

Summary

Scab, caused by *Fusicladium eriobotryae*, is the main disease affecting loquat in Spain and other countries in the Mediterranean basin. This fungus attacks young twigs, leaves and fruits, causing circular olive-colored spots. Scabby fruits are unsuitable for the market, resulting in significant economic losses. *Fusicladium* spp. are the anamorphic stages of the ascomycete genus *Venturia*, but the sexual stage of *F. eriobotryae* has never been found in nature. This genus includes important scab pathogens, such as *Venturia inaequalis* on apple, *V. pyrina* on pear or *Fusicladium oleagineum* on olive.

Although loquat is an important source of income in the main Spanish cultivation areas, *F. eriobotryae* has received little attention by plant pathologists and farmers have been aimed to manage loquat scab according to the information available for apple scab. In this scenario, years with favorable environmental conditions for the disease result in severe economic losses. Therefore, an improvement in the knowledge of loquat scab is needed and has been addressed globally in this thesis with the overall aim of developing specific tools to manage the disease.

First of all, the effect of environmental factors on mycelial growth, conidial germination and the infection of loquat leaves by *F. eriobotryae* was studied, and equations describing these processes were developed. Mycelium of *F. eriobotryae* was able to grow and conidia to germinate in a wide range of temperatures (5-25°C), although more conidia germinated and the mycelium grew faster between 15 and 25°C. Substantial germination of *F. eriobotryae* conidia occurred only after 12 h of wetness and its viability was reduced by dry periods. Infection of loquat leaves by *F. eriobotryae* occurred between 10 and 20°C and with at least 12 h of continuous wetness.

In addition, dispersal of conidia of *F. eriobotryae* was investigated in two loquat orchards in Spain during two growing seasons. *F. eriobotryae* conidia were collected between March and May and 90% of them during rainy periods. Based on ROC and Bayesian analysis, using ≥ 0.2 mm rainfall as a cut-off value resulted in a high probability of correctly predicting actual conidial dispersal, with a low probability of failing. Based on the index of dispersion and the binary power law, the incidence of loquat scab on fruit was highly aggregated in space between and within trees, and aggregation was influenced by disease incidence. The results demonstrated that *F. eriobotryae* is dispersed mainly in rain splash.

The results obtained in the previous research were used to develop a mechanistic, dynamic model to predict infection of loquat fruit by conidia of *F. eriobotryae*. The model simulates scab infection periods and their severity through the sub-processes of spore dispersal, infection, and latency. Change from one state to the following one depends on environmental conditions and on

processes described by mathematical equations. The model was validated by comparing model output with three independent data sets. The model accurately predicts the occurrence and severity of infection periods as well as the progress of loquat scab incidence on fruit (with concordance correlation coefficients >0.95). Moreover, model output agreed with expert assessment of the disease severity in seven loquat-growing seasons.

As a tool for model evaluation, and for further assessments of loquat scab severity, a standard area diagram set (SADs) was developed. The SADs consists of eight black and white images exhibited the typical symptom patterns of loquat scab on fruits. The SADs improve the accuracy and reliability of the estimates by inexperienced rather than experienced raters.

Another valuable tool developed was a nested-PCR protocol for *F. eriobotryae* identification from pure culture or infected loquat tissues. A specific primer was designed in the EF1- α gen, which combined with the universal one EF1-986R, was able to differentiate *F. eriobotryae* from other pathogens belonging to the genus *Venturia* and from fungal species commonly present in loquat tissues. This protocol can be useful for routine diagnosis, disease monitoring programs and epidemiological research.

One of the goals of this thesis was to evaluate the efficacy of the main fungicide classes against *F. eriobotryae*. Thirteen fungicides were evaluated *in vitro* by testing their effect on mycelial growth and conidial germination. The results showed that the fungicides currently recommended in Spain by the regional plant health services against loquat scab are able to reduce both, mycelial growth and conidial germination. Additionally, a growth chamber experiment was conducted to determine the pre- and post-infection activity of five selected fungicides. Difenoconazole and pyraclostrobin applications resulted in relative disease severity (RDS) values lower than 5%, even when applied 7 days before or after inoculation, whereas boscalid and mancozeb showed good pre-infection activity.

Finally, *F. eriobotryae* resistance to the site-specific fungicides difenoconazole (DMI) and thiophanate-methyl (MBC) was determined by inhibition of mycelial growth on fungicide-amended media. To this aim, 249 *F. eriobotryae* isolates were collected from the main loquat production provinces of Spain (Alicante, Almería, Castellón, Granada, and Valencia). A wide distribution of *F. eriobotryae* isolates resistant to difenoconazole, present in 4 out of the 5 provinces surveyed, was found, while isolates resistant to thiophanate-methyl were present only in Alicante province. In this province, almost 15% of the isolates were resistant to this fungicide and *F. eriobotryae* isolates with difenoconazole/thiophanate-methyl multiple resistances were also detected.

Isolates resistant to thiophanate-methyl were molecular characterized by sequencing of the MBC-target encoding the β -tubulin gene. Results showed that all of the *F. eriobotryae* isolates resistant to thiophanate-methyl contained one of the aminoacid substitutions E198K, F200Y or L240F.