# Establishment of black aphid in Macadamia nut trees in Cuba

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## RESUMEN

*El áfido negro* Toxopteraaurantii *(Boyer de Fonscolombe) (Homoptera: Aphididae), es una plaga polífaga que tiene entre sus hospedantes la* Macadamia integrifolia*. La nuez de Macadamia no tiene relevancia económica para Cuba, pero desde 2012 se comenzaron estudios fitopatológicos en una colección* ex situ *de árboles frutales tropicales y subtropicales localizada en Artemisa, Cuba. Durante los estudios detectamos* T. aurantii *afectando árboles de Macadamia. Luego de su identificación taxonómica, detectamos enemigos naturales y fluctuaciones de los diferentes instares en correspondencia con las precipitaciones mensuales. La detección previa de fitoplasma en diez de las doce plantas de nuez de Macadamia fue empleado para corroborar que los áfidos se alimentaban de los árboles. Con esto proveemos evidencias biológicas y moleculares del establecimiento de* T. aurantii *en la nuez de Macadamia en Cuba, constituyendo el primer informe de nuez de Macadamia como hospedante natural de* T. aurantii.

Palabras claves: *Aphididae, insecto, Toxoptera aurantii, fitopatología*

## ABSTRACT

*The Black Aphid* Toxoptera aurantii *(Boyer de Fonscolombe) (Homoptera: Aphididae), is a polyphagous pest that uses* Macadamia integrifolia *trees as its host. In Cuba, Macadamia trees do not have any economic relevance. Nonetheless, since 2012 an agronomic and phytopathology screening started on an* ex situ *tropical and 'subtropical fruit trees collection in Artemisa, Cuba. During the screening it was detected for the first time that T. aurantii affects Macadamia trees. After the taxonomic identification, was detected the presence of natural enemies and fluctuations of different instars due to the different monthly rain. The previous detection of a phytoplasmin ten of twelve Macadamia trees was used to corroborate that* T. aurantii *was indeed feeding from Macadamia trees. We provided biological and molecular evidences of the establishment of* T. aurantii *in Macadamia trees in Cuba. This is the first report of Macadamia nut trees as natural host for* T. aurantii *in Cuba.*

Key words: *Aphididae, insect, pest, Toxoptera aurantii, phytopathology*

## INTRODUCTION

The macadamia nut (*Macadamia integrifolia*) is considered to be the world's finest dessert nut. It belongs to the *Proteaceae* family and is the only native Australian plant to attain the status of a commercial food crop [Cavaletto, 1983]. Orchard plantings of this nut can be found in Australia, South Africa, and California, and more recently in Malawi, Guatemala, Costa Rica and Brazil.

*Toxoptera aurantii* is an extremely polyphagous species, having been recorded in at least 190 genera in 80 families van der Goot (1917); Essig (1949); Bodenheimer (1951); Leonard *et al.* (1971). *Toxoptera aurantii* Boyer de Fonscolombe (1941) has been previously reported in Cuba Jaraslau (1974) and *Toxoptera citricidus* affecting citrus as *Citrus tristeza virus* Batista *et al.* (2008).

Besides the morphologic characteristics of the Aphid, develops of wing because abiotic factors and the presence of natural enemies have been used to describe *T. aurantii* [Rivnay, 1937]. The molecular identification of the feeding source is another evidence of its establishment in this new host. In this study we tried to corroborate the establishment of *T. aurantii* in Macadamia nut trees in Cuba.

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## MATERIALS AND METHODS

### Identification of *T. aurantii*

Several winged specimens were collected frommacadamia nut trees and brought to the laboratory for identification based on their morphology (under stereoscope, 10x). Identification of family and specie was made using the descriptions and keys provided by Cottier (1953), Stroyan (1961), Eastop (1966) and Mondal*et al.* (1976).

### *T. aurantii* density

From January 2013 to December 2013, we evaluated the twelve macadamia nut trees located in an *ex situ* tropical and subtropical fruit trees collection in Artemisa, Cuba. The two methods applied were the collection of young branches and placed sticky yellow traps in trees. Twice a month we collected branches and changed traps. Along with that we correlated the record of rainfalls registered by the Meteorological Station placed in Güira de Melena, Mayabeque, with the density of each *T. aurantii* instar (nymph, wingless or winged).

### Survey of natural enemies

From January 2013 to December 2013 surveys were conducted twice a month to detect natural enemies of *T. aurantii*. The twelve trees were evaluated visually and we collected young infected branches in every survey. After detecting nymphs with parasitic symptoms, they were kept in the laboratory until the parasitoids emerged for their identification based on adult morphology.

