

A novel fluorescence-based multiplex real-time PCR assay for rapid and simultaneous detection of leafminers

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Liriomyza spp. are leafminer pests associated with imported fresh produce, plants and other commodities. Rapid and precise identification of juvenile individuals of *Liriomyza* spp. is of crucial importance to enable appropriate biosecurity decisions to be made at the border or post-border. We have developed a multiplex TaqMan real-time PCR assay that can simultaneously detect *Liriomyza huidobrensis*, *L. sativae* and *L. trifolii* in a single test. Species-specific primers and probes were designed against existing genomic sequences within the mitochondrial cytochrome oxidase I gene. The real-time assay was specific for *L. trifolii*, *L. huidobrensis* or *L. sativae*, both in simplex and multiplex formats. Serial dilution results showed reliable amplification at a 10^{-4} dilution (1 pg of DNA) and generally even at 10^{-5} dilutions (0.1 pg), which allows the possibility of using only a small amount of tissue for DNA extraction.

