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ORIGINAL ARTICLE

Oviposition preference and olfactory response of *Diaphania indica* (Lepidoptera: Pyralidae) to volatiles of uninfested and infested cucurbitaceous host plants

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Key words. Lepidoptera, Pyralidae, *Diaphania indica*, cucumber moth, host volatiles, olfactory response, wind tunnel, oviposition, Cucurbitaceae, *Citrullus lanatus*, *Cucumis melo*, *Cucumis sativus*, *Cucurbita pepo*

Abstract. The cucumber moth, *Diaphania indica* (Saunders) (Lepidoptera: Pyralidae), is a major pest of cucurbitaceous plants. The oviposition preference and olfactory response of larvae, mated and unmated male and female adults to volatiles emanating from uninfested and infested plants of four species of cucurbitaceous host plants and odours of conspecifics were recorded. Also the role of experience in the host finding behaviour of *D. indica* was evaluated. The experiments were done using a wind tunnel, olfactometer attraction assays and oviposition bioassays. The results reveal that fewer eggs were laid on infested plants than on uninfested plants. Females significantly preferred cucumber over squash, melon and watermelon. Cucurbitaceous plants elicited adults of *D. indica* to fly upwind followed by landing on the plants. The effect of experience on the olfactory preference of *D. indica* was dependent on the host plant. Females that had experience of cucumber, squash and melon plants were significantly attracted to the same plant, but not in the case of watermelon. Larvae of this pest were attracted only to volatiles of uninfested cucumber, squash and melon, whereas volatiles of conspecifics, infested plants and intact watermelon did not attract larvae. This study is an initial attempt to investigate the role of volatile infochemicals in the host-finding behaviour of *D. indica*. These results provide baseline information for the development of new control strategies against *D. indica*.

INTRODUCTION

The cucumber caterpillar, *Diaphania indica* (Saunders) (Lepidoptera: Pyralidae), is one of the most important pests of cucurbitaceous plants worldwide (Peter & David, 1991; Namvar & Alipanah, 2002; Nagaraju et al., 2018). The larvae feed on leaves, fruits, flowers and stem and cause considerable yield loss during an outbreak (Nagaraju et al., 2018). The females prefer young leaves over old leaves, petioles and stems for oviposition (Choi et al., 2003). A previous field study shows that *D. indica* prefers to lay eggs on sweet melon (*Cucumis melo* cv Hales Best Jumbo) than on watermelon (*Citrullus vulgaris* cv Charleston Gray) and cucumber (*Cu. sativus* cv Alpha Beta) (Ba-Angood, 1979). Currently control programmes largely rely on the application of chemical insecticides. The overuse of chemical control results in the evolution of resistance to insecticides, toxicity to non-target organisms, environmental contamination and insecticide residues in agricultural products (San Choi et al., 2009). To reduce these effects, alternative control methods are required. Herbivorous insects use various cues to find and identify their host plants (Heisswolf et al., 2007). A combination of olfactory, visual and contact or gustatory cues can eventually lead the insect to its host plant (Heisswolf et al., 2007; Aartsma et al., 2018; Srinivasan, 2019). In most cases plant volatiles are more host-specific than visual cues and olfactory cues are also more reliable for locating host plants, even in complex environments (Bernays & Chapman, 1994; Bruce et al., 2005; Heisswolf et al., 2007; Frérot et al., 2017). Plants can release a variety of volatile organic compounds (VOCs) that play an important role in the host-finding behaviour of phytophagous insects in terms of feeding, mating, egg-laying and aggregation of conspecifics (Metcalf & Metcalf, 1992; Bruce et al., 2005; Cardé & Willis, 2008; Proffit et al., 2011; Silva & Furlong, 2012). VOCs are emitted from different parts of plants including flowers, leaves, stems, fruits and roots and can have different ecological effects such as an attractant or repellent for insects and also activate defences in neighbouring plants (Szendrei & Rodriguez-Saona, 2010; Reinecke & Hilker, 2014; Hatano et

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al., 2015; Frérot et al., 2017). Chemical signals released by plants vary with plant species and cultivar, plant age and physiology, plant part, climatic factors, air pollution and the species of herbivore (Blande et al., 2014; Moghbeli Gharaei et al., 2014; Frérot et al., 2017). Chemical cues from host plants influence host location in various moths, such as *Tuta absoluta* (Lepidoptera: Gelechiidae) (Proffit et al., 2011; Megido et al., 2014; Uzun et al., 2015), *D. nitidalis* (Lepidoptera: Pyralidae) (Peterson et al., 1994), *Anticarsia gemmatalis* (Lepidoptera: Noctuidae) (Meagher & Landolt, 2010), *Plutella xylostella* (Lepidoptera: Plutellidae) (Baoyu et al., 2001), *Helicoverpa armigera* (Lepidoptera: Noctuidae) (Burguiere et al., 2001), *Maruca vitrata* (Lepidoptera: Crambidae) (Zhou et al., 2015), *Epiphyas postvittana* (Lepidoptera: Tortricidae) (El-Sayed et al., 2018) and *Ostrinia nubilalis* (Lepidoptera: Crambidae) (Leppik & Frérot, 2014; Leppik et al., 2014). It is well known that previous feeding experience influences the host finding behaviour of many herbivorous insects (Anderson & Anton, 2014; Hu et al., 2018). In many studies on lepidopteran species, it is documented that, prior experience can influence subsequent responses to plant volatiles (Cunningham et al., 1998; Carlsson et al., 1999; Chow et al., 2005; Pszczolkowski & Brown, 2005; Moreau et al., 2008; Anderson et al., 2013; Megido et al., 2014; Petit et al., 2018). Choosing a suitable host plant for offspring is largely dependent on the oviposition preference of female herbivorous insects (Janz, 2002). In some cases, when suitable food is scarce the larvae of some species of Lepidoptera disperse from the egg-laying site and forage for new host plants. Thus, in so doing they reduce competition, avoid exposure to natural enemies and are less exposed to toxins and plant defence compounds (Singer & Stireman, 2001; Singer et al., 2002; Kakimoto et al., 2003; Singer & Stireman Iii, 2003; Carroll et al., 2006). Understanding the response of *D. indica* to host plant volatiles is essential for the detection and development of an effective attractant for this pest. In this study, we tested the oviposition preference and olfactory response of mated and unmated males and females and larvae of *D. indica* to odours emanating from different cucurbitaceous species, both uninfested and infested, eggs, larvae, pupae and adult conspecifics. Given that the volatile compounds that are induced by the feeding of conspecific and heterospecific herbivores can be different and lead to different responses (Rojas, 1999), responses to plants infested with conspecific larvae, whiteflies and two-spotted spider mites, were also evaluated. We also examined the role of experience in the host finding behaviour of *D. indica* females. The purpose of this study was to improve our understanding of the behavioural characteristics, oviposition preference and olfactory responses of *D. indica* in complex host plant situations, which might be exploited for use in management strategies.

