

Nota científica / Scientific note

## New *Bemisia tuberculata* (Hemiptera: Aleyrodidae) rearing method for studies in cassava

Nuevo método de cría de *Bemisia tuberculata* (Hemiptera: Aleyrodidae) para estudios en yuca

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**Abstract:** The aim of this study was to develop a rearing method for *Bemisia tuberculata* on cassava plants that enables the production and ongoing maintenance of a colony. The initial population of *B. tuberculata* was obtained from leaves collected from plants with high nymph infestations. Emerging adults were transferred from plastic cups to voile cages in different densities. The density of 125 adults of the *B. tuberculata* per leaf was the most suitable for rearing. The rearing method proposed in this study has proven to be effective and is recommended since it allows for population levels to remain stable during experiments.

**Key words:** *Manihot esculenta*, whitefly, laboratory.

**Resumen:** El objetivo de este estudio fue desarrollar un método de cría de *Bemisia tuberculata* en plantas de yuca para la producción y el mantenimiento continuo de una colonia. La población inicial de *B. tuberculata* se obtuvo a partir de hojas recogidas de la planta con alta infestación de ninfas. Los adultos obtenidos fueron transferidos de la taza de plástico a jaulas de tul, en diferentes densidades. La densidad de 125 adultos de *B. tuberculata* por hojas fue el más adecuado para la cría. El método que se propone en este estudio ha demostrado ser eficaz y se recomienda, ya que el nivel de la población se mantuvo estable durante los experimentos.

**Palabras clave:** *Manihot esculenta*, mosca blanca, laboratorio.

### Introduction

Cassava is affected by the occurrence of the whitefly complex. Recently, 12 species were reported to be associated with this culture, among which stand out *Aleurotrachelus socialis* Bondar, 1923 and *Bemisia tuberculata* Bondar, 1923 (Hemiptera: Aleyrodidae) in the Neotropical region, especially the latter species in Brazil (Pietrowski *et al.* 2014; Vásquez-Ordóñez *et al.* 2015).

Whitefly damages in cassava crop are a result of direct suction of the sap (weakening the plant), saliva toxicity (causing chlorosis, deformation and leaf fall), transmission of viruses [*Bemisia tabaci* (Genn.) biotype B in Africa] and the sooty mold development (decreased photosynthesis) (Bellotti *et al.* 2012). However, despite their importance, control methods are scarce, since there are few studies investigating other not chemical control methods, mainly because there is not a rearing method in laboratory aiming to provide insects regardless of its occurrence in field (Rheinheimer *et al.* 2012).

Thus, the development of a rearing method of *B. tuberculata* under laboratory conditions on cassava allows the development of biological and behavioral studies as well as efficient control methods for the management of this pest insect. Therefore, the aim of this study was to develop

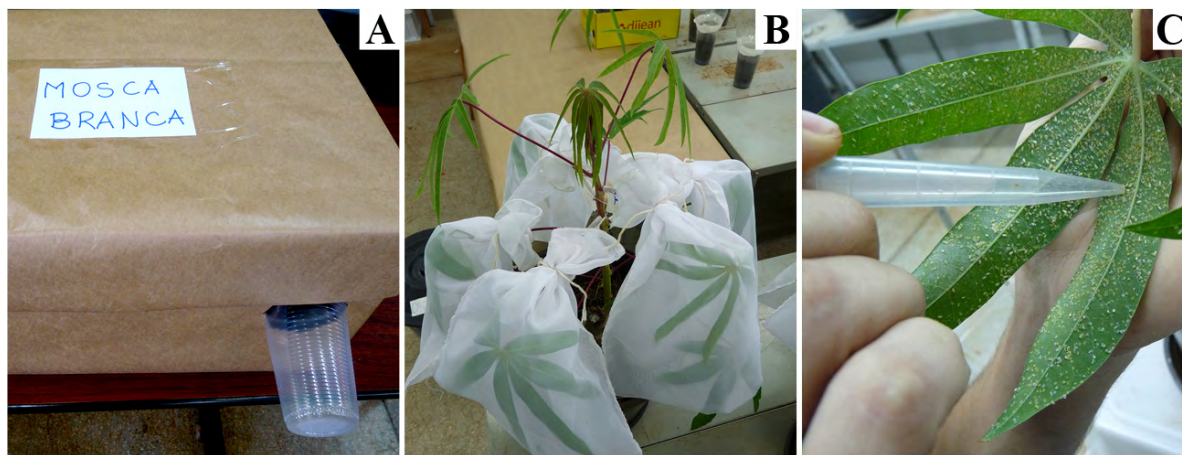
a rearing method of *B. tuberculata* on cassava plants that enables the production and ongoing maintenance of a colony, mostly in the winter when this species is not available in the field.

### Materials and methods

The initial population of *B. tuberculata* was obtained from leaves of cassava, collected from the middle third of the plant, with high infestation of 4th instar nymphs, in commercial plantations. In the laboratory, the leaves were placed in a cardboard box (43 cm long x 30 cm wide x 22 cm height) with a hole on one side, closed with a transparent plastic cup (Fig. 1A). The bottom of the cup was placed facing the outside of the box, in order to collect the adults that are attracted to light (Fig. 1A). To provide durability to leaves, petioles were placed in Petri dishes (10 cm diameter x 2 cm height) and covered with moistened cotton.

