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Development and implementation of cost-effective strategies to manage brown rot of peach trees in Imathia, Greece

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Abstract Development of cost-effective strategies to manage brown rot caused by *Monilinia laxa* of peach implies a better understanding of the susceptibility of different cultivars and pre-harvest contamination. This study investigated the susceptibility of 24 peach and nectarine cultivars to shoot blight caused by *Monilinia* spp. and found various levels of susceptibility, with the nectarine cultivar Tasty Free scored as the most susceptible. Studies on the the existence and detection of latent infections by *Monilinia* spp. in three peach ('A37', 'Andross', and 'E-45') and three nectarine ('Venus', 'Fantasia', and 'Tasty Free') cultivars were also conducted. The results showed that latent infections were detected only in nectarine cultivars when fruit were collected on 23 May and 22 June. In contrast, nectarine fruit collected on 7 June and all peach cultivars tested had no detectable latent infection. This study also indicated that the fungicide thiophanate methyl applied at the pit hardening stage reduced significantly the percentage of latent infection and subsequently preharvest fruit rots. Finally, a disease forecast model to predict blossom blight, caused by *M. laxa*, was evaluated in the Prefecture of Imathia, Greece. Trees, sprayed according to the model predictions, showed a statistically lower percentage of blighted shoots than those of unsprayed trees.

Keywords Blossom blights · Fruit rot · *Monilinia* · Shoot blight

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Introduction

Peach and nectarine are of the most important crops of fruit trees in Greece. According to FAO (2006), the leading peach and nectarine producing countries in European Union are Italy (1664775 tn), Spain (1255600 tn), Greece (711734 tn), and France (400855 tn). The Prefecture of Imathia in northern Greece is considered the largest area producing canning peaches for domestic and foreign markets, although large quantities of fresh market peach—nectarine and other stone fruit are also produced. However, because of the low elevation of this stone fruit production area and its proximity to the sea, it is often subjected to high humidity that favours infection of stone fruit by fungal pathogens.

Brown rot of stone fruit, caused by fungi of the genus *Monilinia*, is one of the most important diseases of stone fruits (*Prunus* spp.) world-wide (Agrios 2005) causing significant yield losses under favourable weather conditions. The brown rot pathogens can cause two disease phases, blossom and shoot blights in early season and fruit rot in mid and late season (Northover and Cerkauskas 1994; Luo and Michailides 2001a). The bloom season of peach and nectarine trees in Imathia Prefecture may last for approximately 1 month, starting in early March (early-bloom cultivars) and ending in early April (late-bloom cultivars). Two species, *M. fructigena* and *M. laxa*, have been reported as the causes of brown rot of stone fruit in Greece, while the sexual form of *M. fructigena* has not been reported yet (Panagopoulos 2007). Panagopoulos (2007) also reported that *M. laxa* is responsible for the brown rot blossom blight of peaches in Greece.

Previous intensive studies on epidemiological features and management of this disease showed a close relationship of blossom and fruit latent infection with fruit rot (Emery et al. 2000; Luo et al. 2005). Michailides et al. (1996) established positive correlations between incidence of latent infections in immature prune, nectarine, and plum fruit and brown rot incidence of fruit at harvest and postharvest. Similarly, a correlation between latent infection in immature plum fruit and incidence of fruit rot at harvest was reported in Ontario, Canada (Northover and Cerkauskas 1994).

Decision-support systems (DSS) for plant protection have been in commercial use and proved reliable in reducing use of plant protection products without affecting net economic returns in many countries (Murali and Secher 1996; Tamošiunas et al. 2000). Factors important for decision-making in plant protection, such as environmental conditions, cultivar susceptibility to diseases, crop management level and their inter-relationships, have been incorporated into many DSS.

