Euphresco

Success story

September 2016

Real-time PCR for the detection of latent infections and the assessment of inocula viability of *Monilinia* spp.

Brown rot caused by *Monilinia laxa*, *M. fructicola* (an EPPO A2 quarantine organism), or *M. fructigena* is a serious fungal disease of peaches. Postharvest losses are typically more severe than preharvest losses, and routinely occur during storage and transport, in some cases even affecting fruit at the processing stage. When the climatic conditions are unfavourable, *Monilinia* infections may remain latent until the conditions for disease development become favourable or the fruit matures and its susceptibility to the disease increases. Latent infections have been described as asymptomatic infections in which a host-parasite relationship has been established or as a dynamic equilibrium between the host, the pathogen, and the environment without any visible sign of disease. The incidence of latent infections ranges between 0 to 30% or even 50% of harvested fruits and most of the fruits are infected the days preceding the harvest. However, most of the latent infections remain asymptomatic until fruits arrive to the markets, which is of especial importance in long distance exports.

Although several molecular tests have been developed to identify and distinguish *Monilinia* species (e.g. van Brouwershaven *et al*. 2010), none of these methods has been used to detect brown rot latent infections. Currently, latent infections are detected using paraquat, an herbicide treatment which causes epidermis senescence and activates latent infection or by freezing the fruits at -20ºC for 48 hours. The disease develops after 5-7 days of incubation; overall, the detection could take up to 10 days.

The main objective of the DIMO project was to test the sensitivity and reproducibility of the real-time PCR test developed by van Brouwershaven *et al*. (2010) to detect brown rot latent infections and distinguish between *Monilinia* spp. on stone fruits.

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The test to detect brown rot latent infection on peaches and nectarines adapted from van Brouwershaven *et al*. (2010), combined with automated DNA isolation allows to obtain results within 2-3 days compared to the time-consuming traditional method. Some *Monilinia* spp. are quarantine organisms in many world countries and speed is of critical importance in the diagnosis of latent infections. The project provides useful information that will support the activities of plant pest diagnostic laboratories.

The real-time PCR proved also useful in the quantification of *Monilinia* conidia viability on different plant material, when combined with a staining dye.

Project ID: Development and validation of molecular tools for detection and identification of European *Monilinia* species (DIMO)

References:

van Brouwershaven, I., Bruil, M., van Leeuwen, G. and Kox, L., 2010. A real-time (TaqMan) PCR assay to differentiate *Monilinia fructicola* from other brown rot fungi of fruit crops. Plant Pathology, 59: 548-555.