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# Effects of Chinese Mustard *Brassica juncea* Volatiles on the Olfactory Responses of *Plutella xylostella*

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Abstract: Many plants under attack by herbivores release chemical reagents that act as a call sign for the enemies of the feeding herbivores. This raises the question of whether these chemical agents can be used by herbivores to find these plants. This case study was attempted to answers that question by recording the olfactory responses of the diamondback moth (DBM; Plutella xylostella (L)) to Chinese mustard (Brassica juncea) volatiles. The Chinese mustard plants used in this study were damaged in five different ways: mechanically, by using detached insect body parts (thorns on the hind legs of grasshoppers), by aphids (Lipaphis ervsimi), by cabbage leaf webber (Crocidolomia binotalis) larvae and by DBM larvae. The results of a Y-olfactometer test showed that DBM females gave a variety of responses according to the type of plant damage. For instance, upon exposure to two types of plants, one was injured by C. binotalis and second one was injured by their larvae, DBM females were attracted more to the plant damaged by C. binotalis than to the plant damaged by their larvae. Similar results were obtained for plants damaged by aphids. However these plants were not preferred when uninjured plants were given like second choice. DBM females showed strong attraction to the plants damaged by C. binotalis larvae in all cases where they have a choice between plants damaged by other insects or C. binotalis larvae. Conversely, only subtle responses were recorded for plants injured mechanically or by detached body parts of insects. Thus, this study shows that a plant emits different volatile organic compounds (VOCs) depending on the type of damage inflicted upon it or on the damage causative. DBM females can use that emission to find hosts that will maximize the survival rates of its offspring and minimize competition for nutrition. This study also confirm that the important of faunal diversity in reducing the insect pest infestation. Introducing of some aphid-damaged plants and plants damaged by C. binotalis larvae randomly in the middle or at the edge of brassica crops field could act as refuges for the DBM and that could also divert more DBM females to lay on them than on the untreated plants. Also, spraying VOCs obtained from aphid-damaged plants or from plants damaged by C. binotalis larvae on some chosen plants in specified area at the brassica crops field could be benefit too. This will further improve the integrated DBM management.

Key words: Plutella xylostella · Crocidolomia binotalis · Crucifer plants · Y-olfactometer · Volatiles compounds

# INTRODUCTION

Plants produce a vast range of compounds that have extremely intricate and complex structures. These products, which so call the 'semiochemicals', help the plant to survive by fulfilling important functions in the interaction between a plant and its biotic and abiotic environment [1, 2]. A wide range of behavioural responses can be produced in insects by a plant's semiochemicals [3]. These volatiles can prove to be either detrimental or helpful for the insect [4]. It is a common defence mechanism of many plants to produce

**Corresponding Author:** Idris Abd. Ghani, School of Environmental and Natural Resource Science, Faculty Science & Technology, Universiti Kebangsaan Malaysia. herbivore-induced volatiles that attract natural enemies of herbivores trying to damage them [5, 6]. Plants belonging to the Brassicacaeae family are usually attacked by diamondback moth (DBM), Plutella xylostella and other insects [7]. In fact, DBM has been the insect that inflicted the most destructive damages to brassicas crop plants worldwide [8, 9]. These plants constitute the most important vegetable crops of tropical Asian countries and therefore finding ways to protect them is paramount [10]. Up to date the DBM has been able to develop resistant to all pesticides used against them including those of microbial and other biopesticides [11]. Other control methods such as mix planting, intercropping and organic farming have also been investigated to have some positive effect to control DBM. However, detail research to understand the behavioural response of DBM populations to plants in such agro-ecosystem is lacking [12]. In fact, DBM may response differently to single host plants attacked by different insect as reported to other insect pests. These might be happened as plant react by producing different volatiles organic compounds (VOC) when attacked by different insect herbivores [13]. These VOCs also have significant impact on the natural enemies of the insect herbivores attacking the host plants. Specialist insect herbivores are often closely associated with plants that produce chemicals with characteristic that make it easy for the insect to locate a host [4]. As a particular plant attacked by herbivores, is prompted to produce a collection of compounds [5, 6]. The herbivore can be identified with the help of these plant volatiles [3, 14, 17]. For example, the reproductive and feeding activities of the DBM can be activated by certain compounds, including glucosinolates, cardenolides, plant volatiles, waxes, as well as the host plant's nutritional quality, leaf morphology and leaf colour, or a combination of these factors [18]. Therefore, the aims of our study were to investigate the effects of VOCs emitted by Chinese mustard (Brassica juncea) plants damaged differently on the olfactory responses of the diamondback moth (DBM; Plutella xylostella (L).

