Effect of Environmental Factors on Mycelial Growth and Conidial Germination of *Fusicladium eriobotryae*, and the Infection of Loquat Leaves

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Abstract

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In Spain, loquat scab, caused by *Fusicladium eriobotryae*, is usually controlled by fungicides when there are favorable conditions for infection. Lacking specific data on the effect of weather conditions on infection by *F. eriobotryae*, infection periods are predicted based on the Mills table for apple scab. Experiments were conducted to determine the influence of temperature, wetness duration, relative humidity (RH), and dry periods on mycelial growth, conidial germination, and infection of loquat leaves by *F. eriobotryae*. *F. eriobotryae* was able to grow and the conidia to germinate in a wide range of temperatures, whereas no germination occurred at RH < 100%. Maximum conidial germination occurred with 24 h of wetness and germination was strongly re-

duced by >10 h of dryness interrupting the wetness period. Loquat infection occurred between 10 and 20°C, and disease incidence and severity increased as the duration of wetness period increased. The combined effect of temperature and wetness duration on conidial germination of *F. eriobotryae* and infection of leaves was described by combining β and Gompertz equations, while the effect of dry periods on reducing the conidial germination was described by a logarithmic equation. The equations developed in this work provided a reasonable fit of the biological processes investigated and could be used for better disease control; they could be further integrated in a disease prediction system for scheduling fungicide sprays against loquat scab.

Loquat (*Eriobotrya japonica* Lindl.) is a fruit tree native of China, which is mainly cultivated in Asia, Australia, South America, the United States, and the Mediterranean basin (3,4,25). In temperate zones of the Northern hemisphere, loquat trees bloom in autumn and develop fruit in winter, which are mature for harvest in spring (34).

In the Mediterranean basin, loquat scab caused by *Fusicladium eriobotryae* (Cavara) Sacc. is the main disease affecting this crop (10,31,32). *F. eriobotryae* can infect leaves, shoots, and fruit, mostly in early stages of their development. The symptoms of loquat scab, which are quite similar to those described for apple scab (31), are generally noticeable and serious on both sides of leaves and on fruit as green to olive-brown spots, which increase in size and can cover almost all their surfaces (29,30). Scabby fruit are unsuitable for the market, resulting in significant economic losses (31).

Fusicladium spp. are the anamorphic stages of the ascomycete genus *Venturia* but the sexual stage of *F. eriobotryae* has never been found in nature (10). The epidemiology of other diseases caused by these pathogens, such as *Venturia inaequalis* (1,2,13,15,19), *V. nashicola* (17,18), *V. pyrina* (33,36,37), *F. carpophilum* (16), *F. effusum* (11), or *F. oleagineum* (23,24,38), is well understood. Results of some of these studies have been used to elaborate epidemiological models (9,26–28) that allow growers to predict the development of the disease, helping them in responding timely and efficiently (7).

Understanding the key factors that affect the development of plant disease epidemics is essential to design and implement effective strategies for disease management (7). Although loquat scab is a well-recognized problem and an important limiting factor for loquat production in the main growing areas of the Mediterranean basin, little is known about the biology of the pathogen, and no

specific research has been conducted to understand its epidemiology or to develop disease models (31). In Spain, the regional plant health services use the Mills-Laplane tables (20) to monitor the risk of infection by *F. eriobotryae* (12). These tables have been elaborated for *V. inaequalis* and disagreement between the risks indicated by the tables and the real infection has been repeatedly found since they were developed (19).

Laboratory and controlled-environment experiments provide information on epidemic components that is crucial to the understanding of epidemics (5). Moreover, the knowledge of these epidemic components is the first step for the development of a disease risk model. Thus, the objectives of this study were to (i) determine the influence of temperature, wetness duration, relative humidity (RH), and dry periods on mycelial growth and conidial germination of *F. eriobotryae*; and (ii) evaluate the infection of loquat leaves by *F. eriobotryae* under different regimes of temperature and wetness duration.

Materials and Methods

Fungal isolates. Six isolates of *F. eriobotryae* (FE-3, FE-6, FE-17, FE-31, FE-36, and FE-40) were obtained in 2008 from diseased loquat fruit collected in several locations of the Comunidad Valenciana region (eastern Spain) and used in this study. These isolates were single spored by means of the serial dilution method (8), stored in 15% glycerol solution at –80°C in 1.5-ml cryovials, and maintained in the fungal culture collection of the Instituto Agroforestal Mediterráneo (Universidad Politécnica de Valencia). Prior to use, a small plug of the colonized agar from each cryovial was transferred to potato dextrose agar (PDA) (Biokar-Diagnostics) and incubated at 20°C in darkness for 2 months. In all experiments, except for the evaluation of mycelial growth, conidial suspensions of 5×10^5 conidia ml⁻¹ were used. Suspensions were obtained by flooding 2-month-old cultures of each isolate with 10 ml of sterile distilled water and scrapping the cultures with a sterile spatula. The resulting conidial suspension was filtered through two layers of cheesecloth and the filtrate was adjusted to 5×10^5 conidia ml⁻¹ with a hemacytometer.

