

## Rapid detection of *Monilinia fructicola* and *Monilinia laxa* on nectarines through loop-mediated isothermal amplification

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*Monilinia laxa* and *Monilinia fructicola* are two of the causal agents of brown rot, one of the most important diseases of *Prunus*, *Malus* and *Pyrus* species. Losses caused by these fungi can reach up 90% of the total production, as the flowers can be infected during blooming, as well as the twigs and fruits, especially during postharvest. Both fungal species have a worldwide distribution occurring in north and south America, Australia and Japan. After its introduction in Europe in 2001, *M. fructicola* became widespread affecting pears, apples, plums, sweet cherries, apricots, peaches, blackberries and nectarines. Due to the high yield losses caused to peaches, apricots and nectarines *M. fructicola* was included in 2005 in the A2 EPPO List of quarantine organisms. Traditionally, *M. fructicola* identification has been carried out using different DNA-based assays. With this study, however, LAMP, a quick method able to identify these pathogens during postharvest was designed and validated, taking into account its specificity, sensitivity, repeatability and reproducibility in accordance with the international EPPO standard (PM7/98). The sensitivity of the technique was checked by monitoring the fruits of two nectarine cultivars stored at two different temperatures. LAMP assays were compared at different time points against the quantification of the inoculum assessed by previous qPCR assays, demonstrating that they can detect a low number of cells. These LAMP methods could be a useful tool for the monitoring of brown rot causal agents in the field and during storage.

## Evaluation of the potential vector role for the alien planthopper *Ricania speculum* in Flavescence dorée phytoplasma epidemiology

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*Ricania speculum* (Walker) is an exotic planthopper, accidentally introduced in Europe and officially reported in Italy (Genoa, Liguria) in 2014. It is now also spread in Tuscany (provinces of Massa Carrara, Lucca, Pisa). *Ricania speculum* is highly polyphagous on herbaceous and woody plants, either wild or cultivated, and can cause damages by sup suction, honeydew emission and egg-laying. This phloem sap-sucking insect often colonizes grapevines and *Clematis vitalba* plants, which are known hosts of Flavescence dorée phytoplasma (FDp), a quarantine plant pathogen causing severe damages to European viticulture. The purposes of this work were to determine if *R. speculum* could be a vector of FDp and assess its eventual impact on epidemiology of this grapevine disease. Nymphs of *R. speculum* were isolated on FDp-infected broad beans to evaluate

acquisition efficiency and then transferred onto grapevines, broad beans and *C. vitalba* plants to determine transmission capability. *Ricania speculum* was able to acquire FDp, but not to transmit it to any of the inoculated plant species. The pathogen multiplication rate measured within *R. speculum* was significantly lower than that observed within the vector *Euscelidius variegatus*. Consistently with absence of transmission, the phytoplasma was almost never detected in salivary glands dissected from *R. speculum* individuals exposed to FDp. Under our conditions, the role of *R. speculum* in spreading FDp is unlikely to be of any concern. Nevertheless, population and spatial distribution of this alien planthopper are rapidly increasing, and therefore attention threshold in monitoring this pest across Europe should be maintained very high.

## Genotyping by high-resolution melting of *Alternaria* species causing citrus brown spot

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*Alternaria* brown spot is one of the most important diseases of tangerines and their hybrids worldwide. To set up effective control strategy, the accurate detection and identification of the species responsible of the diseases is crucial. However, the characterization based on morphology and/or multilocus genetic approaches is time consuming, requires great expertise, and sometimes is not conclusive. Therefore, the setup of a rapid and efficient DNA-based assay might be of paramount importance. The High-Resolution Melting (HRM) analysis represents an interesting tool for the uncovering of nucleotide variations as small as one base difference, and as such, relevant to species characterization. In the present investigation, a HRM assay based on the *Alternaria* barcoding region OPA1-3 was set up. Specimen strains of the main citrus-associated *Alternaria* species and morphotypes generated distinct and normalized profiles, allowing their differentiation when HRM-tested. Moreover, when the assay was used to screen an *Alternaria* collection from citrus fruit and leaves, it distributed the 180 isolates in three independent clusters, readily and consistently resolved. Isolates were identified as belonging to the species *Alternaria alternata* and the species complex *A. arborescens*. Within *A. alternata*, the morphotypes *alternata* (77% of the collection) and *limoniasperae* (17% of the collection) were present. Although further validation experiments will be performed to optimize the assay for a diagnostic use, this HRM approach might represent a rapid, sensitive, and specific method for the detection and identification of *Alternaria* spp. responsible for citrus brown spot disease.

## Pathogenicity and possible biological control of fungi associated to Botryosphaeria dieback of grapevine in western Sicily

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