Meteorological factors play a key role in the fungal infection process. Temperature and leaf wetness duration (WD) are important environmental factors affecting the development of blossom blight caused by *Monilinia* spp. (Wilcox 1989). According to Tamm and Fluckiger (1993), mycelial growth of *M. laxa* was observed at 2.5–30ºC with the optimum calculated at 24.8ºC, while the optimal temperatures for infection of fruit by *M. fructicola* was between 20–25ºC. Koball et al. (1997) determined that severity of blossom blight of sour cherry caused by *M. laxa* increased with increased humidity during the incubation period. Longer periods of leaf wetness could bring about more severe infection of fruit than shorter periods of wetness (Biggs and Northover 1988). According to Tamm et al. (1995) the phenological (blossom) stage of trees is another important factor influencing the development of blossom blight. Wilcox (1989) showed that the incidence of blossom blight of sour cherry was influenced by inoculum concentration. Luo et al. (2001) suggested a disease forecasting model to predict the development of brown rot of French prune caused by *M. fructicola* by considering all the above factors.

A regional database containing disease susceptibility data along with other important cultivar characteristics needs to be incorporated into a DSS (Murali and Secher 1996). In addition, the use of resistant cultivars is the most important available method to control brown rot because fungicide applications can then be reduced or applied more efficiently. However, efficacy of some fungicides can be compromised by the selection of pathogen populations resistant to fungicides.

The main aims of this study were a) to evaluate the susceptibility of 24 peach—nectarine cultivars to shoot blight phase of brown rot, b) to investigate the incidence and management of latent infections in three each peach and nectarine cultivars, and c) to evaluate a disease forecasting model to predict the appearance of blossom blight caused by *Monilinia* spp. in the Prefecture of Imathia, Greece.

Materials and methods

Susceptibility of peach and nectarine cultivars to *Monilinia*

In order to determine the susceptibility to brown rot of the various peach and nectarine cultivars grown in the area of Imathias, Greece, we designed the following experiments during 2006 to 2008. Almost all peach and nectarine commercial cultivars (widely-cultivated in Greece) were used in this study and are listed in Table 1. These cultivars were grafted on the peach rootstock GF677 (a hybrid of *Prunus persica*×*Prunus dulcis*) in 1998, in the experimental field of the National Agricultural Research Foundation, Pomology

Table 1 Susceptibility of peach and nectarine cultivars to development of blosson and shoot blight caused by *Monilinia* spp.

Species	Cultivars	Percentage of blighted shoots
Nectarine	Tasty Free	34.9[y]a[z]
Nectarine	Venus	27.5b
Nectarine	Fantasia	23.8b
Peach	Romea	16.1c
Peach	Catherine	15.5c
Peach	Loadel	11.3d
Peach	Fortuna	9.1[de]
Peach	Spring belle	7.7ef
Peach	A37	7.5ef
Nectarine	Kakamas	6.5fg
Peach	E.M. Crest	6.1fg
Peach	Red haven	5.8fg
Nectarine	Fire bright	4.6fgj
Peach	E-45	4.5fgj
Peach	H.d. hale	4.5fgj
Peach	Everts	4.3fgj
Peach	Spring crest	4.2fgj
Peach	Andross	4.0fgj
Peach	June gold	3.8gj
Peach	Sunglo	3.3j
Peach	Crest haven	2.8j
Nectarine	IB42	2.5j
Peach	M. Bianca	2.2j
Nectarine	Caltesse 2000	1.8j

[y] Values are the mean of three years (effect of years was not significant (P=0.102)). Estimates are based on 3 replicates of 2 trees each (experimental unit).

[z] Values followed by different letters are significantly different (P<0.05) according to Wald Test.

Institute, in Naoussa, Greece. The trees were planted at a space of 4.0×4.5 m, on a silt-clay-loam, alkaline (pH7.5) soil with medium fertility and favourable physical and chemical properties for water and nutrient retention and distribution. The trees were pruned to a vase shape by hand pruning and sprayed once with Bordeaux mixture at the leaf fall stage in the fall. Trees showed similar canopy vigour as determined visually. There were 3 replications of 2 trees for each cultivar as an experimental unit in a randomized arrangement.

To ensure the presence of the overwintering inoculum and natural infections, 3–5 mummies were left on each tree and no fungicide was applied throughout the bloom period. Infection by *Monilinia* spp. was evaluated annually by recording the number of blighted shoots per tree out of 20 shoots randomly marked about one month before the appearance of the disease. Blighted shoots were pruned out after assessment in 2006 and 2007.

