NOTE

Vertical Y-tube Assay for Evaluation of Arthropod Response to Plant Materials[1]

John L. Coffey,[3] Alvin M. Simmons,[2] B. Merle Shepard,[4] and Amnon Levi[3]

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Studying the response of insects to phytochemicals necessitates the use of appropriate tools to quantify insect behavior (Stelinski & Tiwari 2013). Olfactometers are widely used to study relative attraction or repellency of phytochemicals to insects, but the data collected are generally restricted to non-contact of the insect with the source of the odor (Isman 2002). Additionally, hemipterans tend not to exhibit movement readily in olfactometers with a horizontal orientation (Stelinski & Tiwari 2013, Coffey et al. 2015). There is a continued need to develop laboratory monitoring assays to evaluate insect behavior in a manner that is efficient, reliable, and ecologically relevant. Allowing insect test subjects to have physical contact with the plant material to feed and oviposit affords the concurrent collection of data on additional parameters related to behavior, and may increase the ecological relevance of the study. Herein, a simple insect monitoring assay is described that utilizes a vertically oriented glass Y-tube where insect test subjects can freely access leaf samples in a controlled laboratory setting. Adult presence, oviposition, and survival by the sweetpotato whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), were studied on five common vegetable crops and two *Citrullus colocynthis* (L.) Schrader (Cucurbitaceae) accessions using this vertical monitoring assay. This assay intends to simulate natural conditions in a controlled laboratory setting and may be useful for researchers who are interested in the response of arthropods to phytochemicals based on tactile and/or gustatory stimuli in addition to olfactory, including those screening plants for resistance to arthropods.

We evaluated the behavioral responses of adult presence on the leaf surface, oviposition, and survival of *B. tabaci*, a polyphagous insect, on five crops:

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[2] Corresponding author; U.S. Department of Agriculture, Agricultural Research Service, U.S. Vegetable Laboratory, 2700 Savannah Highway, Charleston, South Carolina 29414. E-mail: alvin.simmons@ars.usda.gov.

[3] U.S. Department of Agriculture, Agricultural Research Service, U.S. Vegetable Laboratory, 2700 Savannah Highway, Charleston, South Carolina 29414.

[4] Clemson University, Coastal Research and Education Center, 2700 Savannah Highway, Charleston, South Carolina 29414.

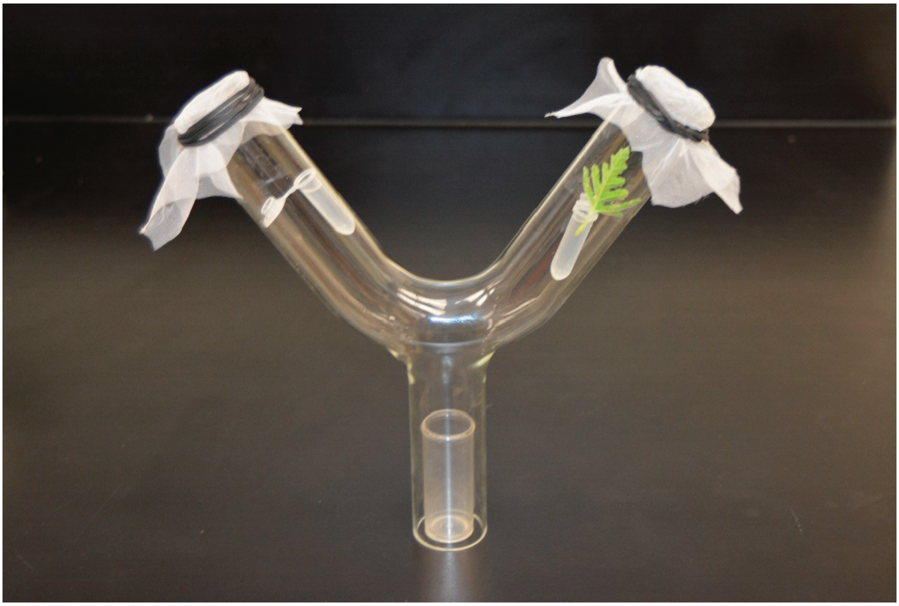

Fig. 1. Novel vertical Y-tube monitoring assay. In this vertical monitoring assay, whiteflies are allowed contact with the leaf sample to feed and oviposit.