**Diamondback moth** (*Plutella xylostella* L. (Leidoptera: Plutellidae)): Diamondback moths (DBM) (larvae and adults) were collected from small farms in Danau Desa and from the Kampong Batu Muda in Kuala Lumpur, Malaysia. DBM larvae were reared on cabbage plants placed in a screen cage  $(30 \times 35 \times 40 \text{ cm})$  and kept under laboratory environment  $[27\pm2^{\circ}\text{C}, 60\pm5\% \text{ R.H})$  in the entomology laboratory at the Malaysian Agriculture

Research and Development Institute (MARDI), Serdang, Selangor, Malaysia. Rearing was carried out in a controlled environment as above, with a photoperiod of 16:8 (L:D). A total of 30 newly emerged males and females were allowed to mate for 2-3 days in 3-4 separate glass containers covered with mosquito netting. Honey solution was used as source of nutrient while fresh cabbage leaves were provided for better oviposition. When the eggs were hatched, the leaves with the first instar larvae were transferred to small plastic cages (20 x 15 x 30 cm) containing fresh cabbage leaves and covered with a mosquito net. The pupae were removed to a small netting cage ( $40 \times 45 \times 45$  cm). The emerged adults were transferred by using insect aspirator to the rearing cages.

**Cabbage Leaf Webber,** *Crocidolomia Binotalis* Zeller (Lepidoptera: Pyralidae): Larvae was collected from the MARDI field at Jalan Kebun and the SENR Research Centre of MARDI in Serdang, Malaysia for the first time and reared on cabbage plants as in DBM above on cabbage plants placed in a screen cage ( $30 \times 35 \times 40$  cm) at the MARDI Entomology Laboratory. The pupae formed were kept in screen cages (45 cm<sup>3</sup>) until adult emergence. Adults were transferred 2 days later to rearing cages of ( $35 \times 40 \times 45$  cm) for mass rearing. A 20% honey solution was provided as food to the adults via a cotton plug and then the 5-7-day-old larvae were used in the experiments.

Aphid, *Lipaphis Erysimi* (Kltb) (Homoptera: Aphididae): Aphids were collected from Chinese mustard plants grown in glasshouses at MARDI and reared on Chinese mustard plants in wooden screen cages ( $70 \times 70 \times 90$  cm) shielded with mosquito netting and kept in the entomology laboratory, MARDI at  $27\pm2^{\circ}$ C,  $60\pm5\%$  R.H.

**Plants:** The seed of Chinese mustard (*Brassica juncea*) and cabbage (*Brassica oleracea* L.) were sown in plastic pots 18 cm in diameter filled with soil. Cabbage plants were used for insect rearing. The seeds were bought from Sing Heng Huat Company, Taman Bukit Puchong, Malaysia. All plants were grown under glasshouse conditions at MARDI. The Chinese mustard plants used in the experiments were grown in separate insect-proof cages ( $2.5 \times 2 \times 8 \text{ m}$ ). These plants were cultivated directly in (12 cm diameter) plastic pots until the 6-leaf stage when they were transferred to the laboratory two days prior to the experiments.

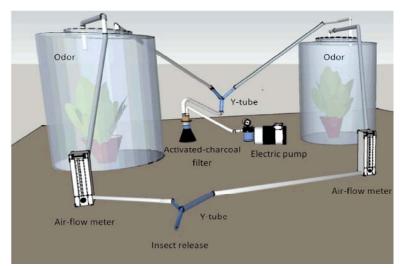


Fig. 1: Perspective view of the Y-Olfactometer.