Effect of temperature on mycelial growth. To determine the effect of temperature on mycelial growth, plugs of agar, 5 mm in

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diameter, were cut from the margins of 2-month-old cultures of *F. eriobotryae* and placed in the center of PDA plates that were incubated in the dark at 5, 10, 15, 20, 25, 30, and 35°C. Six isolates were used in this experiment, with five replicate plates per isolate and temperature combination. After 1 month of incubation, colony diameter was measured along two perpendicular axes and data were converted to daily radial growth (millimeters per day). Each of the culture plates incubated at temperatures in which there was no growth were placed at 20°C to determine whether these temperatures were fungistatic or fungicidal. The experiment was repeated once.

Effect of temperature and wetness duration on conidial germination. The effect of temperature and wetness duration on conidial germination of *F. eriobotryae* was evaluated under six different temperature regimes (5, 10, 15, 20, 25, and 30°C) and six wettings periods (6, 12, 18, 24, 36, and 48 h). Isolates FE-6, FE-31, FE-36, and FE-40 were used in this experiment. For each combination of isolate, temperature, and wetness duration, three 10- μ l drops of the conidial suspensions were placed on a microscope slide and incubated in a moist chamber at 100% RH in darkness. The moist chamber was prepared by filling a hermetic plastic clear box with 80 ml of sterile distilled water, with the slide placed over a plastic grid. One moist chamber was used for each combination of temperature and wetness duration. At the end of each wetness period, the percentage of germinated conidia was determined by examining 200 conidia on each drop by using a microscope ($\times 40$ magnification). A conidium was considered germinated if its germ tube was at least one-half the length of the conidium. The experiment was repeated once.

Effect of RH on conidial germination. In this experiment, the germination of conidia of *F. eriobotryae* was evaluated at five different levels of RH: 100, 98, 95, 90, and 80.5%. To obtain the different RH regimes, the moist chambers described above were filled with 80 ml of different saturated salts solutions (12): $K_2Cr_2O_7$ for 98% RH, Na_2SO_3 for 95% RH, $MgSO_4 \cdot 7H_2O$ for 90% RH, and $(NH_4)_2SO_4$ for 80.5% RH. Prior to use, the chambers were equilibrated at the respective temperatures for at least 12 h. The germination of conidia at each RH was evaluated under three temperature regimes (15, 20, and 25°C) and seven incubation periods (6, 12, 18, 24, 36, 48, and 60 h). Two isolates, FE-6 and FE-40, were used in this experiment. For each combination of isolate, temperature, incubation time, and RH regime, three drops of 10 μ l of conidial suspensions were placed on one microscope slide. Prior to introducing the slide into the moist chamber, the drops with the conidial suspensions were dried in a laminar flow sterile cabinet for 20 min. One slide with three drops of 10 μ l of conidial suspension, which was not dried, was incubated in a saturated atmosphere as a control for each combination of isolate, temperature, and incubation period. One moist chamber was used for each isolate, temperature, and RH regime. The evaluation of conidial germination was performed as described before. The dried drops were rewetted with sterile distilled water to facilitate the microscope observations. The experiment was repeated once.

Effect of dry periods on conidial germination. In this experiment, the effect of five dry periods (6, 12, 24, 48, and 72 h) interrupting two wetness periods on conidial germination of *F. eriobotryae* was evaluated. Two initial wetness periods of 6 and 12 h and two levels of RH for the dry period (20 and 60%) were tested. In all combinations of initial wetness and RH, once the dry period had finished, the conidia were incubated in a moist chamber with saturated atmosphere for a final wetness period of 12 h. The moist chamber for the initial and final wetness period was prepared as described for the experiment of the effect of temperature and wetness duration on conidial germination. Isolates FE-6 and FE-40 were used in this experiment. For each combination of isolate, initial wetness period, dry period, and RH regime during the dry period, three drops of 10 μ l of conidial suspensions were placed on a microscope slide. For the initial and final wetness periods, one moist chamber was prepared for each isolate and RH regime. For the dry period, two different moist chambers were used: with no

water, corresponding to 20% RH (measured by a small data logger [Watchdog 450; Spectrum Technologies, Inc.]), or with 80 ml of a saturated solution of NH_4NO_3 , corresponding to 60% RH (8). Once the initial wetness period of 6 or 12 h had finished, the drops were dried in a laminar flow sterile cabinet for 20 min. After the drops were evaporated, the slides were placed in the chambers at either 20 or 60% RH for the different periods. Following each dry period, conidia on the slides were rewetted with sterile water and incubated in moist chambers at 100% RH for 12 h. Conidia maintained for 18 h under continuous wetting were used as control. All the experiment was conducted at 20°C in darkness. Percentage of conidial germination was evaluated as described before. The experiment was repeated once.

