

LONG PCR AS A SENSITIVE METHOD FOR DETECTING ASIAN GREENING DISEASE IN PARASITIDS UNDERGOING RISK ASSESSMENT IN QUARANTINE

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ABSTRACT. The accidental importation of plant pathogens in or on the bodies of parasitoids imported as natural enemies has been raised as a potential risk of classical biological control projects involving insects that serve as vectors of plant diseases. During quarantine evaluation of two parasitoids, *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae) and *Diaphorencyrtus aligarhensis* (Shafee, Alam and Agarwal) (Hymenoptera: Encyrtidae), imported for classical biological control of Asian citrus psylla *Diaphorina citri* (Kuwayama) in Florida, we were asked to determine that these parasitoids were free of the causal agent of Asian greening disease, the bacterium *Liberobacter asiaticum* (L.) Jack. Preliminary tests using allele-specific polymerase chain reaction (Standard PCR) suggested the assays were prone to false negatives. Another PCR protocol, Long PCR, previously was shown to be more reliable than Standard PCR when screening insects for another bacterium (*Wolbachia*) (Jeyaprakash and Hoy, 2000). The sensitivities of Long and Standard PCR protocols were compared using plasmid DNA containing two DNA fragments from the greening disease agent or plasmid mixed with DNA extracted from host plants, psyllids, or parasitoids.

Results indicated that inhibitors of the PCR were present in both plant and insect DNA, making the Standard PCR relatively insensitive and allowing high levels of false negatives. Long PCR, which incorporates a second DNA polymerase with proof-reading activity, yielded consistent results and was orders of magnitude more sensitive than the Standard PCR. As few as 100 copies of plasmid mixed with either plant or insect DNA consistently could be detected. Long PCR assays conducted on the parasitoids *T. radiata* and *D. aligarhensis*, their psyllid hosts, or their host plants failed to produce any positives over a period of six months, indicating that release of these two parasitoids should elicit little concern that greening bacteria would be introduced accidentally into Florida through this classical biological control program.

The full paper was published as follows: Hoy, M. A., A. Jeyaprakash, and R. Nguyen. 2001. Long PCR is a sensitive method for detecting *Liberobacter asiaticum* in parasitoids undergoing risk assessment in quarantine. *Biological Control* 22: 278-287.