MATERIALS AND METHODS

Plants

Cucumber (*Cucumis sativus*) (cv. Newsun RZ), squash (*Cucurbita pepo*) (cv. Ezra), melon (*Cucumis melo*) (cv. Minoo) and watermelon (*Citrullus lanatus*) (cv. Saturn) plants were cultivated in individual plastic pots (12.5 diameter × 13.0 cm) containing a 1 : 2 : 1 mixture of organic compost, peat moss and perlite. The plants were grown in a greenhouse, under controlled conditions at 27 ± 2°C, 60–80% RH and a 16L : 8D photoperiod. Supplementary lighting was provided by 400-watt sodium vapour lamps, and seedlings that were about twenty days old (4 to 6 leaf stage) were used in the experiments. Fertilizer was applied to the plants daily in the form of an NPK (20-20-20) fertilizer. To obtain infested plants, a series of 8 seedlings of each cucurbitaceous host plant were introduced into each of a series of smaller screen cages (70 × 70 × 70 cm) kept under the same conditions and infested with either 5 third instar larvae of *D. indica*, 5 adults of *Bemisia tabaci* (3 females and 2 males) or 5 adults per plant of *T. urticae*, 48 h prior to the experiment. At the beginning of the experiment, the insects and mites were removed from the infested plants. To obtain healthy plants, seedlings of each species of Cucurbitaceae were placed in aluminium framed screen cages (100 × 100 × 100 cm) in a separate greenhouse.

Insects

Larvae *D. indica* were collected from cucumber in greenhouses, in the County Jiroft in the province of Kerman, Iran (28°63′53.58″N and 57°82′05.50″E) and transported to a laboratory. Four separate stock cultures of *D. indica* were reared and bred on seedlings of each Cucurbitaceae species in each of four aluminium framed screen cages (100 × 100 × 100 cm) kept under the same physical conditions as the plants. Adults were fed by providing them with a piece of cotton wool soaked in honey solution (10%). At least four generations were reared on each type of host plant before starting the experiments. To obtain unmated adults of known age, pupae were separated by gender and kept in separate plastic containers (20 × 17 × 10 cm) until the emergence of the adult insects. To obtain mated adults, pupae of both the sexes were kept in small screen cages (70 × 70 × 70 cm) in an insectary under the same physical conditions as described previously, but with the photoperiod reversed. The newly emerged adults were fed with honey solution as described above. Mated male and female insects, were separated and placed inside a plastic container (5 × 7 cm) with a small piece of cotton wool soaked in a solution of 10% honey and water. Mated 4–5 day old females were used in oviposition preference experiments.

Oviposition bioassay

Long range oviposition experiments were carried out in a wind tunnel made of a transparent acrylic material (180 × 60 × 60 cm) with an airflow of 0.5 m/s. The wind tunnel was housed in a condition-controlled room at 27 ± 1°C, 60–80% RH and 16L : 8D photoperiod. In the first four experiments, the responses to the four different host plants each in the following different conditions: 1 – uninfested, 2 – infested with conspecific larvae, 3 – whiteflies and 4 – two-spotted spider mites, were evaluated. In the last four experiments, the responses to the following four plants: 5 – cucumber, 6 – squash, 7 – melon and 8 – watermelon, were evaluated. All experiments were done during the 8-h dark period, by releasing 5 mated females at a distance of 150 cm from the plants. When all the plants were evaluated simultaneously, insects from each of the four stock cultures were used. In order to avoid the effects of possible learning and adaptation of insects to plants the responses to a particular species of plant or condition of the plant mixtures of moths reared on the other three host plants were used. At the end of each experiment the number of eggs laid on each plant was counted under a binocular microscope. Experiments were replicated 20 times using 100 females for each treatment. The placement of plants in each replication was rotated.

The tunnel and its accessories were ventilated and cleaned with 75% alcohol every day after use.

Role of olfactory stimuli and mating status in host finding behaviour

To determine the role of olfactory stimuli, sex, and mating status in host finding behaviour of *D. indica*, the olfactory responses of 4–5-day old virgin and mated males and females to uninfested and infested host plants were recorded in the wind tunnel. Wind tunnel and physical conditions were similar to those previously described. One hour before the end of the photo phase, moths were placed individually in a cylindrical plastic tube (3 × 6 cm) closed with gauze, in the wind tunnel room. To avoid contamination from the plant pots, each pot was wrapped in aluminium foil. All experiments were carried out 1–5 h after the onset of the scotophase and two red incandescent light sources (5 watts) above the tunnel provided sufficient light for observing the behaviour. The four sets of experiments using four different host plants per experiment in different conditions including: (1) uninfested, (2) infested with conspecific larvae, (3) whiteflies and (4) two-spotted spider mites were evaluated. Insects were released from a 10-cm-high platform 150 cm downwind of the odour source. Insects from each of the four stock cultures were used and the control experiments consisted of recording the response of mated and unmated males and females to dry filter paper. The following steps in the behavioural sequence were recorded: take-off (TO: flight initiation), oriented flight towards the source (OF: flight towards the odour source reaching at least half way along the wind tunnel), close to source (CS: oriented flight at least 10 cm from the source), and landing (L: contact with source). Each treatment combination was randomly tested 30 times. Each insect was tested only once and if it did not respond within 15 min, it was recorded as a non-response.