Effect of temperature and wetness duration on leaf infection. Infection on loquat leaves was evaluated under seven wetness periods (6, 12, 18, 24, 36, 48, and 60 h) and four temperature regimes (10, 15, 20, and 25°C). Six-month-old loquat leaves of the susceptible 'Algerie' were used. Plants were grown in a growth chamber (20°C, 60% RH, 12 h of light, and 12 h of darkness) and were inoculated when they had four fully expanded leaves. For inoculation, the isolate FE-40 was used. A uniform layer of fine droplets of a conidial suspension was gently sprayed on leaves using a hand sprayer (W560; Wagner Spraytech Iberica S.A.). Following inoculation, plants were covered with plastic bags to ensure leaf wetness and placed in growth chambers at 10, 15, 20, or 25°C in darkness. Five random plants were removed from the chambers after each wetness period had passed. These plants were dried by placing them 90 cm from a slow-speed fan, and then were transferred to a growth chamber (20°C, 60% RH, 12 h of light, and 12 h of darkness) for disease development. After 30 days of incubation, disease incidence was assessed as the percentage of leaves with scab lesions per plant. Disease severity was assessed visually on each leaf as the percentage of the leaf area showing typical scab symptoms. The experiment was repeated once.

Data analyses. A homogeneity of variance test was performed for each experiment. A preliminary analysis of variance showed that there were no significant differences between repeated experiments, so that data from different experiments were pooled. Quantitative relationships between environmental variables and mycelial growth, conidial germination, and infection were analyzed using regression analysis. Different linear and nonlinear regression models were fitted to the observed data; the equation parameters were estimated using the nonlinear regression procedure of SPSS (ver. 19.0; SPSS Inc.), which minimizes the residual sums of squares using the Marquardt algorithm. The best model was chosen based on the adjusted R^2 , the number of iterations taken by the Marquardt algorithm to converge on parameter estimates, the magnitude of the standard error of the parameters, and the magnitude and distribution of the standardized residuals (6). The goodness-of-fit was evaluated by considering the properties of the linear regression between real and estimated data (34) and by calculating the following indexes (22): the standard errors of the estimates (SEest), the root mean square error (RMSE) as the square root of the mean square error (RMSE represents the average distance of real data from the fitted line), and the coefficient of residual mass (CRM) as a measure of the tendency of the equation to overestimate or underestimate the observed values (a negative CRM indicates a tendency of the model toward overestimation). For the linear regressions, with each set of results representing pairs of predicated and observed data, the null hypotheses that "a" (intercept of regression line) is equal to 0 and "b" (slope of regression line) is equal to 1 were tested using a *t* test. If the *t* tests for "a" and "b" were not significant, then both null hypotheses were accepted and the model was considered a statistically accurate predictor of the real data (34).

In the experiments where multiple isolates were used, the models were developed for each isolate and differences among isolates were evaluated based on the standard errors of the parameter estimates; because the confidence intervals of the estimated parameters of the different isolates always overlapped, the data from all isolates were pooled.

Results

Effect of temperature on mycelial growth. All isolates of *F. eriobotryae* were able to grow between 5 and 25°C. High radial growth of the colony on PDA occurred between 15°C (average of 0.35 mm/day) and 25°C (0.36 mm/day), with a maximum at 20°C (0.49 mm/day); at 5 and 10°C, the growth of the colonies was slower (0.05 and 0.19 mm/day, respectively). Any isolate was able to grow at 30 and 35°C, and the colonies died after 1 month at 35°C. The effect of temperature on mycelial growth was best described by the β equation, in the form $y = a \times \text{Teq}^b \times (1 - \text{Teq})^c$, where y is the relative growth of the colonies (calculated by dividing the growth rate at any temperature by that at the optimum temperature); a , b , and c are the equation parameters; and Teq is an equivalent of temperature calculated as $\text{Teq} = (T - T_{\min}) / (T_{\max} - T_{\min})$, where T is the temperature regime and $T_{\min}$ and $T_{\max}$ are minimum and maximum temperatures, respectively, at which mycelium is able to grow. With $T_{\min} = 0^\circ\text{C}$ and $T_{\max} = 26^\circ\text{C}$, the β equation fitted the data of single fungal isolates with R^2 between 0.87 and 0.99 (Table 1). Curves for the different isolates had similar shape, as the values of the estimated parameters and their standard errors showed (Table 1). Therefore, the data for the different isolates were pooled in equation 1 and the resulting equation had $R^2 = 0.98$, low standard error of the estimated parameters (Table 2), and the SEest = 0.05. The curve of relative growth increased between 0 and 20°C, had the maximum near 20°C, and then quickly decreased (Fig. 1).

Effect of temperature and wetness duration on conidial germination. Conidia of *F. eriobotryae* germinated at all temperatures tested. After 6 h of wetness, germination occurred at 15, 20, 25, and 30°C, with the highest rate at 20°C (34.4%); at 5 and 10°C, the germination started after 12 h of wetting. Regardless of temperature, there was no increase in germination after 24 h of wetting. The maximum germination was observed at 20°C, with 60% of the conidia germinated. The best fit of the combined effect of tempera-

ture and wetness duration on conidial germination was obtained with the following model that combines a β and a Gompertz equation (Table 1, equation 2; Fig. 2), as follows: $y = a \times \text{Teq}^b \times (1 - \text{Teq})^c \times \exp[d \times \exp(e \times \text{WD})]$, in which y is relative conidial germination referred to the maximum germination observed; Teq is the equivalent of temperature calculated as described before; WD is the wetness duration (in hours); and a , b , c , d , and e are the equation parameters. The equation for pooled data had $R^2 = 0.93$; standard errors of the equation parameter were low compared with the parameter values, except for parameters a and e (Table 1, equation

Fig. 1. Relationship between temperature and relative growth of *Fusicladium eriobotryae* mycelium as predicted by equation 1 of Table 1. Symbols represent observed data and whiskers are the standard errors calculated over different experiments and fungal isolates. Relative growth is calculated by dividing growth (millimeters per day) in each observation by the maximum growth at optimum temperature.

