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Influence of relative virulence and latent infections on the development of *Monilinia* to Greek peach orchards

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ABSTRACT

Brown rot is a very important disease of stone fruit worldwide. Host specificity has important implications for disease management. In this study, the pathogenicity and relative virulence of *Monilinia laxa* and *M. fructicola* isolates from peach on different hosts were evaluated. The results of this study showed that all the isolates of *M. laxa* and *M. fructicola* originated from peach were pathogenic on the excised shoots of cherry, peach, plum, apricot, apple and pear shoot segments and the fruit on plum, apple and pear. The behavior of each species was not the same in different crops and there was no evidence for the predominance of either *Monilinia* species. In addition, the incidence of latent infections on peach and nectarine cultivars was investigated. This study showed that there was no latent infection in bloom stage in 2013. In contrast, the percentage of latent infection during the fruit development stage was between 4 and 13% in peach cultivars and 2–15% in nectarine cultivars. Latent infections were only found in bloom stage of peach cultivars in 2015, but not in the different fruit development stages tested. It is possible that the percentage of latent infections on peach and nectarine flower and fruit may different from year to year depending on the weather conditions and cultivar. Finally, the significance of leaving thinned peach fruits in the orchard on the appearance of latent infection was examined. It was found that the percentage of latent infections on fruit was significantly higher in the untreated control compared with a treatment with thiophanate methyl or when the fruits were thinned. No significant difference was found between fruit treated with thiophanate methyl and thinning of fruits. Generally, the results of this study could help on the better management of brown rot disease.

1. Introduction

Brown rot caused by fungi of genus *Monilinia* is the most important fruit rot disease of peaches in Greece. Field losses of peaches can be extensive if conditions are favorable for disease development during the blossom period or during pre-harvest and harvest periods (Hrustić et al., 2012). This disease, caused mainly from the species M. fructicola, is responsible of up 60% yield losses in high value fresh market nectarine and peach cultivars grown in Australia (Holmes et al., 2011).

Understanding the life cycle of a pathogen has a key role on the management methods applied. In Greece, a recent study showed that M. fructicola and M. laxa were responsible for Brown rot on stone fruit trees at frequencies of 41 and 59%, respectively, while M. fructigena was absent (Papavasileiou et al., 2015). The importance of host specificity has been reported for many pathogens, such as Phytophthora (Thomidis, 2001). Although fungi of the genus *Monilinia* are notorious pathogens for many crops, there are very few reports on the pathogenicity and relative virulence of *Monilinia* isolates from different hosts, and this information is very important on the choosing of the resistance crop and cultivar.

Previous intensive studies on epidemiological features and management of this disease showed a close relationship between blossom and fruit latent infection caused by fungi of the genus *Monilinia* with fruit rot (Emery et al., 2000). Luo et al. (2005) 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, correlation between latent infection in immature plum fruit and incidence of fruit rot at harvest was reported in Ontario, Canada (Northover and Cerkauskas, 1994). Previous works conducted in Spanish peach orchards showed that the incidence of latent infection and that of post-harvest brown rot are positively correlated increasing both of them along the crop season: the average incidence of latent infection during the crop season in Spanish peach orchards explains 55% of the total variation in the incidence of post-

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harvest brown rot (Gell et al., 2008). The effect of environmental factors on the latent infections of peach and nectarine has not been extensively investigated.

The source of inoculum is also a key factor for disease management. Although previous works have show that thinned fruits, mummies, and blighted shoots possibly served as the source of inoculum for Monilinia (Luo et al., 2001, 2005; Michailides et al., 1996), there is little information on the effect of thinned fruit on the latent infections of peach.

The main objectives of this study were to investigate (a) the pathogenicity and relative virulence of 10 isolates of M. laxa and 5 isolates of M. fructicola from peach on peach, apricot, cherry, plum, apple and pear fruits and shoots and (b) the effect of environmental factors and thinned fruit on the appearance of latent infections on different peach and nectarine cultivars.

2. Material and methods

2.1. Pathogenicity of Monilinia laxa and Monilinia fructicola isolates

Five M. fructicola and 10 M. laxa isolates (identification was described by Palavouzis et al., 2014), originating from peach, were used in this study.

The excised shoot assay described by Matheron and Mircetich (1985) was used to examine the pathogenicity and relative virulence of the above isolates. Healthy, uniformly vigorous segments of the current season's fruiting woody shoots (second year of growth), 10 cm in length and 1.5–2 cm in diameter, were collected from the same peach (cv Andross), apricot (cv Bebeco), cherry (cv Burlat), plum (cv Black Star), apple (cv Starking) and pear (cv Williams) tree and disinfested in 10% chlorine bleach (containing around 0.5% sodium hypochlorite) solution for 20 min. Prior to inoculation the 10 cm segments were washed with sterilized water and dried at room temperatures (20-25 °C). One hundred and fifty segments were randomly collected from each host, ten for each of 15 *Monilinia* isolates. Ten more segments from each host, inoculated with PDA without mycelium, were used as control. The inoculum, which consisted of a 6 mm diameter plug from the edge of a 10 days old culture, was inserted in the middle of excised shoot pieces under the bark. The wound was wrapped with adhesive tape to prevent desiccation. Inoculated shoot segments were incubated for 10 days at 25 °C in moist chambers, after which the length of the resulting necrosis (counting by subtracting the length of wound from the total length) was recorded. This experiment was conducted twice.