Role of experience in host finding behaviour

Two-choice olfactory experiments were carried out in the wind tunnel to assess the effect of experience of one species of plant on subsequent attraction to the same and different species of plants. The plants were placed side by side 35 cm apart, at the upwind end of the wind tunnel. Mated females, 4-to-5 days old, were put individually into a cylindrical plastic tube (3 × 6 cm) and released from a 10-cm-high platform 150 cm downwind of the plants. All experiments were done in the dark under the same physical conditions as described previously. Ten mated females were used in each treatment and allowed 15 min for them to respond. A plant was randomly allocated to one side of the wind tunnel at the beginning of each bioassay and replaced after five moths were tested to eliminate potential of positional bias. The duration of the experience was more than five generations.

Olfactory response of larvae

A Y-tube olfactometer with Teflon tubing, was used to measure the response of larvae of *D.indica* to different odours. The Y-tube consisted of a 14-cm long stem and two 10-cm long arms, each with an internal diameter of 2 cm. A glass plug at the base of the stem was used to introduce larvae into the Y-tube. At the end of each arm was a glass chamber, of 3.5 cm internal diameter and 17.5 cm long, in which the odour sources were placed. The Y-tube was housed within a temperature controlled room (27 ± 1°C and 60–80% RH) and oriented vertically with the arms 80 cm below fluorescent lights (~1,600 lux). An air pump was used to provide an airflow controlled by a flow meter (Testo 425 Hot Wire anemometer) of 300 ml/min in each arm. Airflow entering each arm of the olfactometer was drawn through Teflon tubing and was humidified by passing it through a 1,000-ml Erlenmeyer flask with 500 ml of distilled water and filtered by passing it through a 1,000-ml Erlenmeyer flask with activated charcoal before entering the glass tube with the odour source or control. An odour source was randomly allocated to one arm of the olfactometer at the beginning of each bioassay and was changed to the other arm after five larvae to eliminate the potential for positional bias. We harvested approximately 3 g of fresh leaves from either infested or uninfested cucurbitaceous host plants for use as odour sources in the olfactometer. Plants were approximately equal in age and size. In order to eliminate the effects of experience of plant volatiles in each test involving a host plant, a mixture of larvae reared on the other three host plants were used. The fourth instar larvae were starved for 2–6 h by keeping them separately in cylindrical plastic tubes (3 × 6 cm) without food under laboratory conditions in order to enhance their response in the olfactometer. After the introduction of larvae at the base of the stem of the olfactometer, each larva was given 600 s to crawl into one of the Y-tube arms and was scored as responsive if it entered either arm. Individuals that did not enter at least one of the arms were scored as unresponsive. At least 30 larvae were tested in each treatment. Before the tests using host plant odours, preliminary tests were conducted to determine whether the two arms of the Y-tube olfactometer were of equal attractiveness in order to exclude the possibility of positional bias, during which the larvae of *D. indica* were exposed to clean air vs. clean air and also cucumber leaves vs. cucumber leaves. In the first series of assays, leaves of all four species, both uninfested and previously infested (infested by conspecific larvae, whiteflies and two-spotted spider mites separately), were tested individually vs. clean air. In a second series of tests, uninfested leaves were tested against previously infested leaves of the same plant species. In a third series, various conspecific cues including 6 adults (3 females and 3 males), 6 larvae, 6 pupae and 30 eggs were tested vs. clean air. After testing, the olfactometer and connecting tubes were washed with detergent and water, and placed in a drying oven (150°C) for at least 2 h between each treatment.

Statistical analysis

Within treatment analyses were carried out after running normality tests on the results of the oviposition bioassays. Differences in the number of eggs laid on different plants were tested using analysis of variance (ANOVA) (SPSS). If significant differences were detected, multiple comparisons were made using Tukey's HSD Multiple Range Test (P = 5%). Percentage of adult insects responding to plant volatiles and the number of larvae of *D. indica* responding to binary choices in each assay were compared pairwise among experimental treatments using a Chi-square test.

RESULTS

Oviposition bioassays

The results revealed that on uninfested plants *D. indica* laid an average of 15, 13 and 11 eggs per plant on cucumber, squash and melon, respectively. However, it did not lay any eggs on watermelon and significantly preferred cucumber over squash, melon and watermelon ($F_{3,76} = 278.20$, $P < 0.001$) (Fig. 1A). Infestation by conspecific larvae, whiteflies and mites, significantly reduced egg laying ($F_{3,316} = 60.19$, $p < 0.001$) with averages of 3, 4 and 4 eggs per plant laid on plants infested with conspecific larvae, whiteflies and mites, respectively (Fig. 1B). When the host plants in different conditions were evaluated separately, the results revealed that females of *D. indica* significantly preferred uninfested plants over those infested with conspecific larvae, whiteflies and mites, for cucumber plants ($F_{3,76} = 6.00$,

