Notas Científicas

New experimental tools for bioassays with whitefly in laboratory

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Abstract – The objective of this work was to develop an experimental kit for assessments of repellency, deterrence for oviposition, and insecticidal activity on adults of the whitefly *Bemisia tabaci* biotype B. The kit, which consisted of arenas and nebulizer, was effective for conducting bioassays, and the application of aqueous extracts by inhaler was adequate. The techniques are simple, cheap, and may contribute to research on this insect.

Index terms: *Bemisia tabaci*, *Trichilia pallida*, cage, insecticide, nebulizer, oviposition, repellency.

Novas ferramentas experimentais para ensaios com mosca-branca em laboratório

Resumo – O objetivo deste trabalho foi desenvolver um kit experimental para avaliações de repelência, deterrência à oviposição e atividade inseticida a adultos de mosca-branca, *Bemisia tabaci* biótipo B. O kit, constituído de arenas e nebulizador, foi eficaz para realização dos bioensaios, e a aplicação de extratos aquosos com o inalador foi adequada. As técnicas são simples, baratas e podem contribuir para as pesquisas com este inseto.

Termos para indexação: *Bemisia tabaci*, *Trichilia pallida*, gaiola, inseticida, nebulizador, oviposição, repelência.

The whitefly, *Bemisia tabaci* (Genn.) biotype B, is an important pest in worldwide agriculture, affecting more than 600 species of host plants (Oliveira et al., 2001). Besides the direct damage and physiological alterations that it can provoke (Lourenção & Nagai, 1994), the insect is also a vector of more than 100 phytoviruses (Jones, 2003; Xie et al., 2011) that are lethal to plants.

The insect was introduced in Brazil in the 1990s (Valle & Lourenção, 2002) and since then numerous studies have been done to control its infestations. During this period, diverse techniques have been evaluated, mainly assessing the nymphicide effect (Souza & Vendramin, 2001, 2005; Bezerra-Silva et al., 2010; Islam et al., 2011; Zhang et al., 2011). However, great difficulties still remain in the evaluation of the bioactivity of synthetic or plant-derived compounds on adults, such as the large size of the droplets, difficulty in the visualization of the results, and issues related to the target being reached by the product.

In the present study, a conjunction of experimental tools was developed in order to evaluate repellency, oviposition deterrence, and adulticidal activity. Tomato leaflets (cultivar Santa Clara, obtained from Sakata Seed Sudamerica) and aqueous extract from leaves of *Trichilia pallida* Swartz at 3% (weight/volume) – a species with recognized bioactivity against the insect (Souza & Vendramim, 2005; Baldin et al., 2007) – were used. Control treatments were performed with distilled water.

The bioassays were performed under controlled conditions (temperature, 25±2ºC; relative humidity, 70±10%; and photophase, 14 hours) at the Laboratory of Resistance of Plants to Insects and Plant Insecticides of Universidade Estadual Paulista, Botucatu, SP, Brazil, in 2011. Ten replicates were used for each assay modality (repellency, oviposition deterrence, and mortality), in a completely randomized design.

For the bioassays, a cage (Figure 1) was developed, composed of two free parts: one to support the vials containing the seedlings or leaves, and the other for confinement of the insects. The support consisted of a polystyrene plate (12x5x2 cm), with two central orifices for fitting the glass vials (4.0x2.1 cm). This plate was placed on a base, also of polystyrene (19x19x1.5 cm), containing two lateral orifices, one covered with organdy fabric (aeration) and the other

used for releasing the insects. The second part of the cage (confinement) was composed of one transparent plastic container (14x15 cm), with a 2.5 L volume.

For each cage, two tomato leaflets were placed into glass vials (4.0x2.1 cm) containing distilled water for keeping their turgescence. One leaflet was pulverized by a trigger sprayer with 3 mL of *T. pallida* extract, and another with distilled water (control). Five minutes were spent in order to eliminate the pulverization excess. Then, the vials were fitted into the base of the cage and covered by the confinement container. Afterwards, the cage was infested with the insects through the basal orifice. Twenty couples of *B. tabaci* biotype B of 1–2 days of age were used. The number of adults attracted per leaflet and the oviposition were evaluated 24 hours after the release.

Preliminary results showed that the use of trigger sprayers – such as the ones used in laboratory or greenhouses – may not be efficient, due to the variability of droplet size. For larger drops, it was not possible to affirm whether the mortality had resulted from the action of the product or from the size of the droplets that reached the insect.

Since the majority of trigger sprayers produced volume median diameter of droplets ($D_{v0.5}$) ranging from 100 to 600 μm, depending on fluid properties and nozzle design (Giles et al., 2005), a new pulverization methodology was developed using a nebulizer (adapted inhaler) as the pulverization agent – with smaller particles: $D_{v0.5}$ from 1 to 5 μm (Hess, 2000) –, with an outflow rate of 0.15–0.25 mL min^{-1}.

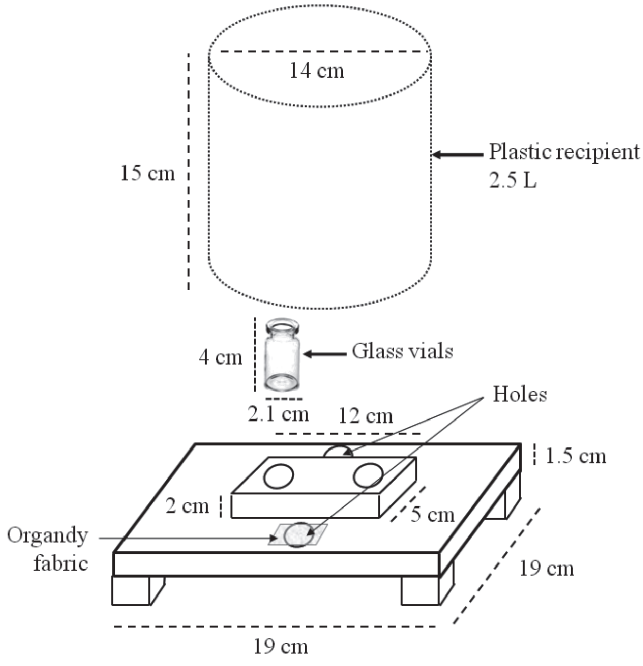

Figure 1. Overview of the cage.

Tomato seedlings (15–20 days) were transferred into glass tubes (9x2.5 cm) containing Plantmax moistened substrate. For the confinement, transparent plastic containers were used (26x10 cm), with a volume of 2 L, covered on the top with organdy fabric.

The bottom surface of the cages consisted of a polystyrene plate (16x16x2.0 cm) perforated in the center – in order to perform the infestation and nebulization, and to fit the seedling –, covered by a black card used to ease the visualization of dead insects.

The bioassay started with 40 adults (1–2 days of age) of *B. tabaci* biotype B, released from the central basal orifice of the polystyrene plate. After the release, the orifice was closed with organdy fabric and fixed with adhesive tape. Five minutes after the release, the nebulization was performed for 30 s. When the fog disappeared (5–10 min after), a receptacle containing the seedling was inserted. The number of dead adult individuals was counted 24 hours after the nebulization.

The cages used in the bioassay of repellency and oviposition deterrence were effective, especially due to the easiness in releasing and visualizing the insects present in the leaflets. The use of the nebulizer allowed the formation of smaller particles when compared to trigger sprayers, not affecting the behavior of whiteflies.

The experimental kit developed presents low cost and is adequate for bioassays with *Bemisia tabaci* biotype B adults. Its utilization can contribute to increase the efficiency of research studies done with this insect under laboratory conditions.

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