The emerging adults were transferred from the plastic cup to voile cages (30 cm long x 25 cm wide), adapted from Bellon *et al.* (2011), on a daily basis. These cages involved the apical leaves of the plants of the cassava variety Baianinha (four to six cages per plant) allowing female oviposition (Fig. 1B). After five days of oviposition, the cages were carefully removed and the insects that were still

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**Figure 1.** Rearing method of *Bemisia tuberculata* in cassava plants. A. Cardboard box with plastic cup adapted to collect adults. B. Voile leaf cage. C. Adults collection with oral aspirator.

alive were collected with the aid of an oral aspirator made with micropipette tips (5 mL) (Fig. 1C) and transferred to new plants as described above, keeping the average number of 50 adults per cage.

The leaves with eggs were identified and, when the nymphs reached the fourth instar ( $\pm 25$  days, according to Barilli *et al.* 2015), were again wrapped inside the cages. As the adult emerged, they were collected every three days and placed on new leaves for oviposition. When more than 50 % of the adults emerged, the leaves were detached from the plants and placed in cardboard box, as described above.

The collected adults were grouped on densities of 25, 50, 75, 100, 125 unsexed individuals per leaf in order to oviposit in the new plants, for a five day period. After that, they were removed and the number of eggs was counted in the five central leaflets of each leaf, in a circle with 1 cm<sup>2</sup> area distant 3cm of the petiole, where the oviposition had greater homogeneity. The *B. tuberculata* densities were randomly assigned in each plant, in apical leaves, and they were replicated in eight plants. Data were submitted to analysis of variance and number of eggs means were compared by Tukey test, both at 5 % significance using the statistical program Sisvar (Ferreira 2011).

### Results and discussion

Among the densities of *B. tuberculata* tested for rearing, the most suitable was 125 insects with an average of 58.0 ( $\pm 2.72$ ) eggs.cm<sup>-2</sup>. This density was significantly different ( $F = 21.7$ ;  $P \leq 0.01$ ) from the 50, 75 and 100 adults per leaves densities which had 24.2 ( $\pm 3.13$ ), 37.6 ( $\pm 4.35$ ), 39.0 ( $\pm 2.35$ ) eggs.cm<sup>-2</sup>, respectively. Leaves with 25 adults presented the lowest mean of eggs (11.7  $\pm 1.31$ ) eggs.cm<sup>-2</sup>).

Considering the number of eggs per individual, it was noted in all treatments mean between 0.4 to 0.5 eggs per adult, indicating that even in the higher population level tested there was no competition for space and/or food. The increasing number of adults per leaf, and therefore the number of eggs, might extrapolate the capacity of leaves support, causing their premature loss, and not allowing the full development of the nymphs, which did not occur on this experiment (Bellotti *et al.* 2012).

Toscano *et al.* (2002) and Campos *et al.* (2005) studying *B. tabaci* biotype B in tomato and cotton plants, observed that there was not a significant difference in the mean number of eggs in the densities of 100 and 150 insects per cage (40 cm diameter x 60 cm height). These authors also noted that the best density would be of 100 insects per cage. Jesus *et al.* (2011) obtained similar result on bean plant, when in the density of 150 adults per cage (2 m x 3 m base x 2 m height) there was an ovipositing reduction, although it did not significantly differ from the density of 100 adults per cage.

As nymphs remained feeding on the leaves for an extended period ( $\pm 25$  days), the plants become debilitated, occurring the attack of mites (*Tetranychus urticae*) as well as the incidence of powdery mildew (*Oidium manihotis*), especially during the winter. Thus, it is necessary control these pests by using products that not affect the whitefly. Specifically for this study, it was used sulfur-based product (Kumulus<sup>®</sup>) for mite control, and raw milk at 10 % solution for powdery mildew control.

Seeking greater efficiency of this rearing method is important that the leaves remain on the plant, taking the following cares: a) use of fertilization and fertile soil as a substrate; b) keeping the plants well watered; c) perform phytosanitary management when necessary, but without harming whitefly nymphs; d) use of strong and healthy leaves preferable the young ones.

The proposed method has the advantage of higher precision in obtaining individuals with a known age due to the limited oviposition period; and, after emerging, adults are trapped in the cages. Furthermore, this rearing method require small spaces, once the use of larger cages is not necessary (Sobrinho *et al.* 2012). However, this rearing method requires constant care of the plants, and before the adult emergency begin the leaves need to be surrounded by the cages in order to prevent adults from escaping.

Keeping all necessary cares it is possible to meet the demand of *B. tuberculata* for conducting experiments in the winter period, time when the leaves of cassava plants in subtropical regions are falling. This method can even be used to the rearing of other species of whiteflies that attack the cassava crop, since provided necessary adjustments, particularly with respect to the density of insects.

### Conclusion

The rearing method proposed in this study has proved to be effective and it is recommended, since the population level remained stable during the conduct of the experiments. It was observed that, despite the high number of eggs per leaf, the insects did develop and reproduced, with high adult emergence for successive generations.

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