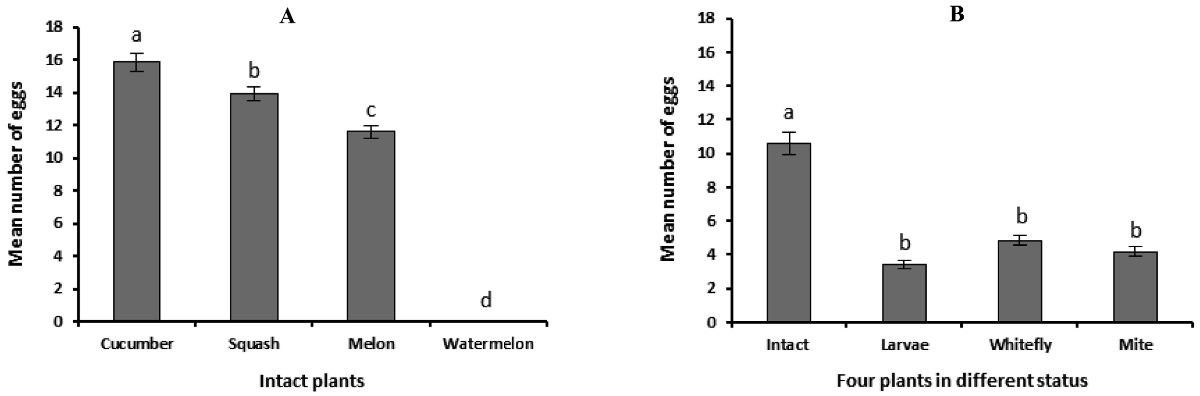

Fig. 1. Mean (± SE) number of eggs laid by *Diaphania indica* on uninfested plants of four host plants (A) and on uninfested plants compared to those on host plants infested with conspecific larvae, whiteflies and two-spotted spider mites (B). Columns topped with same letter are statistically similar (Tukey-HSD test, α = 0.05).

p = 0.001), squash ($F_{3,76}$ = 3.38, p = 0.022) and melon ($F_{3,76}$ = 278.20, p < 0.001) (Fig. 2). For watermelon plants ($F_{3,76}$ = 0.20, p = 0.89) and squash plants infested with whiteflies (Fig. 2), females did not show any preference for plants in particular conditions. Results revealed that volatiles from host plants in different conditions elicit different responses in *D. indica*.

Role of olfactory stimuli and mating status in host finding behaviour

Role of mating status on behavioural responses of males and females of *D. indica* to host plant volatiles in the wind tunnel are presented in Fig. 3. The results reveal that mating status has no statistically significant effect on host finding behaviour of male insects (Fig. 3B, and D). Also no significant differences were recorded in the take-off response of mated and unmated female moths to volatiles from plants in different conditions. However, the percentages of females of *D. indica* that responded when close to the source and landed on the source was affected by mating status and also host plant condition (Fig. 3A, and C). The mean percentage landing on uninfested plants and plants infested with conspecific larvae, whiteflies and mites were 70, 33, 40 and 33%, respectively, for mated females, 30, 20, 23 and 27% for unmated females (χ^2 = 75.8, P < 0.001) (Fig. 3C) and 17, 7, 10 and 13% for mated males and 23, 10, 17 and 17% for unmated males (χ^2 = 15.5, P = 0.03) (Fig. 3D). These results indicate that mated females tended to respond more strongly than virgin females to plant volatiles. Mated females significantly preferred uninfested over infested plants (χ^2 = 37.9, P < 0.001) (Fig. 3C), and of the uninfested plants they significantly preferred cucumber over squash, melon and watermelon (Fig. 4A). Unmated females showed no significant differences in their responses to uninfested and infested plants of all the species tested (χ^2 = 3.09, P = 0.37) (Fig. 3C) and no preference for any of the four host plants (Fig. 4B). For mated (χ^2 = 5.28, P = 0.15) and unmated (χ^2 = 6.07, P = 0.10) males, there were no significant differences in their responses to uninfested and plants infested with whiteflies or mites (fig. 3D) or in their responses to the four host plants (Fig. 4C and D).

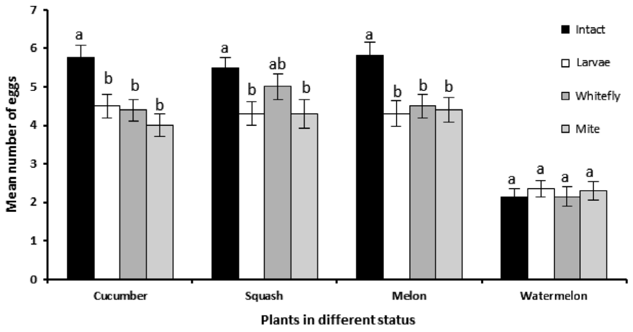

Fig. 2. Mean (± SE) number of eggs laid by *Diaphania indica* on different host plants (cucumber, squash, melon and watermelon) that were either uninfested or infested with either conspecific larvae, whiteflies or two-spotted spider mites. For each plant, columns topped by the same letter do not differ statistically (Tukey-HSD test, α = 0.05).

Role of experience in host finding behaviour

The results reveal that female insects that had fed only on cucumber, squash or melon plants were significantly attracted to the same plant, but those that were reared on watermelon did not show any preference for watermelon over the other plants (Table 1). Also on watermelon the growth of larvae was not as good as on the other three plants and this strain produced few insects. When the effect of the experience of feeding on a particular species of plant on the subsequent attraction to different species of plants were evaluated, the results reveal that females of *D. indica* significantly preferred cucumber over squash, melon and watermelon (Table 1).

Olfactory response of larvae

In the preliminary test using clean air (χ^2 = 0.64, P = 0.42) or cucumber leaves (χ^2 = 1, P = 0.31), there were no significant differences in the choice of arms of the olfactometer. This result reveals that both arms of the olfactometer were equally attractive and there was no positional bias in the tests using different odour sources. In comparison with clean air, the larvae of *D. indica* were attracted to uninfested leaves of cucumber, squash and melon, but not watermelon (Fig. 5). Also larvae did not respond to all of the four cucurbitaceous plants that were previously in-

