Behavioral Responses of Whitefly, *Bemisia tabaci* (Hemiptera: Aleyrodidae), in Relation to Sex and Infestation Status of Their Host Plants


School of Environmental and Natural Resource Sciences, Faculty of Science and Technology, National University Malaysia, 43600 UKM, Bangi, Selangor, Malaysia

Horticulture Research Centre and Strategic Resources Research Centre MARDI Headquarter Persiaran, MARDI-UPM, 43400, Serdang Selangor, Malaysia

Abstract: The whitefly *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is an important vegetables pest in Malaysia. It is a major vector of plant viruses that cause serious crop yield losses. We investigated the behavioral responses of male and female *B. tabaci* to chili (*Capsicum annuum* var. Kulai) plants infested by either male or female *B. tabaci*, using Y-tube olfactometer assays. The results indicated that whitefly females significantly preferred non-infested chili plants over plants infested with whiteflies of the same or opposite sex (*P*<0.05). However, the number of males that responded to chili plants infested by the same and opposite was not significantly different from those that responded to non-infested plants. The findings may help to identify WF (Whitefly) kairomones that may be used for the integrated pest management of WF.

Key words: *Bemisia tabaci* · Chili Plant · Sex · Y-Tube Olfactometer · Infestation Status

INTRODUCTION

Whiteflies (Hemiptera: Aleyrodidae), have long been recognized as an important group of agricultural insect pests that cause serious damage to more than 600 plant species. These insects weaken plants, excrete honeydew and transmit several hundred plant viruses [1]. In Malaysia, two species of whitefly have been found in the lowlands and highlands: sweet potato whitefly, *Bemisia tabaci* (Gennadius) and greenhouse whitefly, *Trialeurodes vaporariorum* (Westwood), respectively. These species were probably introduced from Europe during the importation of ornamental plants. Whitefly infection, especially in tomato, chili and brinjal, causes yield losses of up to 50% [2]. To date, the application of chemical pesticides has been the primary method used to control these pests [3]. In addition to the substantial costs associated with insecticide application, this practice has severe environmental impacts. Moreover, insecticides are ineffective against *B. tabaci* since it develops resistance to the active ingredients. These limitations have caused increased interest in the potential value of plant volatiles compounds that can directly influence insect behavior [4]. Therefore, there is a need for a better understanding of the factors that affect the relationships between insects and their host plants [5]. For example, determining how insects detect and choose their host plants is important. Plant-derived chemical cues are among the important factors affecting the relationships between insects and plants [6]. This study aimed to determine the response of *B. tabaci* males and females to chili plants to examine whether WF are attracted to volatile chemicals associated with chili plants infested by male WF, or female WF. The results are expected to contribute to and improve our understanding of plant-insect interactions and thereby facilitate the development of integrated pest management systems through selecting and breeding chili varieties that have a potential to induce early defense against pests such as WFs.
MATERIALS AND METHODS

Whitefly Rearing: A colony of *B. tabaci* was established and maintained under green-house. The colony was reared on chili plants (cultivar Kulai) that were raised in pots. Before the experiment started, newly emerged WF adults were collected from the chili plants and were sexed under a stereomicroscope (×50). Females could be distinguished from males by their relatively large size and larger abdomen [7]. Insects were confined in individual Erlenmeyer flasks and fasted for 2 h before the start of each experiment.

Host Plants: Chili (*Capsicum annuum* var. Kulai) seeds were obtained from the Malaysian Agriculture Research and Development Institute (MARDI) Station, Jalan Kebun, Klang. Seeds were soaked in pure distilled water for 8 days to initiate germination. Germinated seeds were transferred into hydroponic culture containers that were placed inside plant cups. The plant cups were then lowered into the hydroponic growth medium and maintained on a raft comprising a punched cylindrical piece of polystyrene. Plants with 3-4 stage leaves were used for experiments.

Two-Choice Bioassays: Behavioral observation were conducted with both sexes (male and female) of *B. tabaci* using a small glass tube in the form of (Y-type), with (0.8cm inner diameter, 10 cm in length for the base of the tube and two 10 cm length arms of the tube with an angle of 45 between them), was partially following the methods described by Koschier *et al.* [8] and Davidson *et al.* [9]. Three treatments were prepared: chili plants infested with 50 females, chili plants infested with 50 males and chili plants that were not infested with WFs.

In each trial we placed the plants into two glass chambers (3L in volume) and each chamber was connected to one of the arms of the Y-arm olfactometer via PTFE tubing. One pressure pumps (Cole-Parmer Air cadet vacuum/pressure station, Illinois, U.S.A) pumped air into of the system. Air was passed through an active charcoal filter for purification and then split into two glass chambers passing into an odour source and finally passing into two flow meter connected between the arms of the olfactometer and two glass chambers. Airflow into the olfactometer was set at 60 ml/min. The air was drawn through the Y-tube for 1 min before introducing each WF. One adult WF was introduced at the base of the Y-arm olfactometer at the beginning of each trial. The response time was limited to 10 min per insect. Insects that walked into one of the arms and did not return to the Y-junction within 15 sec were considered to have made a final choice. Responses were considered positive when insects travelled at least 4 cm along an arm connected to a test chamber. Each test WF participated in three separate trials.

