Biology and Management of Septoria Spot of Citrus

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Septoria spot of citrus caused by *Septoria citri* occurs mainly on mature, cold-injured fruit in rainy winter seasons. Due to recent outbreaks, Septoria spot has become a quarantine disease in some export countries for California citrus fruit. Fruit marketed to these destinations have to be certified as disease-free.

Characteristic signs of *S. citri* (i.e., fruiting bodies – pyc- nidia, conidia) on fruit lesions are often not present, and thus the disease is difficult to diagnose. In addition, the fungus also grows very slowly, and *S. citri* colonies are often overgrown by other fungi occurring on citrus. Therefore, we developed a new molecular detection assay.

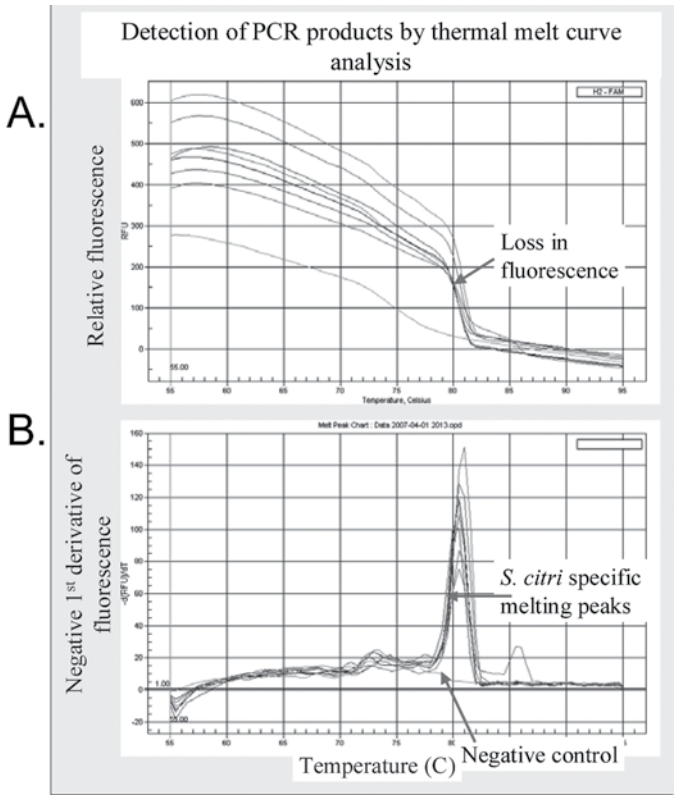

Figure 1. *Thermal melt curve analysis of PCR products using Septoria citri-specific DNA primers. A. Melt curve relative fluorescence over a range of temperatures for 8 samples. B. Negative first derivative of fluorescence melt curve shown in A.*

PCR primers derived from the beta-tubulin gene were designed that specifically amplify *S. citri* DNA in extracts of citrus fruit. Because of the risk of amplicon contamination in a diagnostic laboratory, PCR products are detected non-electrophoretically by thermal melt curve analysis immediately after amplification. PCR and melt curve analysis are done in a single set-up in a real-time thermocycler.

In thermal melt curve analysis of PCR products, the thermal stability of DNA duplexes is measured and graphed as a melt curve (Fig. 1A). The fluorescent dye SYBR Green that is incorporated into the PCR reaction binds to double- stranded DNA molecules. A loss of fluorescence is observed when the dye dissociates from the separating DNA strands at a specific temperature. The negative first derivative of fluorescence is then automatically graphed against a temperature scale and a melt curve is displayed (Fig. 1B). Melt temperatures are specific for different PCR products.

Thus, in samples positive for *S. citri*, specific DNA fragments are produced in PCR amplifications, and their presence is indicated by a peak at a specific melt temperature in a diagram. For verification of DNA quality and to exclude false negatives, a primer pair specific for orange DNA (pro- vided by Dr. M. Roose, UC Riverside) is also used in PCR amplifications. This primer pair produces a second peak at a different melt temperature. The absence of this specific peak in a sample using these orange-specific primers indicates the sample that lacks DNA or has non-amplifiable DNA.

Using both pathogen- and orange- specific PCR primers - with each PCR product having a unique temperature melt curve -a single-step automated procedure was developed to assess suspect lesions for Septoria spot (Fig. 2).

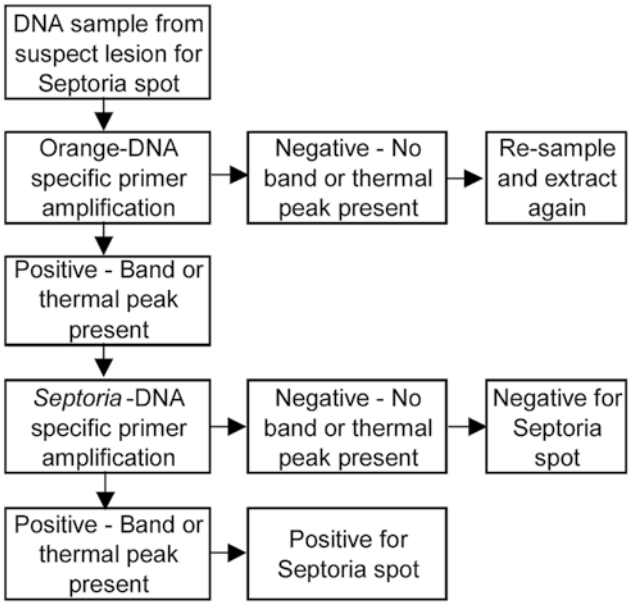

Figure 2. *Flow chart for automation of PCR detection of Septoria spot of citrus.*

This new PCR method was extensively evaluated using orange fruit from the NAVEK (Navel and Valencia Exports to Korea) incubation program at the Kearney Agricultural Center. Samples positive for Septoria spot in fungal isolations were always positive in the PCR assay, whereas samples infected with a range of other fungi never showed a specific melt peak in the assay. Thus, thermal melt curve analysis reliably and rapidly detects *S. citri* in infected citrus fruit, and the procedure will be setting a standard for the detection of this quarantine pathogen.