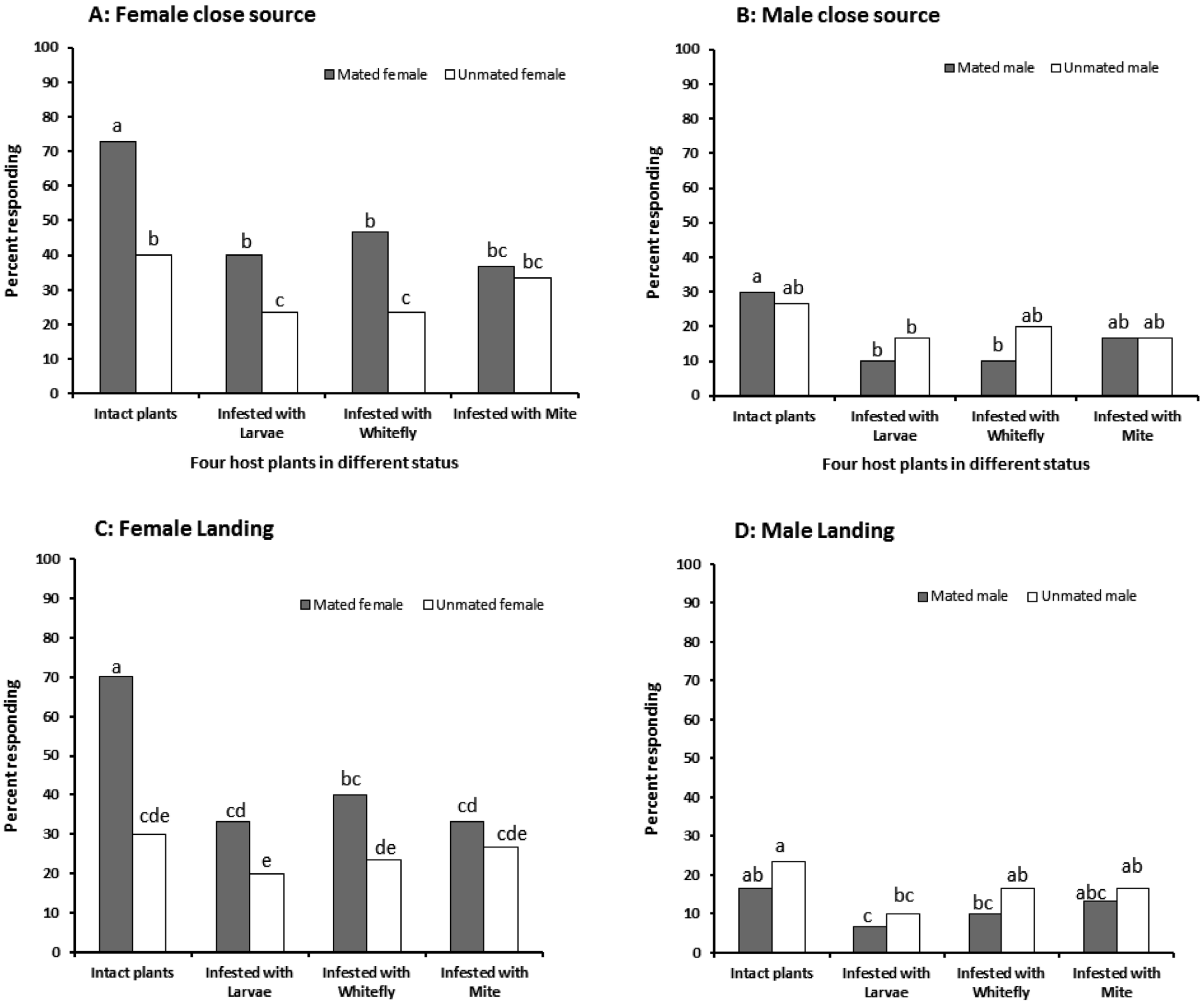

Fig. 3. Percentage of the responses of mated and unmated females and males of *Diaphania indica* to uninfested and infested plants in the wind tunnel. The percentage of individuals close to the source (A – females, B – males) and landing on the source (C – females, D – males) is indicated for each treatment. Columns topped by the same letter do not differ statistically (Chi square test, α = 0.05).

fested with conspecific larvae, whiteflies and mites (Fig. 5). The second assay reveals that volatiles from uninfested plants of cucumber, squash and melon were more attractive to the larvae of *D. indica* than those from previously infested plants, but not in the case of watermelon (Fig. 6). Also larvae did not respond to the odour of conspecifics, whether adults, larvae, pupae or eggs (Fig. 7).

DISCUSSION

Our results demonstrate that adult females *D. indica* distinguish among cucurbitaceous host plants and significantly prefer to oviposit on cucumber over squash, melon and watermelon (Fig. 1). Infestation of host plants by conspecific larvae, whiteflies or mites, significantly reduced egg laying and females significantly preferred uninfested over infested plants (Figs 1 and 2). Several previous studies report that damage to plants can affect their attractiveness for mated females and for egg laying. Herbivore-induced plant volatiles can repel or attract conspecific or heterospecific adult herbivores. Caterpillars induce tobacco to produce volatiles exclusively at night, which are highly repellent to the conspecific female moth *Heliothis virescens* (De Moraes et al., 2001). Mated females of *Trichoplusia ni*, significantly prefer healthy cotton and cabbage plants for oviposition over plants infested with conspecific larvae (Landolt, 1993). Female moths of *Spodoptera littoralis* lay only a third of the number of eggs on cotton plants infested with conspecific larvae than they do on undamaged plants (Anderson & Alborn, 1999). Oviposition choice by females of *T. absoluta* is influenced by the number of conspecific larvae on tomato plants and they significantly prefer uninfested plants or those infested with a few larvae (Bawin et al., 2014). In contrast to our results females of the diamondback moth, *P. xylostella* (Lepidoptera: Plutellidae), lay significantly more eggs on cabbage and canola plants infested with conspecific larvae than on uninfested plants (Silva & Furlong, 2012; Wee, 2016). The advantage of choosing healthy plants for egg laying can be little competition between offspring, a lower level of induced defence in the host plant and/or exposure to natural enemies.