'Georgian' collard, *Brassica oleracea* ssp. *acephala* de Condolle (Brassicaceae); 'Mississippi Silver' cowpea, *Vigna unguiculata* L. Walpers ssp. *unguiculata* (Fabaceae); 'Crookneck' summer squash, *Cucurbita pepo* L. ssp. *melopepo* (Cucurbitaceae); 'Homestead 500' tomato, *Solanum lycopersicum* L. (Solanaceae); and 'Calhoun Gray' watermelon, *Citrullus lanatus* Thunberg. Matsum. & Nakai ssp. *lanatus* (Cucurbitaceae), using a novel vertical monitoring assay. Additionally, two Plant Introduction Accessions (PIs) (PI 537277 and PI 346082) of the wild-type desert melon, *C. colocynthis*, were included. A custom-designed glass Y-tube (Tudor Scientific, Belvedere, SC) was used (Figure 1). Each arm of the vertical Y-tube was 15 cm long and open at the end. The diameter of each open end was approximately 4 cm across. Fine-mesh cloth was placed over each of the two open ends of the Y-tube. An uncapped 2 ml micro-centrifuge tube (snap cap vial) was placed in the upper portion of each of the two compartments of the Y-tube and filled with water. The open vials fitted snugly into the compartments without additional support. The petiole of a leaf sample was placed into one vial. Ten replicate plants for each of the five vegetable crops were started from seed in a commercial peat-based potting mixture (MetroMix 360, Sungro Horticulture, Seba Beach, AB, Canada), in a greenhouse, two weeks before the initiation of the experiment; five replicate plants of each of the two *C. colocynthis* PIs were similarly seeded six weeks before the experiment started. The plants were free of pesticides. For all trials, the third leaf below the apical meristem was used. Whiteflies used in the study were from a greenhouse colony of B-biotype *B. tabaci*. The B-biotype is also known as Middle East-Asia Minor 1 (Dinsdale et al. 2010). The colony of whiteflies originated from a sample collected on sweetpotato, *Ipomoea batatas* (L.) Lam. (Convolvulaceae), and reared continuously on assorted uncaged vegetables (Simmons & Levi 2002). The whiteflies were not exposed to pesticides. For all trials, adult whiteflies were collected into a 35 ml plastic vial from collard plants in the colony using a manual aspirator. Collection was random and no attempt was made to determine the age or sex of the adult whiteflies. Two preliminary control trials were conducted to ensure that environmental factors were not significantly influencing insect behavior: (1) both compartments of the monitoring system were left empty except for a vial of water, and the number of adults that moved to each compartment of the Y-tube was statistically the same; (2) a watermelon leaf from a cultivar known to be highly attractive to whiteflies, 'Calhoun Gray,' was placed in one compartment while the opposite compartment was left empty except for a vial of water, and the number of adults that moved to each compartment was significantly more (86%; $P < 0.001$) for the cultivar as compared with the water check (6.6%). A total of 30 whiteflies were released per control trial, and each trial was repeated six times. A no-choice bioassay was then conducted in a complete randomized design, consisting of 30 whiteflies per trial for each of the five crops and two PIs, totaling 870 adult whiteflies over 29 trials. Each crop was evaluated across three to seven replicate trials (collard, n = 3; cowpea, n = 4; PI 346082, n = 4; PI 537277, n = 3; squash, n = 7; tomato, n = 5; watermelon, n = 3). For each trial, the vertical monitoring Y-tube was placed over an uncapped 35 ml collection vial containing 30 adult whiteflies, and the monitoring cage was left undisturbed for 24 h in the laboratory at 25 ± 2°C with L:D 14:10. Afterwards, whitefly behavioral responses (adult presence on the leaf surface, oviposition, and survival) were recorded. For each trial, numbers of whiteflies on the leaf surface were counted in-situ without disturbing the monitoring apparatus. If a whitefly had failed to move into either compartment of the Y-tube and remained in the base, a no-response result was recorded. Additionally, the number of dead whiteflies was recorded. Leaf samples were examined using a microscope (Leica EZ4D, Buffalo Grove, IL), and whitefly eggs were counted. All statistical analyses for this no-choice experiment were performed using the MiniTab Statistical Package (MiniTab v.16, 2010, State College, PA). One-way analysis of variance (ANOVA) was performed with plant type as the lone fixed treatment effect. The numbers of whitefly eggs on the leaf surface, percent adult survival after 24 h, and percent of adults on the leaf surface were the three response variables. Where the effect of treatment was significant, means for the five crops and two PIs were separated using Tukey's 95% Individual Confidence Intervals ($\alpha < 0.05$). All percentage data were transformed using arcsine transformation before analysis. All means are presented for back-transformed data.