Table 1. Equations used to fit data on mycelial growth (eq. 1), conidial germination (eq. 2), effect of dry period on conidial germination (eq. 3), disease incidence (eq. 4), and severity (eq. 5) of *Fusicladium eriobotryae* as a function of environmental variables

Equation ^y	$T_{\min}/T_{\max}$	Estimated parameters ^x					R^2	Figure ^z
		a	b	c	d	e		
1: $y = a \times \text{Teq}^b \times (1 - \text{Teq})^c$	0/26	2.460 (0.501)	1.557 (0.236)	0.364 (0.076)	...	...	0.98	1
2: $y = a \times \text{Teq}^b \times (1 - \text{Teq})^c \times \exp[d \times \exp(e \times \text{WD})]$	0/35	116.249 (59.597)	4.347 (0.435)	2.882 (0.299)	8.550 (8.620)	0.376 (0.161)	0.93	2
3: $y = a \times \ln(DP) + b$	...	-0.165 (0.015)	0.879 (0.048)	...	...	...	0.98	4
4: $y = a \times \text{Teq}^b \times (1 - \text{Teq})^c \times \exp[d \times \exp(e \times \text{WD})]$	0/25	4.961 (2.944)	1.700 (0.527)	0.771 (0.310)	4.704 (1.381)	0.087 (0.018)	0.94	6
5: $y = a \times \text{Teq}^b \times (1 - \text{Teq})^c \times \exp[d \times \exp(e \times \text{WD})]$	0/25	7.574 (7.629)	2.841 (0.962)	0.769 (0.493)	4.324 (1.048)	0.050 (0.015)	0.93	7

^x Equation parameters were estimated for the data pooled over the different fungal isolates and repeated experiments; standard errors of the estimated parameters are shown in parentheses.

^y Teq = equivalent of temperature, calculated as $\text{Teq} = (T - T_{\min}) / (T_{\max} - T_{\min})$, where $T_{\min}$ and $T_{\max}$ = minimum and maximum temperature, respectively, WD = wetness duration (in hours), and DP = duration of the dry period between two wet periods (in hours)

^z Reference to figures.

Table 2. Parameter estimates and summary statistics of nonlinear regression analysis relating the relative growth of *Fusicladium eriobotryae* at different temperatures

Isolate	Parameter ^x						R ²
	<i>a</i>		<i>b</i>		<i>c</i>		
	Estimate	SE	Estimate	SE	Estimate	SE	
FE-3	1.612	0.698	1.100	0.740	0.179	0.156	0.87
FE-6	2.629	0.469	1.497	0.198	0.414	0.069	0.98
FE-17	1.837	0.602	1.218	0.364	0.220	0.118	0.93
FE-31	6.916	3.026	2.429	0.471	0.930	0.201	0.97
FE-36	1.885	0.457	1.391	0.286	0.218	0.085	0.97
FE-40	3.760	0.477	2.29	0.170	0.487	0.045	0.99

^x Regression equation is $y = a \times \text{Teq}^b \times (1 - \text{Teq})^c$, where y is the relative growth of the colonies (calculated by dividing the growth rate at any temperature by that at the optimum temperature); a , b , and c are the equation parameters; and Teq is an equivalent of temperature calculated as $\text{Teq} = (T - T_{\min}) / (T_{\max} - T_{\min})$, where T is the temperature regime, $T_{\min} = 0^\circ\text{C}$, and $T_{\max} = 26^\circ\text{C}$. SE is the standard error of each parameter.

2). However, the regression of estimated versus observed data had a slope not significantly different from 1, and the intercept was different from 0 at $P = 0.02$ (Fig. 3). The CRM was -0.013 , which showed a small overestimation of real germination; RMSE was 0.088 and SEest was 0.086. The model showed an increase of the germination rate of *F. eriobotryae* between 0 and 20°C, and then a decrease at higher temperatures, with $T_{\max} = 35^{\circ}\text{C}$. Regardless of temperature, germination increased with wetness duration until 18 h (Fig. 2).

Effect of RH on conidial germination. Compared with germination in free water, germination at 100% RH was reduced by a 24.9% on average. At 100% RH, less than 10% of the conidia germinated, except for 20°C and 36 h of incubation (14.1%). Germination at RH between 81 and 99% was lower than 2% for all temperatures and incubation periods tested, also at the optimum temperature of 20°C.