Detection of latent infection

To determine the importance of latent infection on disease levels at harvest, the incidence of latent infections of immature fruit was determined in selected peach and nectarine cultivars. The overnight freezing incubation technique (ONFIT; Luo and Michailides, 2001a,b) was used to detect latent infections in three peach ('A37', 'Andross', and 'E-45') and nectarine ('Venus', 'Fantasia', and 'Tasty Free') cultivars located in three different locations (Episkopis Anthemion, Mesi Verias, and Pomology Institute). There were 5 replicated trees in a randomized arrangement for each cultivar in each experimental field. The spraying programme followed in these orchards was that of growers and included one application of wettable sulphur and captan in April and a second wettable sulphur spray in May.

Fruit samples were collected at random from all around of each tree canopy on 23 May (pit hardening), 7 June, and 23 June. Twenty fruit of similar size per replicate tree were chosen in each collection. The fruit samples were brought to the laboratory and subjected to the following procedure. Plastic containers (40×24×12 cm) and screens were sterilized by soaking in 10% commercial bleach for at least 8 h. For each sample, fruit were surface sterilized in a chlorine solution (32 ml of 0.525% sodium hypochlorite, 32 ml of 95% ETOH, and 0.01 ml of surfactant Tween 20 in 2 l of water) for approximately 15 to 20 min. The fruit were washed with sterile distilled water 10 times and placed on a sterilized plastic screen in a container with 150 ml of water at the bottom (fruit of each tree were placed in separate containers). The containers were placed in a freezer at −16°C for 10 h initially and subsequently incubated on a laboratory bench at 23±2°C for 5 days. By this time, fruit covered with sporulation of *Monilinia* was recorded, and incidence as a percentage of fruit with brown rot was calculated for each sample.

Management of latent infection

To determine whether management of latent infection can affect disease levels and help make decisions on timing of fungicide sprays, sprays (by using a hand boom sprayer) with the fungicide thiophanate methyl (THIOPHANATE-METHYL 70WP, 70%, Inagro E.P.E., Athens, Greece) at label rate of 800 mgL^{-1} were applied to six trees of each nectarine cultivar ‘Venus’, ‘Fantasia’ and ‘Tasty Free’ on each 16 May and 25 May (pit hardening). All these trees were located in the same experimental field as described above. The fungicide plot was a randomized design for each date of spray application. The spraying programme was that of the grower’s programme and included one application of wettable sulphur and captan in April and a second spray with wettable sulphur in May. Six untreated trees of each cultivar were used as a control for this experiment.

Samples of 15 fruit each were collected on 20 June from around the canopy of each tree. The ONFIT method, described above, was used to determine the incidence of fruit with latent infection

In addition, the percentage of preharvest fruit brown rot was recorded for all treatments by recording the number of fruit showing brown rot symptoms of a total 50 fruit per tree, randomly collected around the canopy.

Model evaluation

Because stone fruit growers in the area of Imathias, Greece, desire to use fungicides more efficiently, we evaluated a modified version of the disease forecasting model developed by Luo et al. (2001) for *M. fructicola* causing brown rot of French prune in California. In comparison to the original model, temperatures that ranged from 5 to 20ºC instead of 10 to 25ºC were used in order to obtain equations more suitable for describing the effect of temperatures on *M. laxa* development according to the results of Tamm et al. (1995). This modified model was evaluated to forecast brown rot blossom blight on peaches rather than French prune and cherry in previous studies conducted by Luo et al. (2001) and Tamm et al. (1995), respectively. Cultivar susceptibility was not considered because of insufficient amount of information relevant to its role on the occurence of infection.

Experiments were conducted in the experimental field of the Pomology Institute, Naoussa (Prefecture of Imathia) for two consecutive years (2007 and 2008), where a telemetric meteorological station was installed. To ensure the presence of the pathogen’s overwintering inoculum, a nectarine orchard (cv. ‘Arm King’) with 12-yr-old trees, that had shown blossom blight and preharvest fruit rots in the previous season, was selected and no fungicides were applied either at leaf fall or at the end of winter. In addition, mummies (3–5 per tree) from the previous year were left on the trees. The trees were cultivated traditionally, had a vase-shaped system, and received common cultural practices representing commercial practices followed in the area for stone fruit orchards.