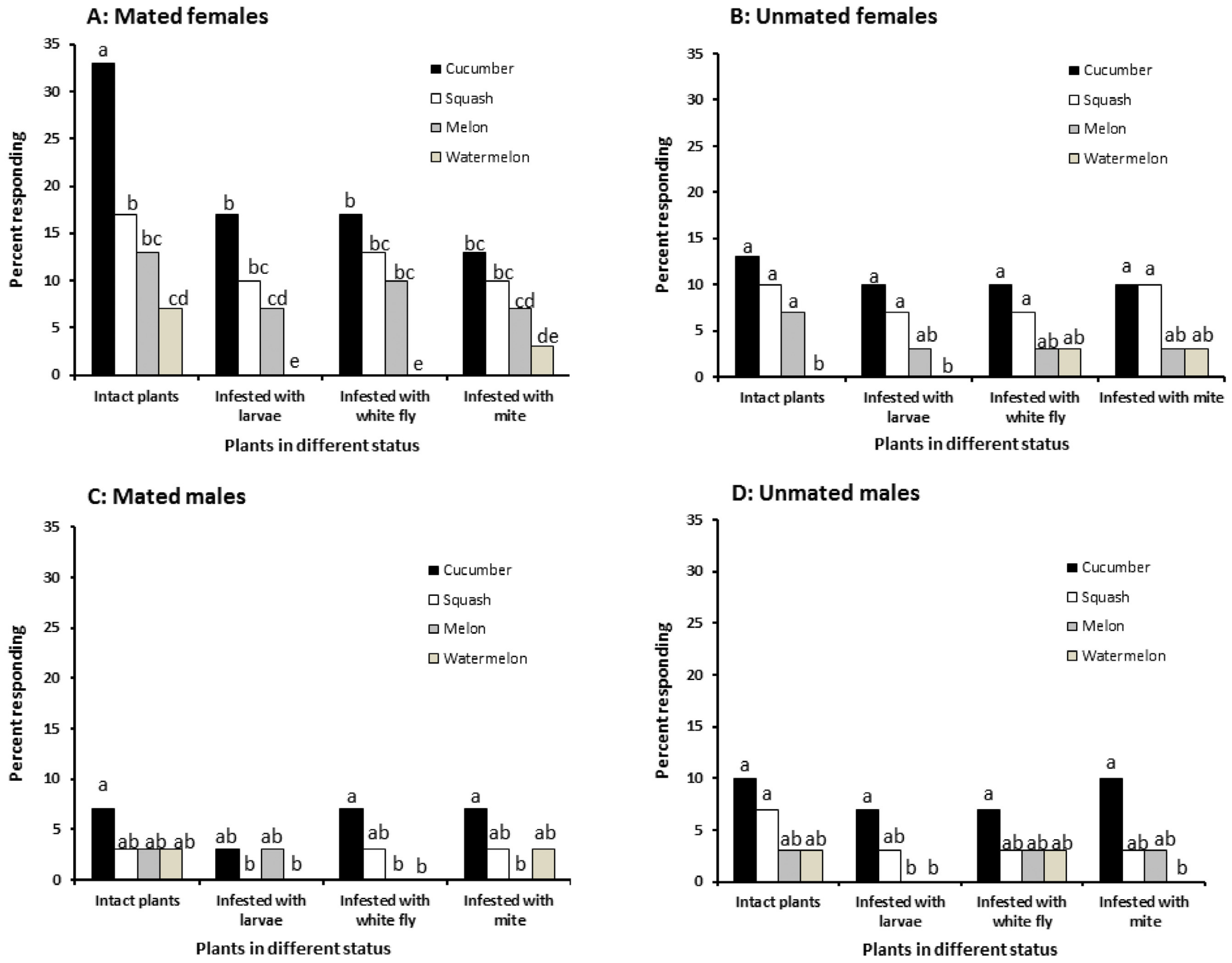

Fig. 4. Percentage attractiveness of the different host plants to (A) mated females, (B) unmated females, (C) mated males and (D) unmated males of *Diaphania indica* in the flight tunnel. Columns topped by the same letter do not differ statistically (Chi square test, α = 0.05).

Table 1. The effect of previous experience on host finding behaviour of females of *Diaphania indica* (n = 10 in each choice, Cu – Cucumber, Sq – Squash, Me – Melon, Wa – Watermelon).

Rearing source	Plant A	Plant B	Percentage of insects responding	Preferred plant	Percentage responding to the preferred plant	Statistical analyses	p
Cu	Cu	Sq	90	Cu	89	$\chi^2 = 60.84$	< 0.001
Sq	Cu	Sq	80	Sq	75	$\chi^2 = 25.00$	< 0.001
Me	Cu	Sq	70	Cu	71	$\chi^2 = 17.64$	< 0.001
Wa	Cu	Sq	70	Cu	85	$\chi^2 = 49.00$	< 0.001
Cu	Cu	Me	90	Cu	78	$\chi^2 = 31.36$	< 0.001
Sq	Cu	Me	80	Cu	75	$\chi^2 = 25.00$	< 0.001
Me	Cu	Me	90	Me	67	$\chi^2 = 11.56$	< 0.001
Wa	Cu	Me	80	Cu	75	$\chi^2 = 25.00$	< 0.001
Cu	Cu	Wa	80	Cu	88	$\chi^2 = 57.76$	< 0.001
Sq	Cu	Wa	90	Cu	78	$\chi^2 = 31.36$	< 0.001
Me	Cu	Wa	70	Cu	86	$\chi^2 = 51.84$	< 0.001
Wa	Cu	Wa	80	/	50	$\chi^2 = 0$	1
Cu	Sq	Me	100	Sq	60	$\chi^2 = 4$	0.045
Sq	Sq	Me	90	Sq	89	$\chi^2 = 60.84$	< 0.001
Me	Sq	Me	80	Me	75	$\chi^2 = 25.00$	< 0.001
Wa	Sq	Me	60	/	50	$\chi^2 = 0$	1
Cu	Sq	Wa	70	Sq	71	$\chi^2 = 17.64$	< 0.001
Sq	Sq	Wa	90	Sq	78	$\chi^2 = 31.36$	< 0.001
Me	Sq	Wa	70	Sq	71	$\chi^2 = 17.64$	< 0.001
Wa	Sq	Wa	70	Wa	57	$\chi^2 = 1.96$	0.16
Cu	Me	Wa	80	Me	62	$\chi^2 = 5.76$	0.016
Sq	Me	Wa	70	Me	86	$\chi^2 = 51.84$	< 0.001
Me	Me	Wa	80	Me	88	$\chi^2 = 57.76$	< 0.001
Wa	Me	Wa	70	Me	57	$\chi^2 = 1.96$	0.16

