Widespread Occurrence of an Endosymbiont Bacterium in *Brevipalpus phoenicis* Populations from Citrus in the Southeastern Region of Brazil

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ABSTRACT. *Brevipalpus phoenicis* mites are vectors of Citrus leprosis virus (CiLV), the causal agent of leprosis, an important viral disease of citrus. *Brevipalpus* individuals harbor an endosymbiont bacterium belonging to the phylum *Bacteroidetes*, genus *Cardinium*, which causes feminization in these mites. We determined the prevalence of these endosymbionts in *B. phoenicis* mite populations from different citrus production areas in southeastern Brazil. PCR was performed with primers specific for the amplification of a fragment within the 16S rDNA gene of the endosymbiont. All *B. phoenicis* populations tested yielded bands of the expected size when analyzed by agarose gel electrophoresis and analysis of the sequence of the amplified DNA confirmed the presence and identity of the endosymbiont in *B. phoenicis* populations from different citrus production areas.

Brevipalpus phoenicis (Acari: Tenuipalpidae) mites are known to transmit Citrus leprosis virus (CiLV), the causal agent of leprosis, which is considered the most important viral disease of citrus in Brazil due to the severe symptoms it induces and the high cost involved in controlling the mite vector. The presence of an endosymbiont bacterium belonging to the (phylum *Bacteroidetes*, genus *Cardinium*) was recently reported in a *B. phoenicis* population from coffee trees in São Paulo, Brazil (2, 4).

Several studies have shown that this endosymbiotic bacterium is able to induce a number of reproductive effects in its hosts such as cytoplasmic incompatibility, partheno-genesis and fecundity enhancement in arthropod species. In some cases, e.g. in *B. phoenicis*, the bacterium causes feminization of the mites (2). Here, we report the first effort to determine the prevalence of this endosymbiont in *B. phoenicis* mite populations from different citrus production areas of Brazil.

The populations of *B. phoenicis* were collected from 10 different citrus production areas in the southeastern region of Brazil: Piracicaba, Cordeirópolis, Conchal, Matão, Gavião Peixoto, Barretos, Onda Verde, Bauru, and Monte Alegre do Sul, São Paulo State, and from Teresópolis, Rio de Janeiro State. All populations were maintained at the Entomology Laboratory at the Citriculture Center, Cordeirópolis, SP, Brazil.

Total DNA was extracted as described by Weeks et al. (1) and suspended in 12 µl of sterile distilled water. For polymerase chain reaction (PCR) amplification analysis of mite populations, a region within the 16S rDNA was amplified using primers specific for this sequence in CFB bacteria (2). All PCR reactions contained 4 µl of template DNA, 2.5 µl of 10× PCR buffer, 0.9 µl of 50 mM $MgCl_2$, 0.5 µl of a 10-mM dNTP mixture, 5 pmoles of each primer and 0.2 µl of *Taq* DNA polymerase (5 U/µl) in a total volume of 25 µl. Amplifications were

performed with an initial denaturation at 95°C for 5 min, followed by 30 cycles at 95°C for 30 s, 55°C for 45 s, 72°C for 30s, with a final extension cycle at 72°C for 5 min. Aliquots of PCR products were analyzed by electrophoresis in 1% agarose in TAE buffer.

All *B. phoenicis* populations tested yielded bands of the expected size (832 bp) and sequence analysis of the PCR products from two populations confirmed they were amplified from the correct region of the 16S rDNA gene of the endosymbiont. This data confirmed the identity and the presence of the endosymbiont in *B. phoenicis* populations collected from citrus in the southeastern region of Brazil.

Weeks et al. (3) showed that CFB also infects a significant number of arthropod species, and may be horizontally transmitted. Consequently, other false spider mite populations from citrus are being tested for the presence of the bacterium and more sequencing is in progress in order to assess the genetic variability of this organism using both 16S rDNA and the internal transcribed spacer (ITS) region. In *B. phoenicis* is not clear whether the endosymbiont has any effect besides feminization of the mites, but studies are being carried out to determine if the endosymbiont plays a role in CiLV acquisition and transmission to citrus plants.

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