Effect of dry periods on conidial germination. The germination of *F. eriobotryae* conidia diminished as the duration of the dry period between two wetness periods increased. When the initial wetness period was 6 h long, the germination decreased from 17.6% (0 h of dry period) to 2.9% (72 h of dry period) at 20% RH during the dry period; germination decreased to 8.8% at 60% RH. When the initial wetness period was 12 h long, the germination decreased from 27.3 to 4.1% at 20% RH during the dry period and 5.2% at 60% RH. The overall effect of dryness on germination was described by a logarithmic equation (Table 1, equation 3; Fig. 4) in the form $y = a \times \ln(DP) + b$, where y is the relative germination rate referred to the maximum observed, a and b are the equation parameters, and DP is the duration of the dry period between two wet periods (in hours). The equation fitted the data with $R^2 = 0.98$; standard errors for the estimated parameters were low (Table 1, equation 3). The germination curve continuously decreased as the length of dry period increased, with 50% of reduction in the first 10 h of dryness (Fig. 4).

Effect of temperature and wetness duration on leaves infection. *F. eriobotryae* conidia caused infection at temperatures between 10 and 20°C. No infection was observed at 25°C. At 10, 15, and 20°C, infection occurred with a minimum of 12 h of wetness; both disease incidence and severity increased as the wetness dura-

tion increased. Disease incidence (percentage of leaves with scab lesions) was 28.3 and 30.0% after 18 h of wetness at 15 and 20°C, respectively; incidence was 53.0 and 55.0% after 60 h for the two temperatures, respectively (Fig. 5A). At 10°C, only 3.3% of leaves were infected after 18 h and 37.7% after 60 h. Disease severity (percentage of leaf area with scab lesions) showed a similar trend, with 30.7 and 31.9% after 60 h of wetness at 15 and 20°C, respectively (Fig. 5B). At 10°C, disease severity after 60 h of wetness was 17.4%.

The effect of temperature and wetness duration on either disease incidence or severity was described by the combination of β and Gompertz equations in the form described for conidial germination (Table 1, equations 4 and 5; Figs. 6 and 7). For disease incidence and severity, the model provided $R^2 = 0.942$ and 0.931, respectively (Table 1, equation 4 and 5). The regression equations of estimated versus observed data had slopes and intercepts not significantly different from 1 and 0, respectively (Fig. 8). Moreover, SEest was 0.085 for incidence and 0.073 for severity, and RMSE was 0.007 for incidence and 0.006 for severity. However, CRM

Fig. 3. Predicted versus observed data on relative germination of *Fusicladium eriobotryae* conidia. Data are predicted by equation 2 of Table 1. Whiskers are the standard errors calculated over different experiments and fungal isolates.

Fig. 2. Relationship between temperature, wetness duration, and relative germination of conidia of *Fusicladium eriobotryae* as predicted by equation 2 of Table 1. Relative conidial germination is calculated by dividing percentage of germinated conidia in each observation by the maximum germination found in each experiment.

was negative in both cases (-0.019 for incidence and -0.033 for severity), which express the tendency of the equations to overestimate the infection. The model developed showed, in both cases, that the relative percentage of affected leaves increased with temperature and wetness duration (Figs. 6 and 7). The lag phase was longer for disease severity than incidence but maximum values of incidence and severity occurred at the same conditions (i.e., 60 h of wetness at 20°C).

Discussion

This is the first published study that investigates the effect of environmental factors on mycelial growth and conidial germination of *F. eriobotryae*, and on the ability of the pathogen to infect loquat leaves. These results increase the current knowledge of the epidemiology of this pathogen and of its life cycle, which are both essential steps to develop a disease prediction system.

Mycelium of *F. eriobotryae* was able to grow and conidia to germinate in a wide range of temperatures. Temperature requirements obtained are similar to those of other pathogens in the genus *Venturia*, such as *V. inaequalis* (2), *V. nashicola* (18), *F. carpophilum* (16), and *F. oleaginum* (23). *F. carpophilum* showed, like *F. eriobotryae*, a slow growth at 10°C and low percentage of germination at 15°C (16). However, for *V. inaequalis*, *V. nashicola*, and *F. oleaginum*, the rate of germination was higher at low temperatures compared with *F. eriobotryae*. For *V. inaequalis* and *V. nashicola*, almost all the conidia germinated at 10°C and 50% of them at 5°C (2,18); 40% of *F. oleaginum* conidia germinated at both temperatures but no germination occurred at 30°C (23). In general, in the above pathogens, temperature had no decisive influence on the germination of conidia because high germination was achieved within a wide range of temperatures (5 to 30°C). In this work, the temperature range was similar, although more conidia germinated and the mycelium grew faster between 15 and 25°C, indicating that the temperature range for optimal development of *F. eriobotryae* is narrower, as in the case of *F. carpophilum* (16).

Conidial germination of *F. eriobotryae* was strongly influenced by moisture and substantial germination occurred only in free water. Obanor et al. (32) observed that conidia of *F. oleaginum* did not germinate after 48 h of incubation at 60 and 80% RH. No germination of conidia of *F. carpophilum*, *V. inaequalis*, and *V. nashicola* was observed when they were incubated with less than 98% RH (18,19) but germination occurred between 98 and 100% RH, showing that these pathogens are able to germinate in the absence of free water.

