 2016, only 149 mm rain was recorded, from 1 January to 23 March (the period when the model was operated for cv. *Algerie* and *Peluche*), and 148 mm from 2 February to 15 April (the period for cv. *San Filipparo*) (Figures 5a and b). Although the number of infection periods were similar to those in 2014 (60 periods for cv. *Algerie* and *Peluche*, and 44 for cv. *San Filipparo*), the estimated severities of these periods (i.e., the value of LULI) were in general less than those observed in 2014 (Figures 2b and 5b). Consequently, low values of Σ LULI were predicted at the end of the 2016 season (Figures 2c and 5c), and only one important peak was predicted for cv. *San Filipparo* in the middle of March (Figure 5c). In this year, the lowest incidence of loquat scab was observed in all the cultivars (Figure 3). Scab symptoms began to appear in February on cv. *Algerie* and *Peluche*, and in March on cv. *San Filipparo*, and 40 to 50% of fruits were affected at the end of the season (Figure 3).

Overall, based on the AUDPC values, disease incidence was less in 2016 than in 2014 and 2015 (Figure 3). Cultivars *Algerie* and *Peluche* had similar AUDPC values in 2014 and 2015, although in 2016 fruit scab incidence was greater in *Algerie* (28%) than in *Peluche* (19%) (Figure 3).

The linear regression of model predictions (Σ LULI) versus observed data (loquat scab incidence) had high values of R^2 and CCC for all the cultivars (Figure 6). For all the years, R^2 was greater than 0.85, and CCC was greater than 0.9. For both parameters, the greatest values were obtained for cv. *Peluche* ($R^2 = 0.895$, CCC = 0.942). Moreover, for all three linear equations, the slopes were not significantly different from 1, and the intercepts were not significantly different from zero (data not show). Low values of CRM confirmed the goodness of fit of the datasets (Figure 6).