Luo et al. (2001) calculated the relative risk for brown rot in French prunes based on several parameters from a linear regression and developed a risk assessment table in which the model forecasts (model prediction of the risk for flower infection) were grouped into: a) no risk, b) low risk, c) moderate risk, and d) high risk. The aim of this work was to estimate the percentage of the blighted shoots in each group. In the second year, trees sprayed with thiophanate methyl at a rate of 800 mgL^{-1} (by using a hand boom sprayer) 3 days before full bloom were used as control. The orchard was partitioned into completely randomized blocks. Trees of the groups ‘low risk’, ‘moderate risk’ and ‘high risk’ were sprayed with thiophanate methyl at the above rate only when there was a respective forecast by the model during bloom. Trees included in the ‘no risk’ group were not sprayed with the fungicide. In general, growers of the region do not spray for shoot blight caused by *Monilinia* spp; they only apply fungicides against brown rot of fruit, 8–10 days before ripening of fruit (preharvest sprays).

All treatments, consisted of three trees each (experimental unit), were randomly arranged in four randomized blocks. Results were collected by recording the number of blighted shoots out of a total of 20 shoots per tree randomly marked about one month before the appearance of the disease. Blighted shoots were pruned out after assessment in 2007.

Statistical analysis

The data expressed in percentages were angular transformed before statistical analysis. To analyse the transformed data for significant differences at α= 0.05, the Generalized Linear Model (Wald's Chi-Square) was applied (SPSS Grad Pack 16 for Windows) and the means presented in the Tables are the back transformed values.

Results

Susceptibility of peach and nectarine cultivars to *Monilinia*

The nectarine cultivar 'Tasty Free' was the most susceptible of all those tested (Table 1). High levels of susceptibility were also found in the nectarine cultivars 'Venus' and 'Fantasia'. The peach cultivars 'Romea' and 'Catherine' had significantly fewer blighted shoots than the nectarines 'Fantasia' and 'Venus', but more than the peaches 'Loadel' and 'Fortuna'. No significant differences were found between 'Loadel' and 'Fortuna'. Furthermore, the cultivars 'Fortuna', 'Spring Belle', and 'A37' showed similar levels of susceptibility. A number of peach cultivars and a single nectarine cultivar showed moderate susceptibility to shoot blight while fewer peach and nectarine cultivars were the least susceptible (Table 1).

Detection of latent infection

In both years, latent infections were detected only in nectarine cultivars when fruit were collected on 23 May and 22 June in all three experimental fields (Table 2). In contrast, no latent infections were detected on fruit collected on 7 June. The three peach cultivars did not show any latent infection in any of the sampling dates (Table 2).

In both years, the cultivar 'Fantasia' showed the highest percentage of latent infection, while the cultivar 'Venus' the least, regardless of the sampling date and the location (Table 2). The cultivar 'Tasty Free' had significantly lower percentage of latent infection than that of 'Fantasia' but higher than 'Venus'.

Management of latent infection

A fungicide spray with thiophanate methyl reduced significantly both the percentage of latent infections and percentage of fruit rot (Table 3). No significant differences were found in the reduction of the percentage of latent infections between the dates of spray applications. However, the spray applied on 25 May was more effective than that applied on 16 May in reducing the percentage of fruit rot.

Model evaluation

In the first year, only the treatments 'low risk' and 'moderate risk' were sprayed on the same date (12 March 2007) (there was no model prediction for high risk during bloom), while, in the second year, the treatments 'low risk', 'moderate risk' and 'high risk' were sprayed again approximately on the same date (14 March 2008). In both years, the unsprayed trees had significantly higher percentages of blighted shoots than the sprayed trees (Table 4). In the first year, the high risk treatment was not sprayed so it was similar to the results for the unsprayed trees. In the second year, trees sprayed according to the model predictions showed a similar incidence of blighted shoots with that of the sprayed control trees.

