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Morphological and molecular identification of all developmental stages of four whitefly species (Hemiptera: Aleyrodidae) commonly intercepted in quarantine

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Abstract

Whiteflies are inadvertently, but commonly, transported in international plant trade. Rapid, accurate identification is the essential first step when such insects are intercepted by quarantine authorities. Whitefly taxonomy, and identification, is almost entirely based on the fourth-larval instar or puparium, but often only the eggs, early larval instars or adults are detected. Morphological descriptions of the egg, first three larval stages and adult are presented for four species commonly detected in trade, *Bemisia afer* (Priesner & Hosny), *B. tabaci* (Gennadius), *Trialeurodes ricini* (Misra) and *Trialeurodes vaporariorum* (Westwood). Morphological characters are provided that enable most life stage/species combinations in these four species to be distinguished. The structure of the antenna is a reliable and simple character for separating the four larval instars. Phenotypic plasticity, previously only reported in the puparial stage, also occurs in the second and third-larval instars. Where morphological separation of two species is sometimes inconclusive, or impossible, identification can be achieved using four real-time PCR assays, designed and validated to distinguish between the four species. The assays are generic in their set-up and can be multiplexed to form two reactions allowing discrimination of *B. afer* and *B. tabaci* in one well, and *T. ricini* and *T. vaporariorum* in another.

Keywords: *Bemisia afer*, *B. tabaci*, *Trialeurodes ricini*, *T. vaporariorum*, identification, adults, larval instars, real-time PCR assay, dichotomous keys

Introduction

Whiteflies are plant sap-sucking insects placed in the family Aleyrodidae in the superfamily Aleyrodoidea (Martin & Mound, 2007). Many species are important agricultural pests and a small number are vectors of plant pathogenic viruses, most notably *Bemisia tabaci* (Gennadius), which is recorded transmitting 111 plant viruses (Jones, 2003). Unfortunately for the agricultural and horticultural industries, the Aleyrodidae is one of the most frequently transported arthropod families in international plant trade. This is partly because the eggs and larval stages are small, sessile, firmly attached to the host plant for most of their development, often inconspicuous, difficult to detect during phytosanitary inspections, and difficult to control. *Bemisia tabaci* is listed in the plant health legislation of the European Union (European Plant Health Directive 2000/29/EC as amended, Annex designations I/A1 and I/B) and is included in the A2 list of organisms of quarantine concern for the European and Mediterranean Plant Protection Organisation. It therefore needs to be identified quickly and accurately when encountered in order for National Plant Protection Organisations (NPPOs) to decide upon the most appropriate course of action.

The two whitefly species most commonly found moving in trade are the cosmopolitan species *B. tabaci* and *Trialeurodes vaporariorum* (Westwood). For both species, a congeneric species with which it might be particularly confused is also being increasingly commonly intercepted, *Bemisia afer* (Priesner & Hosny) and *Trialeurodes ricini* (Misra) respectively. Apart from occasional greenhouse infestations of *B. tabaci* and

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