# SCIENTIFIC NOTE

# Occurrence of *Wolbachia* in Brazilian Samples of *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)

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Ocorrência de *Wolbachia* em Amostras Brasileiras da Mosca-do-Mediterrâneo, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae)

RESUMO - Bactérias *Wolbachia* foram detectadas por PCR seguido de seqüenciamento de um segmento do gene ribossomal 16S em uma amostra de população natural e em duas colônias de laboratório de *Ceratitis capitata* (Wied.) do Brasil. O seqüenciamento do fragmento amplificado mostrou que este é compatível com os de *Wolbachia* tipo A, encontrada em numerosas espécies de insetos. Esta é a primeira descrição de uma infecção natural desse hospedeiro por *Wolbachia* desde que, em linhagens de *C. capitata* provenientes de outras regiões, não foram encontradas essas bactérias.

PALAVRAS-CHAVE: Bactéria, endossimbionte

ABSTRACT - *Wolbachia* bacteria were detected by PCR followed by sequencing of a fragment of the 16S ribosomal gene, in a natural population sample and in two laboratory colonies of the medfly, *C. capitata* (Wied.), from Brazil. Sequencing revealed that the fragment was compatible with the *Wolbachia* type A group found in several insect species. This is the first description of a natural *Wolbachia* infection in *C. capitata*, since several other samples from different regions so far analyzed seemed to be free of infection.

KEY WORDS: Bacteria, medfly, endosymbiont

*Wolbachia* are intracellular alpha-proteobacteria which infest a very large number of arthropods usually affecting their reproductive biology, inducing feminization, parthenogenesis, male killing and cytoplasmic incompatibilities (Werren 1997). The bacteria are transovarially inherited, but may be transmitted horizontally between taxa (Vavre *et al*. 1990, Heath *et al*. 1994). Moreover, artificial transfection has been successfully achieved among several insect species (Braig *et al.* 1994, Sasaki *et al.* 2002, Riegler *et al.* 2004). Due to their ability to manipulate their hosts' reproduction, causing incompatibilities among strains, the use of these bacteria as agents for insect-pest biological control or population suppression has been suggested as an alternative to the sterile insect technique (SIT) (Beard *et al.* 1993, Sinkins *et al.* 1997).

*Wolbachia* was reported to infest species of the tephritid fruit flies (Werren *et al.* 1995, Kittayapong *et al.* 2000, Selivon *et al.* 2002, Riegler & Stauffer 2002), but has not been found in several established strains of the medfly *Ceratitis capitata* (Wied.) (Bourtzis *et al.* 1994). Zabalou *et al.* (2004), successfully infected a medfly strain with *Wolbachia* derived from the tephritid *Rhagoletis cerasi* (L.). Laboratory tests showed that the bacteria induced cytoplasmic incompatibilities in the new host indicating its potential use as an alternative method for the control of these insect-pest populations.

The medfly was introduced in Brazil, where it was first detected in 1901 by Ihering. Since then, it has spread over the country and became a major pest of fruit culture, attacking a large number of commercial fruits (Zucchi 2001). During a screening for *Wolbachia* in Brazilian fruit flies, we found samples of *C. capitata* which were infected by this endosymbiont. For our knowledge, this is the first report on a natural infection of *C. capitata* by *Wolbachia*. Since this information might be useful for control programs of this insect-pest (see Zabalou *et al*. 2004), we report herein the data on *Wolbachia* naturally infecting the medfly in Brazil.

Three samples of *C. capitata* were examined for the presence of *Wolbachia*. One sample came from a natural population collected in oranges from São Bento do Sapucaí,

State of São Paulo (22°46'S/ 45°04'W), and the others, from laboratory colonies maintained at the Centro de Energia Nuclear na Agricultura (CENA/USP) and in our laboratory at the Universidade de São Paulo, all originally collected in Brazilian regions.

Total DNA of *C. capitata* was extracted from adult females and embryos according to the technique described by Jowett (1998). Detection of *Wolbachia* was done by PCR in 10 individual specimens of each sample, using the primers WSpec-f 5´-CATACCTATTCGAAGGGATAG- 3´ and WSpec-r 5´-AGCTTCGAGTGAAACCAATTC- 3´, which amplify a 438 bp fragment of the 16S rDNA gene (Werren & Windsor 2000). The PCR reaction was carried out with 3µl DNA sample, 2.5µl 10x buffer, 0.75µl $MgCl_2$ (50mM), 1µl nucleotide mix (5mM each), 0.35µl (20mM) of each primer, 0.25µl Taq Polymerase (Gibco BRL Life Technologies), completed with distilled deionized water to a final volume of 25µl. The W-Spec amplification conditions consisted of two cycles of two min at 95°C, one min at 60°C, one min at 72°C, 35 cycles of 30 s at 95°C, one min at 60°C, 45 s at 72°C and a final extension of five min at 72°C (Werren & Windsor 2000).The presence of the amplified fragment was checked by electrophoresis on 1.5% agarose gel. The PCR-amplified DNA fragments were sequenced using the two primers above decribed and the Big Dye system (Applied Biosystems) in an automated DNA sequencer ABI377 (Applied Biosystems). Sequence analyses and alignments were made using the software Sequence Navigator (Applied Biosystems) and BLAST at NCBI (National Center of Biotechnology Information, www.ncbi.nih.gov/BLAST).

The *Wolbachia*-specific 16S rDNA 438bp fragment was detected in all individuals from the three samples of *C. capitata.* Seven amplified fragments were totally sequenced, being four from the natural population sample and three from our laboratory colony. A reliable internal portion of 365 bp of the sequence was used in the following sequence analysis. The sequences were identical within and among samples and are available at the GenBank website (www.ncbi.nih.gov, accession # AY910011). The sequence obtained from the fragment showed 95% to 98% identity with *Wolbachia* type A sequences from a large number of insect species available at the GenBank database. Eleven bacterial sequences reached the condition e-value = 0, among which the most similar to the *Wolbachia* sequence here found were from coleopterans [*Diabrotica virgifera* Le Conte, *D. cristata* Le Conte, *D. lemniscata* Le Conte (acession # AY007551, AY007550, AY007547, respectively)], hymenopterans [*Nasonia longicornis* Darling, *N. giraulti* Darling (acession # M84691, M84690, respectively), and the dipteran *Phlebotomus papatasi* (Scopoli) (acession # U80584). The data on *Wolbachia* from tephritid flies (Werren *et al.* 1995, Kittayapong *et al.* 2000, Riegler & Staufer 2002), including brazilian samples of the *Anastrepha* sp2 *aff. fraterculus* (Selivon *et al.* 2002) and from the lepidopteran parasitoid *Trichogramma atopovirilia* Oatman & Platner (Ciociola *et al.* 2001) could not be used for these comparisons since fragments of different genes were amplified.

For our knowledge, this is the first record of *Wolbachia* being naturally hosted by the fruit fly *C. capitata*. The fact that *Wolbachia* was not found in many other strains of *C. capitata* (Bourtzis *et al.* 1994, Zabalou *et al.* 2004) suggests that this insect might have acquired a neotropical strain of *Wolbachia* after its introduction in Brazil. Unfortunately, the thirty sequences (including the above mentioned), ranging from 95-98% similarity to the sequence here found in *C. capitata,* were from insects sampled outside the neotropical region and could not be used to test this hypothesis. An alternative hypothesis would be that the introduced flies derived from populations which were infected with *Wolbachia*, although this infection was not yet detected. Further analysis on neotropical insects could provide clues on the origin of *Wolbachia* infection in Brazilian strains of *C. capitata*.

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