Survival of *B. tabaci* was significantly ($F = 30.37$, df = 6, 22; $P < 0.001$) lower on the wild-type *C. colocynthis* melon PIs 537277 and 346082 (54.4 ± 1.9% and 60.8 ± 6.8% of adults surviving after 24 h, respectively) as compared with all other plant types evaluated (Figure 2). Conversely, survival was highest (92.9 ± 2.9%) on summer squash, a plant well-known to be attractive to whiteflies (Simmons 1994), collard, and watermelon (Figure 2). Presence of adult whiteflies on the leaf samples was significantly ($F = 20.81$, df = 6, 22; $P < 0.001$) higher for squash (77.6 ± 3.2%), compared with all other entries except collard (65.6 ± 5.6%), which was statistically not different from squash (Figure 2). Collard and squash were associated with the highest oviposition rates (87.1 ± 18.8 and 89.7 ±

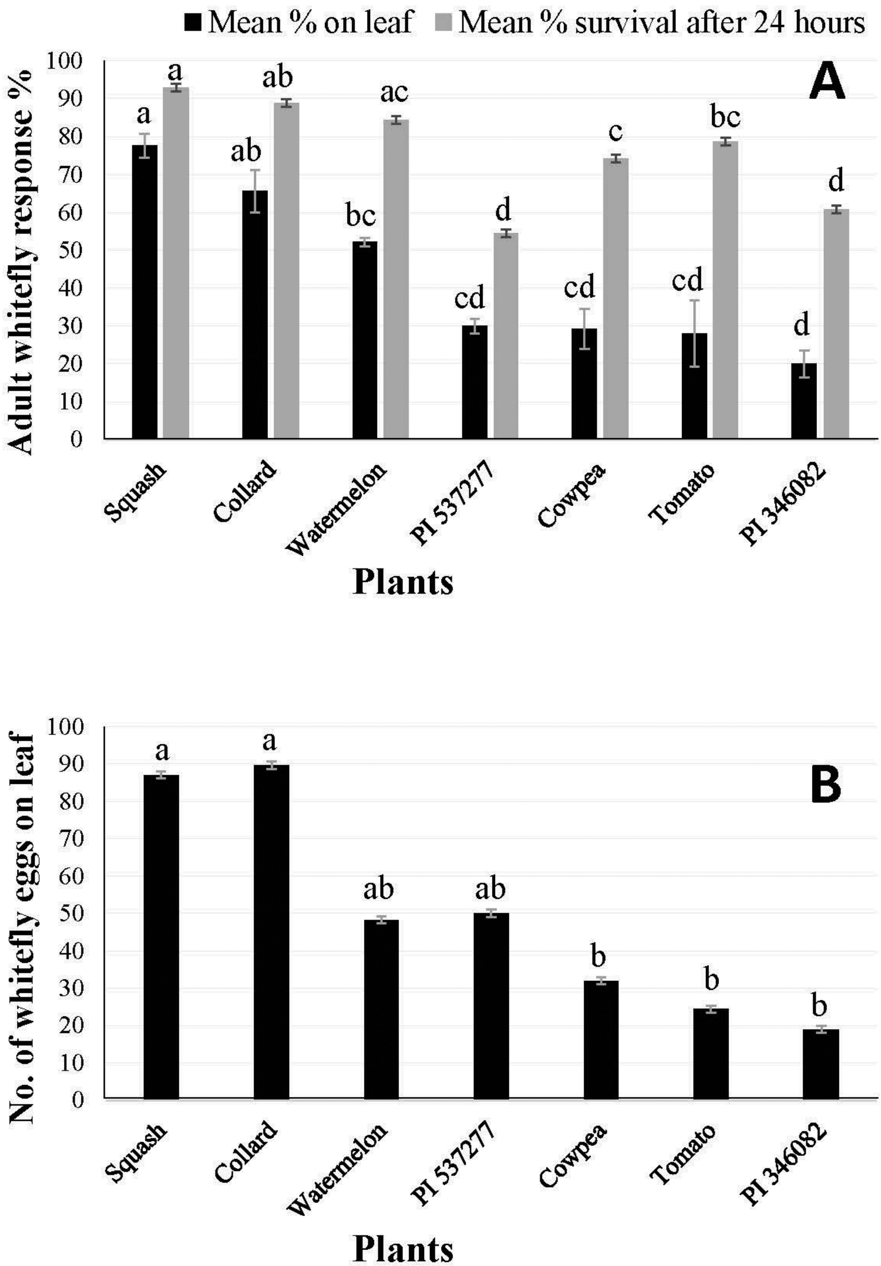

Fig. 2. (A) Mean % (± SEM) of *Bemisia tabaci* adults on leaves and adults surviving after 24 hours, and (B) mean no. (± SEM) of eggs oviposited on leaf after 24 h in no-choice assays with a vertical Y-tube (n = 870 adult whiteflies). Means followed by the same letter are not significantly ($P < 0.05$) different according to Tukey's 95% Simultaneous Confidence Intervals.

12.1 eggs per 24-h trial, respectively). Overall, 32.5% of the whiteflies exhibited no-response, where the insect failed to move into either of the compartments of the vertical Y-tube. The lowest percentage of no-response results was associated with squash (15.2%) and collard (23.3%), while the highest percentage of no-response results was associated with tomato (50.7%), cowpea (45.0%), and *C. colocynthis* PI 346082 (40.8%).

In a recent study (Coffey et al. 2015), a horizontal Y-tube olfactometer was used to evaluate relative preference of *B. tabaci* for 30 genotypes of *C. colocynthis* and the heirloom watermelon cultivar 'Calhoun Gray'. However, the majority of trials in that study yielded a no-response result, in which the insect failed to move toward either sample of plant material and remained near the entrance of the horizontal Y-tube olfactometer. Many other insects display negative geotaxis and positive phototaxis (Simmons 1994, Saad et al. 2013, Stelinski & Tiwari 2013), and hemipterans tend not to exhibit movement readily in horizontally-oriented olfactometers (Stelinski & Tiwari 2013, Coffey et al. 2015). In addition to the relatively large number of no-response results in our previous study using a horizontal Y-tube olfactometer assay (Coffey et al. 2015), the genotype that was associated with the highest relative preference, *C. colocynthis* PI 537277, was associated with low whitefly oviposition and survival in subsequent greenhouse assays (Coffey et al. 2015). This wide difference between initial attraction and extended