### Molecular testing

We detected previously a ´*Candidatus* Phytoplasma asteris´ affecting ten of twelve macadamia nut trees [Pérez-López *et al.*, 2013] in the survey area. The detection of this phytoplasma from *T. aurantii* could be used as an indirect evidence of the feeding from macadamia nut trees.

DNA was extracted from 10 black aphids collected from every macadamia plant. The aphids were macerated with liquid nitrogen and incubated 30 minutes with 2.5 ml of extraction buffer (100 mMTris-base pH 8, 150 mMNaCl, 25 mM EDTA, 8 mMPolyvinylpyrrolidone and 50 mMCetyltrimethyl ammonium bromide). For avoid oxidation, we supplied buffer with 0.2 % β-Mercaptoethanol. After incubation we precipitated by centrifugation and transfered 900 µl into 2.0 mL clean Eppendorf tube. We added 900 µL of Chloroform: Isoamylalcohol (24:1 proportion), shaking in vortex and centrifugation to separate phases. Transferred 800 µl of into 1.5 mL clean Eppendorf tubeand added 500µL of Isopropanol. After 2 hours we centrifuged, remove supernatant and washed pellet with 70 % Ethanol (twice). After dry the pellet we eluted in 30 µL of water tri distilled sterile. The DNA obtained was used as a template for a nested PCR assay.

Universal primer pairs that target the phytoplasma 16S rRNA gene, P1 [Deng andHiruki, 1991] and P7 [Schneider *et al.*, 1995] were used for the first reaction, and R16F2n/R16R2 [Gundersen and Lee, 1996] for the nested reaction. Nested PCR products of expected size (approximately 1250 bp) were obtained from *T. aurantii* collected from ten symptom-bearing plants. PCR products were purified (Wizard SV Gel and PCR Clean-Up System, Promega, Madison, WI, USA), cloned (pGEMT-Easy Vector, Promega), and two individual clones per aphids collected in every infected plant were sequenced using Macrogene Inc. Sequencing Service. Phylogenetic relationships were established between the «Toxoptera aurantii» phytoplasma and those of 16SrI and other phytoplasma groups (Mega 5.0, USA).

## RESULTS

### Identification of *T. aurantii*

The winged *T. aurantii* are immediately distinguishable even in the field, but not so wingless and nymphs. We were able to identified *T. aurantii* throw morphologic winged keys such as wings architecture Cottier (1953); Stroyan (1961); Eastop (1966); Jaraslau (1974) and Mondal*et al.* (1976).

### *T. aurantii* density

We observed an inverse relation between higher rains accumulates and winged instar, while wingless number increased with the higher rains accumulates (*Fig. 1*). The higher number of winged was 154 aphids in February (second month with lowest rain accumulates), while the higher number nymphs and wingless was 181 aphids in October (month with higher rain accumulates).

Survey of natural enemies

Young branches collected and the sticky yellow traps allowed detect natural enemies such as the Aphi-

dophagous insects *Cycloneda sanguine* Limbifer Casey (Coleoptera: Coccinellidae). For the identification process we used the descriptions provided by Gordon (1985) and Peck (2005).