Conidia germinated after 6 h of continuous wetness between 15 and 30°C and after 12 h at 5 and 10°C. These wetness require-

ments were slightly different than those reported for *V. inaequalis* (2) and *V. nashicola* (18), in which 5 h of continuous wetness were enough for germination at 5 and 30°C and 2 h for germination at 15 to 25°C. In the case of *F. oleaginum*, conidial germination required 24 h of wetness at 5°C and 9 h at 20°C (23). For all these pathogens, conidial germination increased with increasing wetness duration (2,18,23); for *F. eriobotryae*, no increase in germination occurred after 24 h of wetness, regardless of the temperature.

Conidia of *F. eriobotryae* were susceptible to dry periods interrupting two wet periods. Li et al. (17) reported 70% of mortality of conidia of *V. nashicola* after 10 h of dryness at high temperatures (28°C) and a reduction of 40% at 20°C. Also, conidia of *F. oleaginum* and *V. pyrina* were affected by dryness. In the former fungus, a reduction of 50% in the number of lesions in olive leaves which have been inoculated with conidia was observed after 10 h of dryness and no lesions developed after 24 h (70% RH during the dry period) (24). In the case of *V. pyrina*, no lesions developed when inoculated leaves remained dry for more than 12 h at RH <70% (36). On the contrary, Becker and Burr (1) reported that, in the case of *V. inaequalis*, the viability of ungerminated conidia was not affected by exposure to a dry period of less than 24 h, regardless of the RH level (60 and >90%), and decreased only slightly after 96 h of dryness.

The infection of loquat leaves by *F. eriobotryae* was influenced by both temperature and wetness duration. The minimal wetness period required for infection (12 h) is in agreement with that observed for *F. oleaginum* (24,38) whereas, for *V. inaequalis*, *V. nashicola*, and *V. pyrina*, at the optimal temperature of 20°C, scab symptoms appeared after 6, 9, and 9 h, respectively (13,17,33,37). *F. effusum* showed infection after 2 h of wetness at temperatures of 10 and 15°C but needed 9 h of wetness for 20 to 30°C (16). For all the above pathogens different from *F. eriobotryae*, symptoms developed also at 25°C. In *F. eriobotryae*, the increase of leaf wet-

Fig. 4. Relationship between length of dry periods and relative germination of *Fusicladium eriobotryae* conidia as predicted by equation 3 of Table 1. Symbols represent observed data and whiskers are the standard errors calculated over different experiments and fungal isolates. Relative conidial germination is calculated by dividing percentage of germinated conidia in each observation by the maximum germination found in each experiment

Fig. 5. Effect of temperature and wetness duration on scab **A**, incidence and **B**, severity in loquat leaves inoculated with *Fusicladium eriobotryae*. Each column represents the mean disease incidence of two experiments with five replicate plants each. Incidence was assessed as the percentage of leaves with scab lesions per plant and severity as the percentage of leaf area with scab lesions. Whiskers represent the standard error.

ness duration resulted in higher disease incidence and severity of loquat scab until 60 h of wetness. Also for *F. effusum*, *F. oleaginum*, and *V. nashicola*, the development of scab symptoms increased until 48, 96, and 50 h of wetness, respectively (11,17,24,38). Hartman et al. (13) and Villalta et al. (37) reported, at the optimal temperature of 20°C, no increase in scab development after 14 and 30 h of wetness for *V. inaequalis* and *V. pyrina*, respectively.

The results obtained in this study indicate that mild temperatures and long wet periods are necessary for loquat scab infection to occur. Moreover, viability of conidia is substantially reduced by dry periods. In the Mediterranean areas where loquat is grown, temperature seems not to be a limiting factor for the disease in late fall and spring, because medium values range between 15 and 25°C (14). In contrast, moisture might be the limiting factor for infection by *F. eriobotryae*; for instance, only six periods with

Fig. 6. Relationship between temperature, wetness duration, and relative scab incidence of *Fusicladium eriobotryae* on loquat leaves as predicted by equation 4 of Table 1. Relative disease incidence is calculated by dividing incidence for each plant by the maximum incidence found in the experiment.

Fig. 7. Relationship between temperature, wetness duration, and relative scab severity of *Fusicladium eriobotryae* on loquat leaves as predicted by equation 5 of Table 1. Relative disease severity is calculated by dividing percentage of severity for each plant by the maximum severity found in the experiment.

more than 60 h of interrupted wetness occurred in the loquat production area of the Comunidad Valenciana (eastern Spain) in the 2011–2012 period (14).

The information obtained in this study shows that *F. eriobotryae* has temperature and moisture requirements similar to those of *F. oleaginum*, causal agent of olive scab, and another pathogen of Mediterranean crops (23,24,38). In contrast, *F. eriobotryae* has shown substantial differences compared with other members of Venturiaceae that affect crops from different climatic zones, such as apple (*V. inaequalis*), pear (*V. nashicola* and *V. pyrina*), or pecan (*F. effusum*). In the latter pathogens, the germination rate of conidia was higher at low temperatures (5 to 15°C) and the wetness requirement for germination to start or infection to occur was shorter (2 to 6 h) (11,13,33,37). Moreover, conidia of *V. inaequalis* and *V. nashicola* were able to germinate also in the absence of free water (18,19).