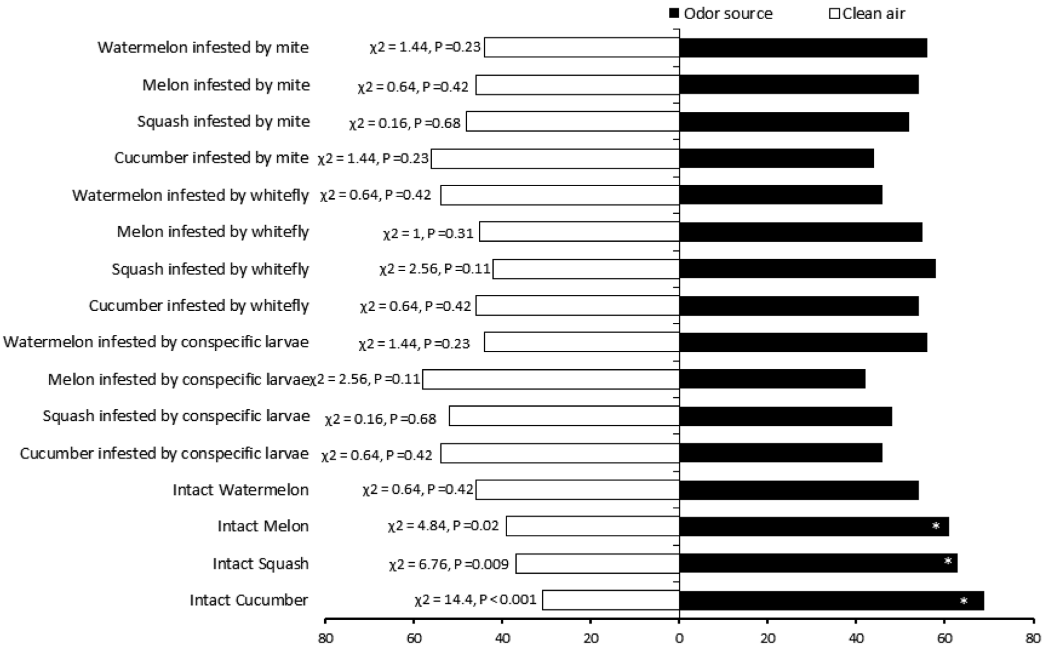

Fig. 5. Percentages of *Diaphania indica* larvae responding to uninfested or previously infested leaves of four cucurbitaceous host plants species vs. clean air in a Y-tube olfactometer. Asterisks indicate choice significantly different from 50 : 50 (χ^2 test, P ≤ 0.05).

The results of the present study on the olfactory responses of adults reveal that mated females had a stronger response than unmated females to the odours of uninfested and infested plants (Fig. 3C), which indicates that the response to host plant volatiles is associated with the physiological state of the female. However, there was no significant difference in the response of mated and unmated males to plant volatiles (Fig. 3D). Both male and female insects were more attracted to uninfested than infested plants. The differences in the responses of mated and unmated females indicate that mated females of *D. indica* are more responsive to cucurbitaceous plant volatiles than are unmated females and this is important for mated females as their choice of plants determines their fitness. It is reported that physiological changes in female moths after mating enhance the response of gravid females to host plant odours, because they need to lay eggs (Anton et al., 2007; Wee, 2016). Similarly, Saveer et al. (2012) show that mating, modifies the olfactory physiology of females of the cotton leaf worm, *S. littoralis* (Lepidoptera, Noctuidae), leading to a shift in olfactory preference from lilac flowers (*Syringa vulgaris*) to the green leaves of the larval host plant cotton. Attraction to volatile compounds is not only dependent on their chemical structure, but might change with the physiological status of the recipient (e.g., age, hormone levels, feeding status or mating status) or environmental conditions (Anton et al., 2007; Martel et al., 2009). The odour of tomato leaves elicit an upwind orientation

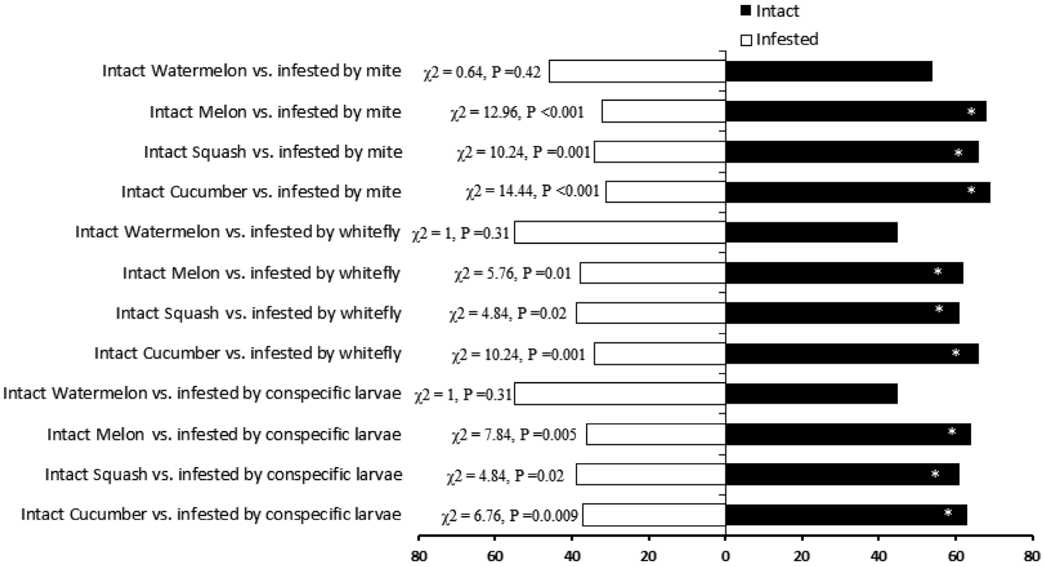

Fig. 6. Percentages of *Diaphania indica* larvae responding to intact vs. previously infested leaves of four cucurbitaceous host plants in a Y-tube olfactometer. Asterisks indicate a choice significantly different from 50: 5 0 (χ^2 test, P ≤ 0.05).