Discussion

Disease forecasting has become an established component of quantitative epidemiology. Although it is difficult to predict disease incidence as an exact value, estimating a possible range of disease intensity (risk) can be relatively easy. This estimation will provide decision makers with valuable information. The model used in this study was validated by Luo et al. (2001) to predict the incidence of blossom blight in French prune orchards of California. A similar study on phenological analysis of brown rot blossom blight of sweet cherry, caused by *M. laxa*, was conducted by Tamm et al. (1995). Although the present study showed that this model works well in the environmental conditions of the Prefecture of Imathia, Greece, it was impossible to make any correlation between the different levels of risk (low, moderate, high) and the percentage of blighted shoots to make a conclusion for when spray application would be economically acceptable. This occurred because, in the first year, the model prediction suggested low and moderate risk on the same date; while, in the second year, the model predictions suggested all the levels of

Table 2 Percentage of latent infections (no latent infection detected in peach) caused by *Monilinia* spp. in three nectarine cultivars in Imathia, Greece in 2007 and 2008

Cultivars	Percentage of latent infections					
	23/5/2007	7/6/2007	22/6/2007	23/5/2008	7/6/2008	22/6/2008
Pomology Institute						
Fantasia^{x}	20.8^{y}a^{z}	0.0	25.6a	23.2a	0.0	29.2a
Tasty free	13.5b	0.0	14.7b	16.6b	0.0	17.3b
Venus	6.6c	0.0	10.2c	10.1c	0.0	11.4c
Episkopie Anthemion						
Fantasia^{x}	24.1a	0.0	29.6a	22.6a	0.0	24.7a
Tasty Free	12.7b	0.0	11.9b	10.8b	0.0	13.6b
Venus	8.2c	0.0	8.8c	4.5c	0.0	6.5c
Mesi Verias						
Fantasia^{x}	26.3a	0.0	28.4a	27.0a	0.0	29.1a
Tasty free	16.1b	0.0	18.7b	15.5b	0.0	19.4b
Venus	5.7c	0.0	9.3c	4.7c	0.0	7.9c

^{x} Only the results from nectarine cultivars are presented because no disease was observed on peach cultivars in any of the locations.

^{y} Estimates are based on 5 replicates of 20 fruit each.

^{z} Values in the same column followed by different letters are significantly different ($P<0.05$) according to Wald Test.

risk on the same date. In the last 10 years, Greek growers have stopped spraying their peach trees against blossom blight in order to reduce costs of fruit production. However, when weather conditions are favourable for development of brown rot, serious blossom blight and fruit damage can occur in peach orchards of this region. The modified model (using the 5 to 20ºC range of temperatures) may help growers to spray peach trees only in the years with high risk for the development of blossom blights and thus make savings in production costs from avoiding sprays that are not needed.

Improvement of disease forecasting models can be made by incorporating some other factors such as cultivar susceptibility to brown rot (Safran and Levy 1995). According to Rogers and Stevenson (2006), host susceptibility of carrot plants affected the efficacy of weather-based spray programs, resulting in longer spray intervals and fewer fungicide applications on the resistant cultivar Bolero when compared with the susceptible cultivar Fontana. This study reported that the peach and nectarine cultivars showed different level of susceptibility to blossom blights caused by *Monilinia* spp. Similarly, Corazza (1983) tested the susceptibility of peach cultivars to blossom blight and found differences, with the earliest blooming

Table 3 Effect of early spray applications in controlling latent infections and fruit rots caused by *Monilinia* spp. in three nectarine varieties

Cultivars	Control	16 May^{x}	25 May^{x}
Percentage of latent infections			
Venus	11.5^{y}c^{z}	8.2def	1.8 g
Fantasia	20.7b	10.3cde	5.7f
Tasty Free	26.1a	12.2c	7.2ef
Mean	19.43A	7.57B	4.9B
Percentage of Rotted Fruit (%)			
Venus	28.6a	23.2b	16.2c
Fantasia	20.4b	15.1c	11.6d
Tasty free	30.8a	20.2b	16.5c
Mean	26.6A	19.5B	14.8C

^{x} Date of spray with thiophanate methyl.

^{y} Estimates are based on 6 replicates. Treatment × year interaction effects were not significant (P=0.301), so data were combined.

^{z} Values followed by different letters are significantly different ($P<0.05$) according to Wald Test.