In the main loquat cultivation areas of Spain, the regional plant health services use the Mills-Laplane tables (20) to estimate the risk of infection by *F. eriobotryae* (12). These tables have been developed to control apple scab (19) and have been used for other diseases such as pear scab (37) and grape powdery mildew (35). This work has shown that conidia of *F. eriobotryae* require longer times for leaf infection to occur than those described by the Mills-Laplane tables for *V. inaequalis*, and the temperature range in which infection occurs is quite different. Therefore, the Mills-Laplane criterion might lead to an overestimation of the infection periods of *F. eriobotryae* and, consequently, to unjustified alarms provided to growers, which might result in unnecessary spray of fungicides. Therefore, to improve the accuracy and robustness of

infection period estimation for *F. eriobotryae*, specific equations need to be developed and used in the control of loquat scab.

In the present research, equations which describe the growth, germination, and infection of *F. eriobotryae* at different weather conditions have been developed, which provided a reasonable fit to the biological processes investigated, as demonstrated by the goodness-of-fit. The equations were first fit to individual fungal isolates; after analyzing the equation parameter estimates for the different strains and their confidence intervals, the data of all strains were pooled in a unique equation. Therefore, the equation parameters estimated for the pooled data were consistent over the different isolates and the equations are supposed to estimate the epidemiological parameters of *F. eriobotryae* robustly, regardless of the strains (21,23,24,36,38). The possibility of using these equations to predict scab occurrence in the field needs to be tested in further experiments.

The equations developed in this work could be further integrated in a disease prediction system for scheduling fungicide sprays against loquat scab. In order to develop a comprehensive loquat scab prediction model, further studies are necessary. In particular, information on factors influencing production and dispersal of *F. eriobotryae* conidia and on changes of fruit susceptibility at different growth stages is required. This is currently under investigation.

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Literature Cited

1. Becker, C. M., and Burr, T. J. 1994. Discontinuous wetting and survival of conidia of *Venturia inaequalis* on apple leaves. *Phytopathology* 84:372-378.
2. Boric, B. 1985. Influence of temperature on germinability of spores of *Venturia inaequalis* (Cooke) Winter, and their viability as affected by age. *Zastita Bilja* 36:295-302. (In Serbian)
3. Caballero, P., and Fernández, M. A. 2002. Loquat, production and market. *Opt. Mediterr. Ser. No.* 58:11-20.
4. Calabrese, F. 2006. Origen de la especie. In: *El cultivo del níspero japonés*. M. Agustí, C. Reig, and P. Undurraga, eds. Pontificia Universidad Católica de Valparaíso, Chile and Universidad Politécnica de Valencia, España.
5. Campbell, C. L., and Madden, L. V. 1990. *Introduction to Plant Disease Epidemiology*. Wiley, New York.
6. Clewer, A. G., and Scarisbrick, D. H. 2001. *Practical Statistics and Experimental Design for Plant and Crop Science*. John Wiley and Sons Ltd., Chichester, UK.
7. De Wolf, E. D., and Isard, S. A. 2007. Disease cycle approach to plant disease prediction. *Annu. Rev. Phytopathol.* 45:91-918.
8. Dingra, O. D., and Sinclair, J. B. 1985. *Basic Plant Pathology Methods*. CRC Press, Inc. Boca Raton, FL.
9. Eikemo, H., Gadoury, D. M., Spotts, R. A., Villalta, O., Creemers, P., Seem, R. C., and Stensvand, A. 2011. Evaluation of six models to estimate ascospore maturation in *Venturia pyrina*. *Plant Dis.* 95:279-284.
10. Gladieux, P., Caffier, V., Devaux, M., and Le Cam, B. 2010. Host-specific differentiation among populations of *Venturia inaequalis* causing scab on apple, pyracantha and loquat. *Fungal Genet. Biol.* 47:511-521.
11. Gottwald, T. R. 1985. Influence of temperature, leaf wetness period, leaf age, and spore concentration on infection of pecan leaves by conidia of *Cladosporium carygenum*. *Phytopathology* 75:190-194.
12. GVA. 2012. Butlletí d'Avisos—Diciembre núm. 12/2012. Generalitat Valenciana, Conselleria d'Agricultura, Pesca i Alimentació, València, Spain.
13. Hartman, J. R., Parisi, L., and Bautrais, P. 1999. Effect of leaf wetness duration, temperature, and conidial inoculum dose on apple scab infections. *Plant Dis.* 83:531-534.
14. IVIA. 2012. Instituto Valenciano de Investigaciones Agrarias. <http://riegos.ivia.es/>
15. James, J. R., and Sutton, T. B. 1982. Environmental factors influencing pseudothecial development and ascospore maturation of *Venturia inaequalis*. *Phytopathology* 72:1073-1080.
16. Lawrence, E. G., Jr., and Zehr, E. I. 1982. Environmental effects on the development and dissemination of *Cladosporium carpophilum* on peach. *Phytopathology* 72:773-776.
17. Li, B.-H., Xu, X.-M., Li, J.-T., and Li, B.-D. 2005. Effects of temperature, and continuous and interrupted wetness on the infection of pear leaves by conidia of *Venturia nashicola*. *Plant Pathol.* 54:357-363.
18. Li, B.-H., Zhao, H.-H., Li, B.-D., and Xu, X.-M. 2003. Effects of temperature, relative humidity and duration of wet period on germination and infec-

Fig. 8. Predicted versus observed data on relative A, incidence and B, severity of *Fusicladium eriobotryae* infection on loquat leaves. Data are predicted by equations 4 and 5, respectively, of Table 1.