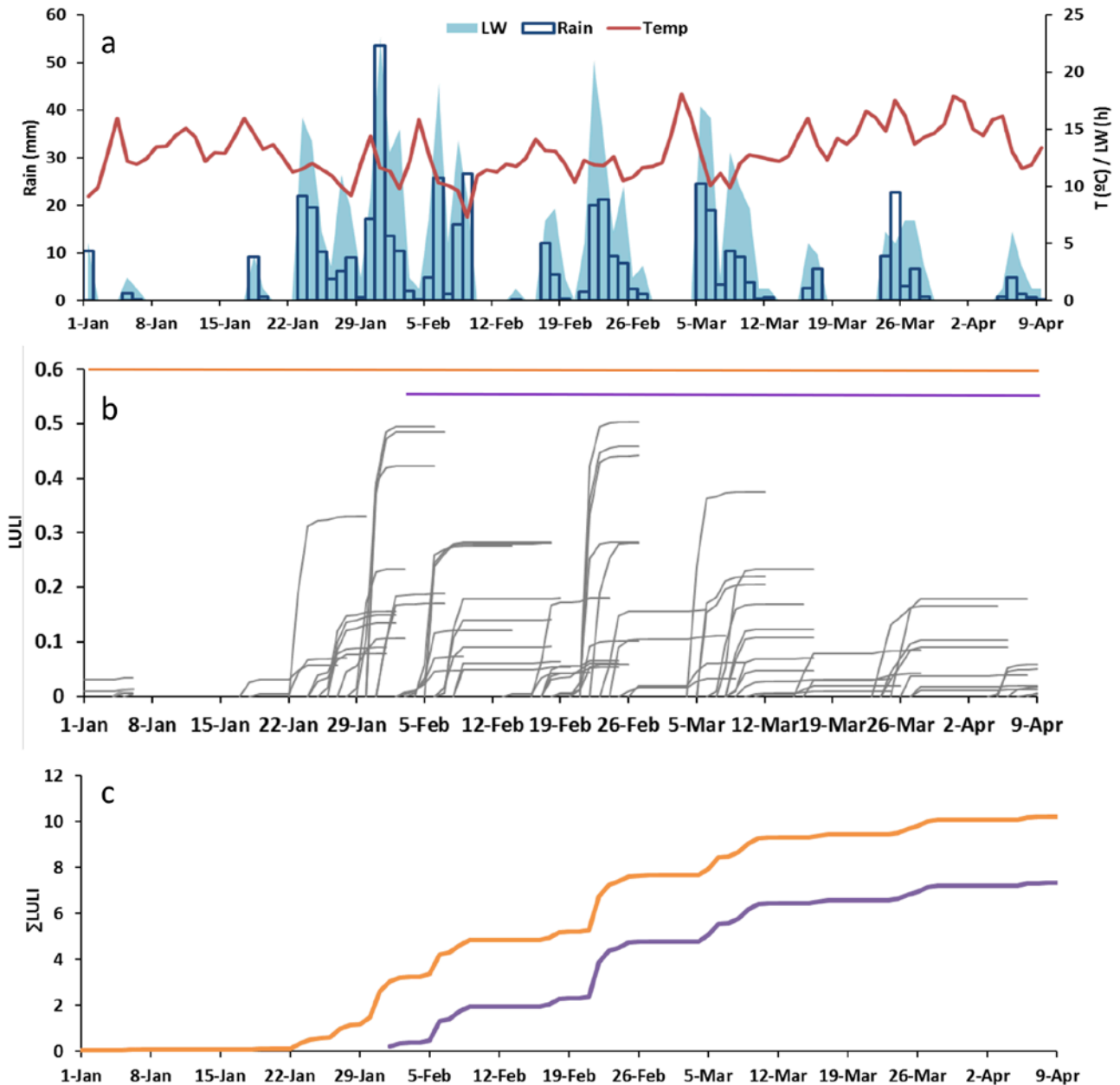


Figure 4. Weather data and model output for 2015. a: daily weather data; b: predicted increase of latent infections (LULI) for each infection period; c: predicted seasonal dynamics of the cumulative values of LULI (ΣLULI) for cv. Peluche and Algeria (orange line) and for cv. San Filipparo (purple line). Coloured lines at the top in b represent the periods in which the model was operated for cv. Peluche and Algeria (orange line) and for cv. San Filipparo (purple line).

Discussion

In this study, a recently published model (González-Domínguez *et al.*, 2014b) that predicts infection periods of loquat scab was validated during

a 3-year period (2014 to 2016), by comparing model output with disease development on fruits in a loquat orchard in Sicily, Italy, and on three loquat cultivars. The scab epidemics observed were different