In each trial, the WF was presented with a choice between (female-infested vs. non-infested, male-infested vs. non-infested and female-infested vs. male-infested chili plants). Insects that did not make a choice within 10 min were excluded from the data analysis. The experiment was replicated on three different experimental days with new sets of chili plants and insects. Ten adult females and ten adult males were included in each replicate. All bioassays were conducted under the same laboratory conditions at 24 ± 1°C and 60–65% RH.

Statistical Analyses: *T*-tests were used to evaluate differences between the number of male and female whiteflies choosing the different sides of the Y-tube among the three treatment groups. Differences with *p*-values < 0.05 were considered significant. All data was analyzed using the Minitab Statistical Package (v. 16).

RESULTS AND DISCUSSION

Male and female *B. tabaci* differed from one another in their response ratios for each treatment group (female-infested vs. non-infested, male-infested vs. non-infested and female-infested vs. male-infested chili plants). WF females significantly preferred non-infested plants to plants infested with female WF (*t* 40 = 0.1667, *P* < 0.047) or male WF (*t* 57 = 0.267, *P* < 0.035). In contrast, there was no significant difference in the response of males to non-infested plants and plants infested with females (*t* 57 = 0.100, *P* < 0.399) or to non-infested plants and plants infested with males (*t* 57 = 0.067, *P* < 0.605) (Fig.1, 2). Neither females (*t* 53 = 0.167, *P* = 0.122) nor males (*t* 57 = 0.067, *P* = 0.600) responded differently to plants infested with female-infested than male-infested chili plants (Fig. 3).
Our results suggested that male and female whiteflies respond differently to chili plants infested with WFs of the same or opposite sex. Females were more strongly attracted to non-infested plants than to plants that were infested by either the same or the opposite sex. In contrast, the responses of male whiteflies did not vary significantly among plants infested with WFs of the same, or opposite sexes. It is likely that the preference for non-infested plants shown by *B. tabaci* females was dictated by the need to avoid conditions that are potentially threatening to their offspring.

The female whiteflies may use volatile cues to discriminate between infested and non-infested plants in order to avoid such conditions. Female insects typically search for a specific location to lay their eggs, often on material that will become food for the emerging offspring [10, 11]. For example, Rothschild and Schoonhoven [12] described how *Pieris brassicae* females are able to be very selective regarding their choice of host plants for oviposition and prefer plants with no eggs or conspecifics, thus avoiding inter-specific competition and the attraction of natural enemies. Similarly, there is cross-talk among different plant response pathways to induce resistance of plants to insects can develop due to a previous attack by the same or another organism and this repels further herbivore colonization [13, 14]. For example, aphids are attracted to wheat seedlings without herbivore damage, but are repelled from wheat seedlings that produce odors when attacked by a high density of aphids [15]. Other studies have reported that feeding by the aphids *Myzus persicae*, *Aphis gossypii*, or *Rhopalosiphum padi* induce resistance to the same aphid species [15-18].

On the other hand, plants infested with herbivores may emit more volatile compounds than non-infested plants, as indicated by the preference of *B. tabaci* for non-infested tomato plants over tomato plants infested by conspecifics for feeding and oviposition [19]. Further, some sesquiterpenes and monoterpenes from tomato plants were found to elicit a response after coming in contact with the receptors on female *B. tabaci* antennae and these volatiles had a strong effect on the preference of *B. tabaci* as revealed by free-choice bioassays conducted by Bleeker *et al.* [20]. These factors might have enhanced the female *B. tabaci* preference for non-infested
plants compared to those infested with either B. tabaci of the same or opposite sex.

The olfactometer bioassays revealed that male whiteflies showed no difference in their response to infested and non-infested plants. This could be because they were unable to identify volatiles from chili plants as indicated by no significant difference in response to the three bioassays (chili plants occupied by B. tabaci of the same, opposite and both sexes) tested. Females of many insect species are reported to show a greater response than males to plant volatile compounds. [21-25]. For example, in comparison to males, female mirids have a stronger electroantennogram response to most monoterpenes [26, 27]. Our results are consistent with the findings of these studies in that whitefly females were more responsive to host plant odors than males. Hence, this sex-related difference in response might be related to a female’s need to determine a suitable host plant for oviposition. Gender-specific olfactory responses and responses to volatile compounds during WF host selection are relatively poorly studied. It is possible that pheromones elicit higher responses from male insects than from female insects [28], whereas chemicals released by host plants are thought to mainly affect females [29].

In summary, our olfactometer experiments suggest that WF show differential preferences among host plants infested with WF of a single sex, both sexes of WF and non-infested host plants. This suggests that volatile compounds emitted by the host plant affects the WF host selection process. Further studies are warranted to identify and characterize the chemical compounds and the structure of volatiles released by the plants infested by B. tabaci males, or females. This may help to identify WF kairomones that may be used for the integrated pest management of WF.

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REFERENCES