Table 4 Incidence of blighted shoots caused by *Monilinia* spp. in the experimental field sprayed according to model signals

Treatments	Percentage of blighted shoots	
	2007	2008
Unsprayed trees	23.5^{y}a^{z}	15.8a
Low risk	7.2b	2.6b
Moderate risk	6.4b	1.5b
High risk	20.1a	1.9b
Sprayed control		2.9b

^{y} Estimates are based on 4 replicates, each of 3 trees.

^{z} Values within each column followed by different letters are significantly different ($P<0.05$) according to Wald Test.

cultivars 'Camden', 'Favorita Morettini III', and 'Springtime' being the most susceptible, while the nectarine cultivar 'Nectared 3' seemed to be the least susceptible. Bassi et al. (1998) also found different level of susceptibility of peach cultivars on fruit rots caused by *M. laxa.* In other studies, Wagner et al. (2005) did not find a correlation between the percentage of infected flowers and the average percentage of fruit surface area infected by *M. fructicola*, or between percentages of infected flowers and incidence of infected fruit after testing 27 peach cultivars and selections.

The relationship between the incidence of latent infections caused by *Monilinia* and the incidence of brown rot of peach fruit has been established previously (Luo and Michailides 2001a; Gell et al. 2008). Emery et al. (2000) suggested that latent infections can serve as a source of inoculum for subsequent fruit rot in peach orchards in Georgia. Luo and Michailides (2001a) demonstrated the usefulness of detection of latent infections for risk analysis in a decision support system for disease management in French prunes. In this study, latent infections were detected only in nectarine cultivars and only at the pit hardening stage and one month later. In contrast, no latent infection was detected 15 days after pit hardening stage. These results are in good agreement with those published by Luo and Michailides (2001a,b) who showed that the susceptibility to latent infection at bloom stage was at a moderate level, increased to reach to the highest level at about pit hardening stage, and subsequently decreased, reaching the lowest level in early June at embryo growth; it then increased again with fruit development and maturity until harvest. In this study, latent infections were not detected in peach cultivars. A possible explanation is given by Lee and Bostock (2007) who suggested that quiescence and development of *M. fructicola* infections in *Prunus* can be influenced by phenols present in host tissue and that changes in the redox environment may influence gene expression and differentiation of structures associated with infection by the pathogen. More investigation should be conducted to find if these peach cultivars remain free from latent infections year after year, or perhaps the years in which this study took place were not condusive to the development of latent infection on the peach cultivars tested. In addition, it must be examined if the phenols described by Lee and Bostock (2007) exist in peach cultivars, but not in nectarine cultivars, explaining the presence of latent infections in nectarines. Furthermore, the layer of trichomes present on peaches, but absent on nectarines may prevent the close contact of the conidia of *Monilinia* spp. with the cuticle of fruit, and thus making spore germination and penetration more difficult on peach than the smooth surface of the nectarine fruit. In addition, the cuticular microcracking on fruit surface that particularly develops in nectarines (Gibert et al. 2007) may facilitate infection by *Monilinia* spp.

The incidence of latent infections affected the percentage of fruit rot showing the importance of developing methods to control latent infections. However, more research is required to understand the importance of latent infections, their effects on fruit rot, and their efficient management. According to Michailides et al. (1995, 1996) sprays in early summer were effective in reducing brown rot of French prunes at harvest and suggested possible effects of these sprays on latent infections that occurred early in the season.

Conclusion

Generally, based on the results of this study, the forecast model described by Luo et al. (2001) can be used (after slight modification) in the Prefecture of Imathia, Greece, to predict the severity of shoot blight caused by *Monilinia* spp. Thus, Greek growers have the option now to spray the peaches only when the model predicts risks of infection. More investigations should be conducted to determine possible correlations between risk levels (low, moderate, high) and the percentage of blighted blossoms and shoots and determine the number of spray applications which could be economically acceptable. The different level of susceptibility between the most important commercial peach and nectarine cultivars shows that this factor is very important and more work should be conducted to help improve (adapt) the forecast model after taking in consideration the most commonly used commercial cultivars of peach and nectarines in Imathia, Greece. In addition, this study demonstrated the importance of latent infection by *Monilinia* spp. for fruit rot development in nectarine cultivars. Growers could make a spray application at the pit hardening stage to possibly reduce the percentage of latent infections and subsequently lower the incidence of fruit rot at harvest.

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