survival demonstrates the complexity of interactions between phytochemicals and insect performance and suggests that the initial response by an insect as observed in the laboratory may not necessarily be representative of its actual preference and performance in a greenhouse or field setting. Moreover, this variability underscores the need for ecologically relevant laboratory assays that allow extended insect-plant physical contact, in a manner conducive to eliciting a natural behavioral response.

Almost 90 y ago, McIndoo (1926) reported on his invention of a horizontal Y-tube apparatus, named an insect olfactometer, which he used to study insect response to odors. Since then, laboratory assays involving whiteflies and Y-tube apparatuses have elucidated several aspects of whitefly behavior, which can lead to improved management efforts. For example, Zhang et al. (2004) demonstrated a repellent effect on whiteflies when tomato leaf disks were dipped in oil derived from ginger, *Zingiber officinale* Roscoe (Zingiberaceae), and placed in a single-tube vertical olfactometer in which contact with whiteflies was prevented. Subsequently, Legaspi et al. (2011) demonstrated repellency of mustard, *Brassica juncea* (L.) Czern. (Brassicaceae), volatiles to whiteflies using a horizontal Y-tube olfactometer. More recently, Saad et al. (2013) found that *B. tabaci* females preferred non-infested chili pepper, *Capsicum annuum* L., var. Kulai (Solanaceae), plants over plants infested with whiteflies using a horizontal Y-tube olfactometer assays in a laboratory.

The assay described herein employed a Y-tube that is consistent in shape with the Y-tube components used in many traditional horizontal olfactometers, but features a change in orientation from horizontal to vertical. This vertical orientation takes advantage of the negative geotaxis and positive phototaxis exhibited by many insects, and is particularly well suited for studies involving hemipterans, such as whiteflies, which tend not to exhibit movement readily in horizontally oriented olfactometers (Stelinski & Tiwari 2013, Coffey et al. 2015). This assay allows for the collection of data based on the response of the insect to plant odors as well as data on oviposition and survival. This technique also combines the benefits of a controlled laboratory setting with the increased ecological relevance afforded by allowing the insect to have physical contact with the leaf sample to feed and freely oviposit. The vertical Y-tube monitoring assay described here is a reliable assay for rapid testing of arthropod response to phytochemicals and may be useful for a wide range of assays, including assays for screening plants for resistance to insect pests.

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