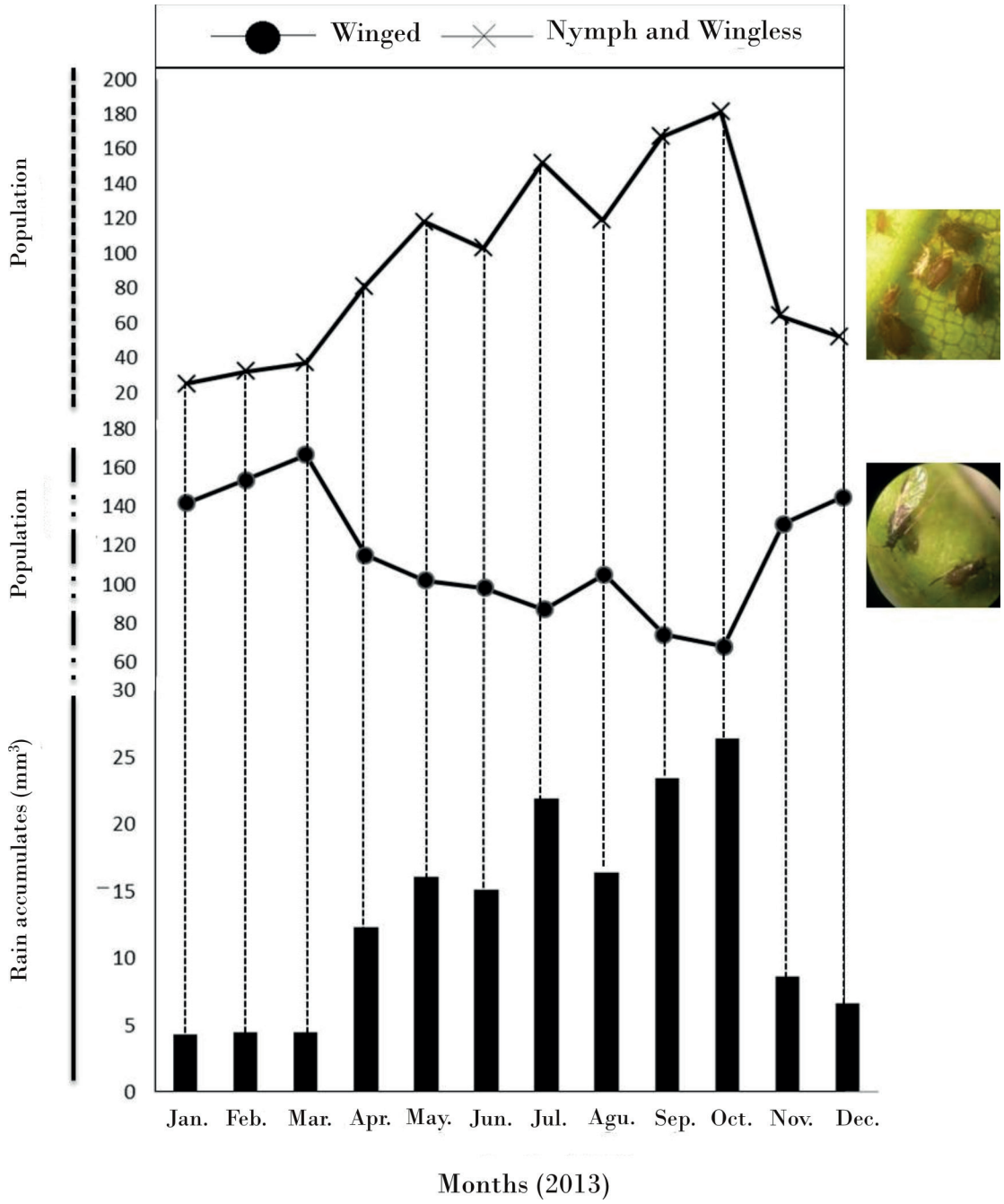


*Figure 1.* Relation between 2013 monthly rain accumulates and aphid number detected.

The presence of mummified nymphs was an indicium of parasitoids affecting *T. aurantii*. After emergence from *T. aurantii* and adult development we classified the parasitoid as a member of *Lysiphlebus* genus. We took into consideration the basis of the fore, the wings venation pattern and the number of maxillary and labial palpomeres [Atanassova *et al*., 1998; Traugott*et al*., 2006].

**Molecular testing**

The R16F2n/R16R2 sequencesof phytoplasmaswere amplified by aphids collected from symptom-bearing

plants. All sequences amplified were 100 % identical to each other. The consensus sequence (1255 nt) of the phytoplasma amplified from aphid was deposited in GenBank (Accession No. KF992835) and showed 99 % of sequence identity with those of the 16SrI group '*Candidatus* Phytoplasmaasteris', including phytoplasma strains AY-CVB (AY265212) and ACLR-AY (AY265211), and showed 100 % of sequence identity with macadamia phytoplasma (Accession No. KC513772), all members of subgroup 16SrI-F. The result of sequence was confirmed by phylogeny (*Fig. 2*) since the aphid phytoplasma grouped within the cluster corresponding to the 16SrI group, closely related to those of subgroup 16SrI-F.