In a second experiment, healthy plum (cv Black Star), apple (cv Starking) and pear (cv Williams) fruits were collected at harvest time and disinfested in 10% chlorine bleach (containing around 0.5% sodium hypochlorite) solution for 10 min. Fruits were washed with sterilized water and dried at room temperatures (20-25 °C). Then, the fruits were artificially inoculated with one of the above *Monilinia* isolate (10 days old culture grown on PDA). For inoculation, a sterilized inoculation needle was used to make a small wound (6 mm diameter x 5 mm deep) on the surface of each fruit, and a 5 mm mycelial plug taken from the edge of colony of each *Monilinia* isolate was placed onto the wound and cover with adhesive tape to avoid dehydration. One hundred and fifty fruits from each host were used, 10 for each isolate. Ten more fruits from each host, inoculated with PDA without mycelium, were used as control. Inoculated fruits were then incubated at room temperatures (about 20–25 °C) for 5 days after which the diameter (in cross axes with the average reported) of resulting rot was recorded. This experiment was conducted twice.

2.2. Incidence of latent infection

The possibly effect of environmental conditions on the incidence of latent infection on flowers and immature fruit was determined in selected peach and nectarine commercial cultivars established in the experimental field of Pomology Institute, Naoussa, Greece. A meteorological station (ADCON Telemetry, Scientact AE – Thessaloniki, Greece) was established in the experimental field to record the climate conditions every 12 min. The flower incubation technique (FIT; Michailides et al., 2007) and the overnight freezing incubation technique (ONFIT; Luo and Michailides, 2001a,b) were used to detect latent infections on flower and immature fruit of 6 peach cultivars (Springcrest, June Gold, Loadel, Everts, Andross, Fayette) and 5 nectarine cultivars (May Grand, Adriana, Venus, Redgold, Tastyfree) in 2013 and 2015. There were 5 replicated trees in a randomized arrangement for each cultivar. The spray programme followed in the experimental field included only three applications (2000 L spray solution/1 ha) of wettable sulphur (Thiovit 80 WG, Syngenta Hellas, 800 g/kg SULPHUR (S) present as elemental sulphur) starting in the mid of April and repeating every 12–15 days.

Flower samples were randomly collected at the growth stage of popcorn and full bloom in both years of experiments. Twenty flower samples from each replicated tree were brought to the laboratory and subjected to the following procedure: surface sterilization of blossoms with 3% commercial bleach for 3 min then washing with sterile water twice and finally incubating at 23–25 °C for 5 days. Results were collected by recording flowers covered with sporulation of *Monilinia*.

Fruit samples were collected at random from all around of each tree canopy on 25 May, 13 June, 28 June, 15 July in 2013 and 7 May, 2 June, 16 June, 6 July 21 July in 2015. 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, fruits were surface sterilized in a chlorine solution (32 ml of 0.525% sodium hypochlorite, 32 ml of 95% ethanol, and 0.01 ml of surfactant Tween 20 in 2 L of water) for approximately 15 min. The fruits were washed with sterile distilled water 3 times and placed on a sterilized 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 24 h initially and subsequently incubated on a laboratory bench at 23 ± 2 °C for 5 days. After this time, fruits covered with sporulation of *Monilinia* were recorded, and disease incidence, as percentage of fruit with brown rot, was calculated for each sample.

2.3. Significance of thinned peach fruit on the appearance of latent infection

All the experiments were conducted in a commercial peach orchard (cv Suncrest; 10-yr-old) located in Episkopie Naoussa, Imathia Greece, for two years (2013 and 2015). The ONFIT method described above was used to detect latent infections in the immature fruit. The orchard was divided in three parts (treatments), 6 × 6 trees each: (a) thinned fruit were completely removed from the ground of plots, (b) thinned fruit were not removed, but sprayed with the fungicide thiophanate methyl (NEOTOΨIN 70 WG, Efthimiadis Hellas) at a rate (80 mg/L) recommended by the manufacturer, and c), the untreated control. Five replicated trees were selected in a randomized arrangement for each treatment. Twenty fruit of similar size were randomly collected from each of the five replicated trees (around of each tree canopy) at 25th May and 2nd June of 2013 and 2015 respectively.