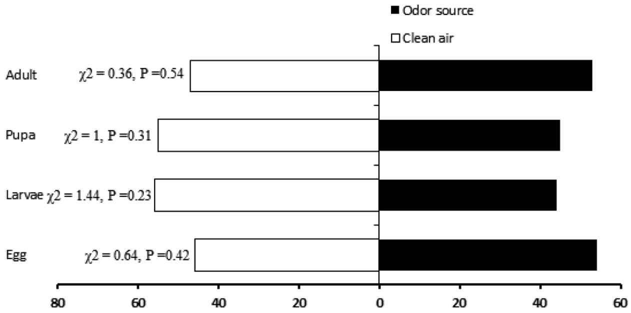

Fig. 7. Percentages of *Diaphania indica* larvae responding to different conspecific life stages vs. clean air in a Y-tube olfactometer. Asterisks indicate a choice significantly different from 50 : 50 (χ^2 test, P ≤ 0.05).

flight followed by landing and egg laying in mated females of the tomato leaf miner *T. absoluta*, but not in unmated females (Proffit et al., 2011). Mated females of the moth *Pectinophora gossypiella* (Lepidoptera: Gelechiidae) are more readily attracted to cotton flowers than are unmated females or male moths (Wiesenborn & Baker, 1990). Mated females of *Lobesia botrana* (Lepidoptera: Tortricidae) are attracted to host plant volatiles, but unmated females and mated and unmated males are not attracted (Masante-Roca et al., 2007). It is also reported that mating, increases antennal detection in *Dioryctria abietivorella* (Lepidoptera: Pyralidae) (Shu et al., 1997). In contrast to our results, mating status in the diamondback moth has little effect on the female's response to host odour and female moths are significantly more attracted to cabbage plants infested with conspecific larvae (Wee, 2016). It is also reported that virgin females of *S. littoralis* (Lepidoptera: Noctuidae) are more responsive to host plant volatiles than mated females (Martel et al., 2009).

The results of the present study indicate that the effect of experience on the olfactory preference of *D. indica* is dependent on the host plant. Females that had previous experience of cucumber, squash or melon plants were significantly more attracted to the same plant. But females that were reared on watermelon did not show any preference for watermelon over the other plants. Inexperienced females of *D. indica*, preferred cucumber over squash, melon and watermelon, suggesting that females have an innate olfactory preference for cucumber (Table 1). In other studies the results depend on the plant and insect species. For example, females of *H. armigera* inherently prefer tobacco for oviposition even after experiencing tomato, cotton or hot pepper plants (Hu et al., 2018). A one generation experience is enough to induce a preference for vanillin in *S. nonagrioides*, whereas even two generation experience failed to induce a response in adults of *Busseola fusca* and *B. nairobica* (Petit et al., 2018). It is reported that larval host plant experience modulates both mate finding and oviposition choice in *S. littoralis* (Anderson et al., 2013). In a variable environment, dependence on experience rather than innate preference could be advantages (Dukas, 2008) in terms of responding to environmental changes and increase in insect fitness (Anderson et al., 2013).

Few studies report the attractiveness of host plants for larvae. Our bioassays demonstrate that, the larvae of this pest can orient to plant volatiles. The volatiles from leaves of uninfested cucumber, squash and melon in the olfactometer attracted the larvae and enabled them distinguish between these host plants (Fig. 5). However, volatiles emitted by either conspecifics, uninfested watermelon or plants infested with either conspecific larvae, whiteflies or mites were not attractive for larvae of this pest (Figs 5, 7). When suitable food sources are exhausted the larvae of some species can leave the original oviposition site and forage for other host plants (Singer & Stireman, 2001; Zalucki et al., 2002; Carroll et al., 2006). Larvae can use visual and olfactory cues to find host plants. The role of volatile compounds in the host finding behaviour of larvae, has been documented far less frequently than for adult moths. The few studies on their feeding behaviour report similar results for larvae of *S. littoralis* that are attracted and feed more on undamaged cotton plants than on leaves of herbivore damaged plants (Anderson et al., 2001). It is reported that the larvae of *S. exigua* prefer undamaged plants to damaged plants and this is due to the leaves producing a feeding deterrent when damaged by larvae (Alborn et al., 1996). In contrast to our results, Carroll et al. (2006) found that both infested and intact maize plants are attractive for larvae *S. frugiperda* and this pest prefers odours from infested plants over those from uninfested plants. First instar larvae of *L. botrana* (Lepidoptera: Tortricidae) are attracted by plants infested with the fungus (*Botrytis cinerea*) (Mondy et al., 1998). Larvae that select an infested host plant may encounter induced plant defence, competition, cannibalism and also natural enemies.

In conclusion, our results indicate that the attractiveness of cucurbitaceous host plants for *D. indica* depends on the species and condition of the plant (uninfested and infested) and sex, mating status and experience of the insect. This study investigated the effects of plant volatile infochemicals in attracting and determining the oviposition behaviour of *D. indica.* For improving the monitoring and management of this pest it is now important to identify the behaviourally active volatiles involved and breed more resistant plants.

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