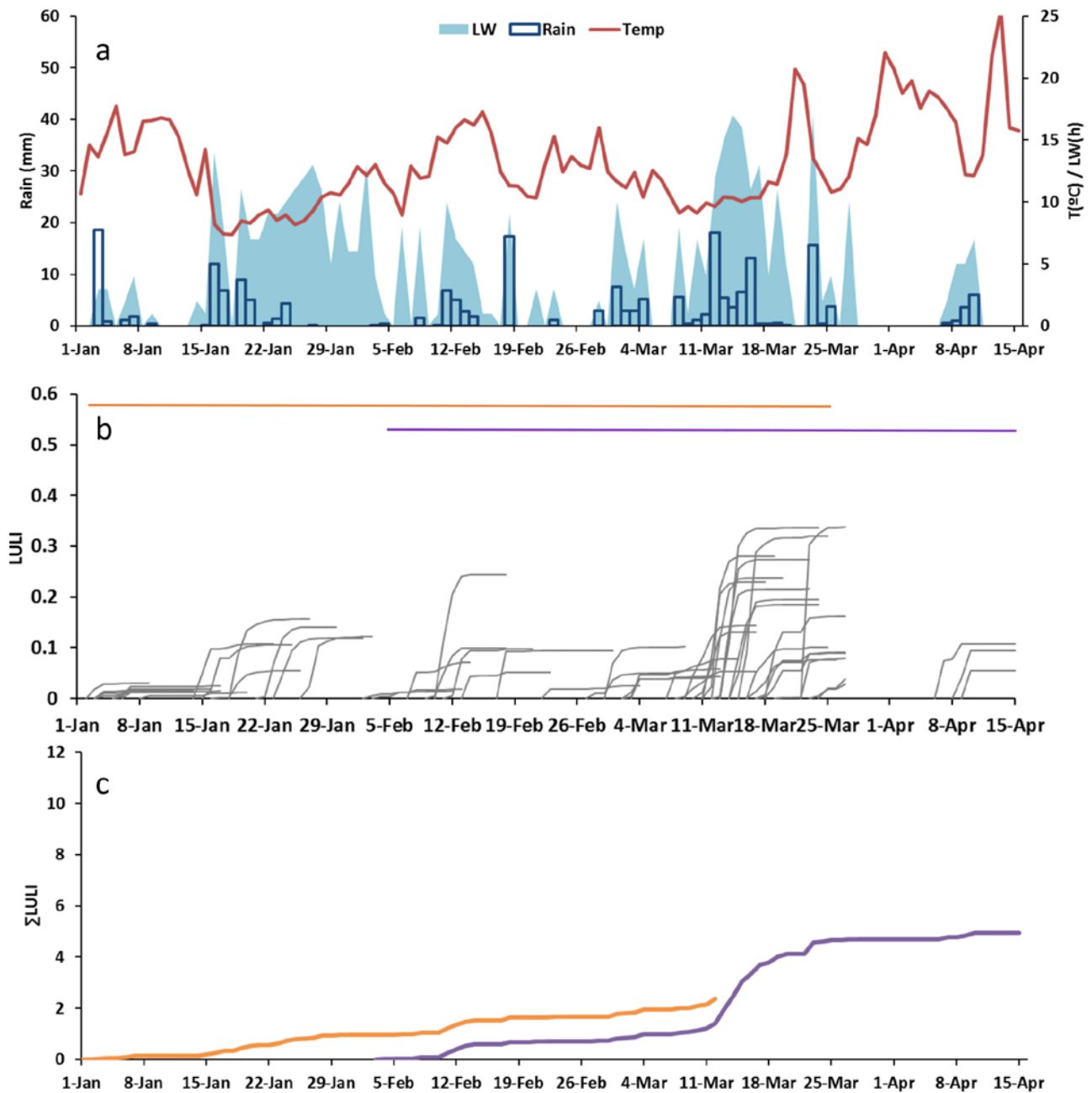


Figure 5. Weather data and model output for 2016. a: daily weather data; b: predicted increase of latent infections (LULI) for each infection period; c: predicted seasonal dynamics of the cumulative values of LULI ($\Sigma LULI$) for cv. Peluche and cv. San Filipparo (purple line). Coloured lines at the top in b represent the periods in which the model was operated for cv. Peluche (orange line) and for cv. San Filipparo (purple line).

in the different years and cultivars, so they represent a suitable data set for model validation. The model correctly predicted loquat scab, as demonstrated by

the goodness of fit between model predictions and observed disease incidence on fruits. These results from Italy, together with those previously reported