- tion of conidia of pear scab (*Venturia nashicola*). Plant Pathol. 52:546-552.
19. MacHardy, W. E. 1996. Apple Scab: Biology, Epidemiology, and Management. American Phytopathological Society, St. Paul, MN.
20. Mills, W. D., and Laplante, A. A. 1951. Diseases and insects in the orchard. Cornell Ext. Bull. 711.
21. Mwakutuya, E., and Banniza, S. 2010. Influence of temperature and wetness periods on the development of Stemphylium blight on lentil. Plant Dis. 94:1219-1224.
22. Nash, J. E., and Sutcliffe, J. V. 1970. River flow forecasting through conceptual models part I. A discussion of principles. J. Hydrol. 10:282-290.
23. Obanor, F. O., Walter, M., Jones, E. E., and Jaspers, M. V. 2008. Effect of temperature, relative humidity, leaf wetness and leaf age on *Spilocaea oleagina* conidium germination on olive leaves. Eur. J. Plant Pathol. 120:211-222.
24. Obanor, F. O., Walter, M., Jones, E. E., and Jaspers M. V. 2010. Effects of temperature, inoculum concentration, leaf age, and continuous and interrupted wetness on infection of olive plants by *Spilocaea oleagina*. Plant Pathol. 60:190-199.
25. Reig C., Mesejo C., Martínez-Fuentes A., Iglesias D. J., and Agustí M. 2013. Fruit load and root development in field-grown loquat trees (*Eriobotrya japonica* Lindl.). J. Plant Growth Regul. 32:281-290.
26. Rossi, V., Giosuè, S., and Bugiani, R. 2007. A-scab (Apple-scab), a simulation model for estimating risk of *Venturia inaequalis* primary infections. EPPO Bull. 37:300-308.
27. Rossi, V., Salinari, F., Patteri, E., Giosuè, S., and Bugiani, R. 2009. Predicting the dynamics of ascospore maturation of *Venturia pyrina* based on environmental factors. Phytopathology 99:453-461.
28. Roubal, C., Regis, S., and Nicot, P. C. 2013. Field models for the prediction of leaf infection and latent period of *Fusicladium oleagineum* on olive based on rain, temperature and relative humidity. Plant Pathol. 62:657-666.
29. Sanchez-Torres, P., Hinarejos, R., and Tuset, J. J. 2007. *Fusicladium eriobotryae*: hongo causante del moteado del níspero en el mediterráneo español. Bol. Sanid. Veg. Plagas. 33:87-98.
30. Sánchez-Torres, P., Hinarejos, R., and Tuset, J. J. 2007. Identification and characterization of *Fusicladium eriobotryae*: fungal pathogen causing Mediterranean loquat scab. Acta Hort. 750:343-348.
31. Sánchez-Torres, P., Hinarejos, R., and Tuset, J. J. 2009. Characterization and pathogenicity of *Fusicladium eriobotryae*; the fungal pathogen responsible for loquat scab. Plant Dis. 93:1151-1157.
32. Soler, E., Martínez-Calvo, J., Llácer, G., and Badenes, M. L. 2007. Loquat in Spain: production and market. Acta Hort. 750:45-47.
33. Spotts, R. A., and Cervantes, L. A. 1991. Effects of temperature and wetness on infection of pear by *Venturia pyrina* and the relationship between pre-harvest inoculation and storage scab. Plant Dis. 75:1204-1207.
34. Teng, P. S. 1981. Validation of computer models of plant disease epidemics: a review of philosophy and methodology. J. Plant Dis. Prot. 88:63-49.
35. Thomas, C. S., Gubler, W. D., and Leavitt, G. 1994. Field testing of a powdery mildew disease forecast model on grapes in California. (Abstr.) Phytopathology 84:1070.
36. Villalta, O., Washington, W. S., Rimmington, G. M., and Taylor, P. A. 2000. Influence of spore dose and interrupted wet periods on the development of pear scab caused by *Venturia pyrina* on pear (*Pyrus communis*) seedlings. Australas. Plant Pathol. 29:255-262.
37. Villalta, O., Washington, W. S., Rimmington, G. M., and Taylor, P. A. 2000. Effects of temperature and leaf wetness duration on infection of pear leaves by *Venturia pyrina*. Aust. J. Agric. Res. 51:97-106.
38. Viruega, J. R., Roca, L. F., Moral, J., and Trapero, A. 2011. Factors affecting infection and disease development on olive leaves inoculated with *Fusicladium oleagineum*. Plant Dis. 95:1139-1146.