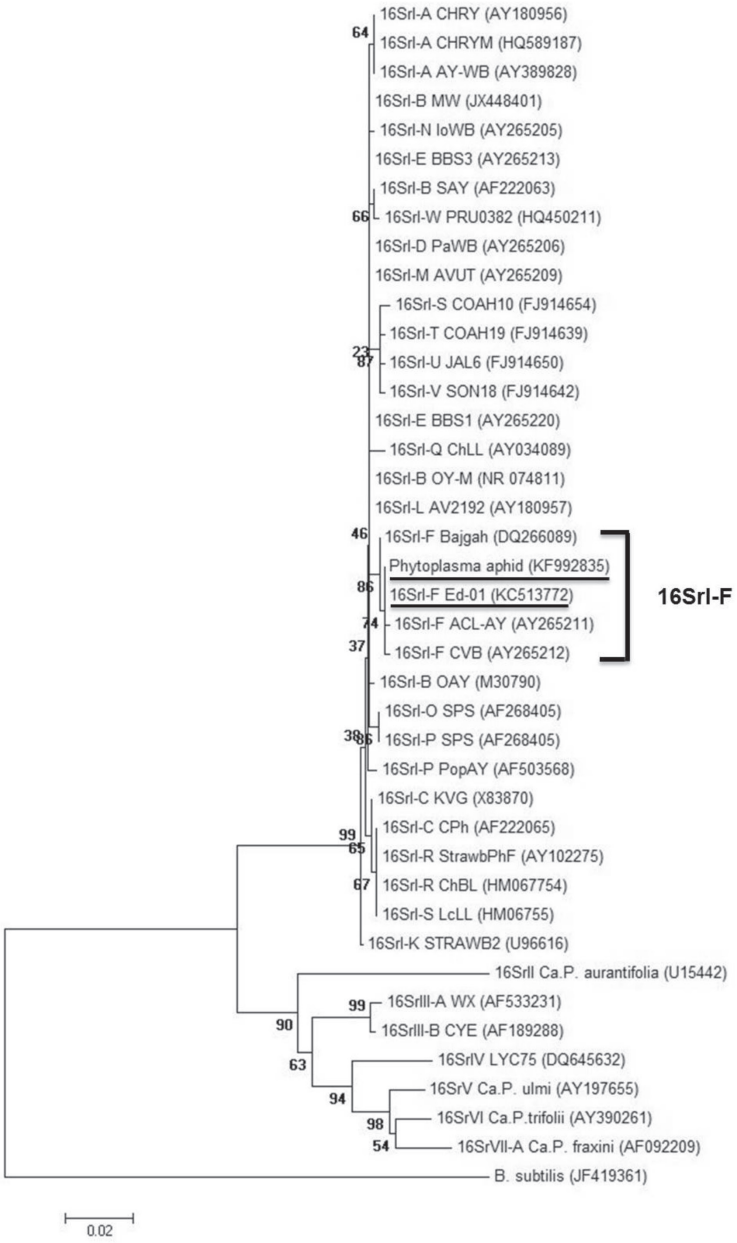


*Figure 2*. Phylogenetic tree based on the 16S rDNA sequences of the phytoplasma identified in *Toxoptera aurantii* (underlined), phytoplasma isolated from Macadamia nut (underlined) of 16SrI subgroups and other 16Sr reference phytoplasma groups. Bootstrap values obtained for 1000 replicates are shown above the branches. *Bacilus subtilis* was used as the outgroup to root the tree.

## DISCUSSION

The rain accumulates and the young shoot emerging has a positive relation, with higher production of young shoots in macadamia nut shoots when precipitations were higher too. The aphid density survey and it relation with the rain accumulates, gave us information about the biology of the aphid, and was used to confirm it establishment in macadamia trees. This behavior has been previously described, and the changes in food availability are a well-known influencing factor in *T. aurantii* wing develops [Rivnay, 1937; Yokomi and Oldfield, 1991]. We do not detect *T. aurantii* affecting inflorescences as previous reported Van der Goot (1918); Stary (1965); Calilung (1967); Anon(1975).

The development, survival and reproduction of the black aphid (*T. aurantii*) are affected also by temperature Wang and Tsai (2001). Wang and Tsai (2001) observed in a temperature ranged from 15 to 20 °C increase female longevity and the number of progeny per female. For other hand, to 32 °C, female longevity and number of female progeny have lowest values. Cuba is a tropical island with a very stable hot weather reason why we were unable to determine the effect of temperature in the aphid biology.

The presence of both insect from aphidophagous genus (*Cyclodena* and *Lysiphlebus*) in Macadamia nut trees is evidence of the establishment of *T. aurantii* in this host. Both genus have been reported in Cuba, Milán*et al*. (2008) and considered as tools in biological control of *T. citricide* and *T aurantii*.

The phloem-feeding insects from genus *Cicadellidae*, *Fulgoromorpha* and *Psyllidae* [Weintraub and Beanland, 2006], has been reported as phytoplasmas vectors, but phytoplasmas has been reported also from aphids (Homoptera: Aphididae) [Cainelli*et al*., 2007]. With this results we can confirm that *T. aurantii* observed and collected from macadamia nut trees has been used this Macadamia nut tress as food and host.

## CONCLUSIONS

- With this study we detected for first time *T. aurantii* in Macadamia nut trees in Cuba. The corroboration of the establishment of the black aphid is key for the future propagation of the fruit and it management.

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