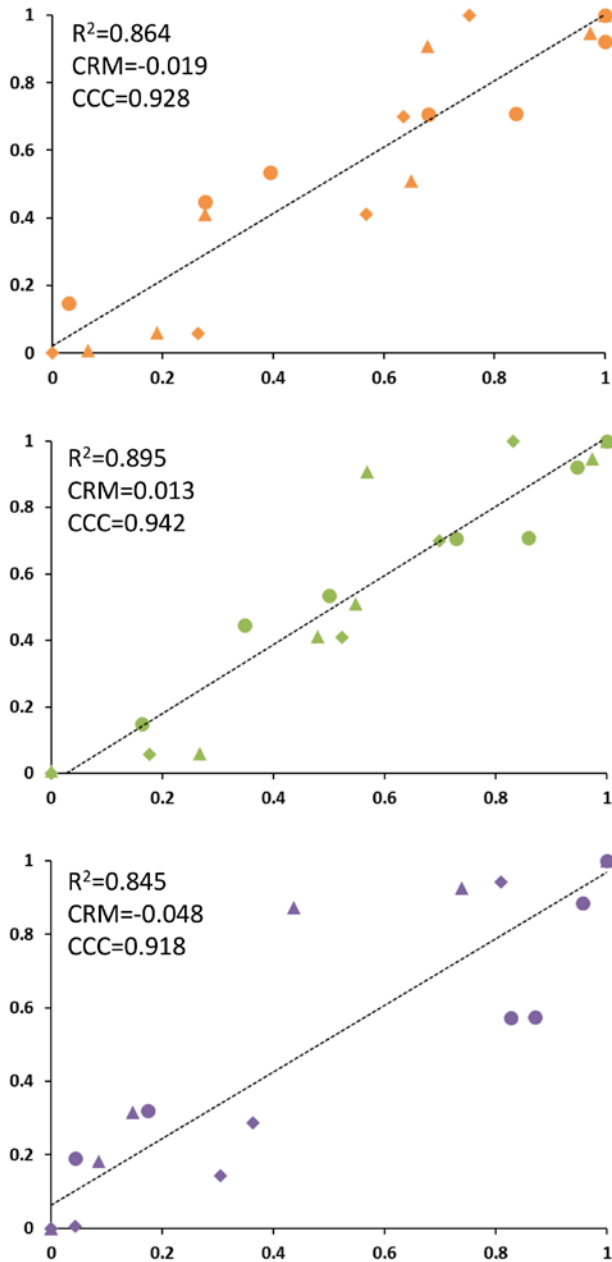


Figure 6. Plots of predicted versus observed values of loquat scab fruit infection for cv. Algerie (orange), Peluche (green) and San Filipparo (purple) in 2013 (dot symbols), 2014 (triangle symbols) and 2015 (diamond symbols). Goodness of fit parameters (R^2 , CRM and CCC) are indicated for each cultivar.

by González-Domínguez *et al.* (2014b) in Spain, confirm the accuracy and robustness of the model for predicting loquat scab development in orchards.

Mechanistic disease prediction models are known to provide more accurate and robust outputs than empirical models (Rossi *et al.*, 2010). A mechanistic approach includes multiple sub-stages of a disease cycle, and each of these is modelled independently by extracting information from experiments in controlled conditions (De Wolf and Isard, 2007). This approach lets the model integrate all the complexity simulated in laboratory experiments. Empirical models, in contrast, are developed using data collected under specific field conditions so that their accuracy and robustness are very dependent on the extent by which the datasets represent all possible situations in which a pathogen can develop and affect its host (Caffi *et al.*, 2007). Results from the present study reflect the robustness and accuracy of mechanistic models developed for other plant diseases, including as downy mildew (Caffi *et al.*, 2007) or black-rot (Onesti *et al.*, 2016) of grapevine.

This study also demonstrates that the loquat scab model may be used to predict development of the disease in three different loquat cultivars, Algerie, Peluche and San Filipparo, which are among the main cultivars grown in Spain and Italy (Caballero and Fernández, 2002; Farina *et al.*, 2016). The differences observed in disease development, mainly between Algerie and San Filipparo, are probably related to their differences in periods of fruiting, and the model has been able to account for these.

In the absence of specific information, the loquat scab model validated here assumes that inoculum sources are always present in scab-affected loquat orchards, and that viable conidia of *F. erobotryae* are always present at fruit set (González-Domínguez *et al.*, 2014b). Specific field experiments are under development to identify the main sources of *F. erobotryae* inoculum at fruit set and before (i.e., during flowering). Because of its mechanistic structure, this information could be easily incorporated into the model, once obtained (Rossi *et al.*, 2010).

Even without this improvement, the present model can contribute to the practical management of loquat scab in Sicily, the main cultivated area loquat in Italy. Currently, control of loquat scab in Sicily is based on copper or dodine fungicide applications. At least one application is carried out at flowering. Extra applications, if considered by the growers, are applied after the appearance of disease symptoms, and are scheduled by the calendar, or when growers empirically consider that the environmental condi-

tions are suitable for disease development (Calabrese, 1993). Consequently, these management practices can lead to unnecessary fungicide applications and/or inefficient control of the disease. The use of the present model for fungicide scheduling against *F. eriobotryae* may improve the management of the loquat scab, reducing the number of fungicide applications required for effective disease control.

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