No-Choice Test: Chinese mustard plants previously injured by five different ways, mechanically, using detached insect body parts (thorns on the hind legs of grasshoppers), aphids, cabbage leaf webber larvae and DBM larvae. The 2-3-day-old DBM mated females were tested individually (no-choice test) using a Y-olfactometer (Figure 1). This instrument had a Y shape and made of transparent Plexiglas (2.5 cm ID; stem 13 cm, arms 10 cm; stem-arms angle 120°). The arms of the Y-olfactometer were connected to two glass containers (22 L) in which the plants (odour source) were placed. An electric pump (Cole-Parmer Air cadet vacuum/pressure station, Illinois, USA) connected to the olfactometer pushed filtered air via silicon tubes into both containers through activated charcoal and the air then passed through two separate flow meters that regulated the flow rate of air to 0.6 l min ?<sup>1</sup>. To avoid cross contamination between experiments by volatiles come from tested plants, all materials used in the test were thoroughly washed in soap and water, rinsed in 70% ethanol and dried after every three runs. Each insect was allowed to respond for 8 min and used only once. A total of 16 plants were tested in each experiment. The control plant was changed every three runs.

In the paired comparison experiments, the plants were damaged by larvae of DBM, larvae of *C. binotalis* or aphids. Basically, the test was conducted to compare the responses of a DBM female to the volatile organic compounds (VOCs) emitted by plants damaged by larvae, either by chewing (*C. binotalis*) or by sap-feeding (aphid). In this comparison experiments, for each pair of odour sources, 16 plants were used per experiment, i.e. eight plants for each type of insect damage and a total of

40 female DBMs adults were individually tested (five in each run). All experiments were done at  $26\pm2$  C° and at 60-75% relative humidity. The Y-olfactometer was positioned on a white table with overhead lighting by fluorescent lamps.

The Chinese mustard plants (*B. juncea*) were moved to the laboratory two days before the experiments where they were kept individually in small netting cages  $(20 \times 20 \times 30 \text{ cm})$ . For each of the five ways of plant damaged, eight separate plants were studied and 30 individual of second- and third-instar DBM larvae, 15 second-instar *C. binotalis* larvae or 150 aphids were placed on the leaves of each plant by using a soft brush. To inflict mechanical damage, a quarter of each of three leaves per plant was cut with scissors. For the damage by detached insect body parts, the upper surface of the leaves was scratched by using the thorns of the detached hind legs of grasshoppers. Plants were impaired again mechanically and by detached insect body parts one hour prior to the start of experiments.

A paired t-test was used to compare the difference in responses of naïve DBM females to VOCs emitted by undamaged Chinese mustard plants and their responses to the different types of damage on individual host plants under laboratory conditions.

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

#### RESULTS

No significant difference (t = 1.05, P = 0.05, df = 57) was observed between the mean number of DBM females adult responded to plants damaged by DBM larvae and the mean number of DBM adult females that responded to undamaged plants (Figure 2- A).

In respect of the effects of the VOCs of Chinese mustard plants damaged by *C. binotalis*, a high significant difference (t = 3.83, P < 0.01, df = 60) was observed between the mean numbers of DBM females attracted to plants damaged by *C. binotalis* larvae and to undamaged plants (Figure 2-B). No significant difference (t = -1.00, P = 0.2, df = 67) between the mean number of DBM females attracted to the VOCs from aphid-damaged plants and that of females attracted to the control (undamaged) plants was observed (Figure 2-C).

There was also no significant difference (t = 1.59, P = 0.116, df = 81) between the number of DBM females responded to plants damaged by detached insect body parts and the control (undamaged) plant (Figure 2-D). Similar results (t = 2.16, P= 0.35, df = 63) were obtained when DBM females were offered a choice between mechanically damaged plants and an undamaged plant (Figure 2-E). There was a very high significant difference (t = -3.31, P = 0.002, df = 58) between the mean

number of DBM females attracted by the plants damaged by *C. binotalis* larvae and the mean number attracted to the plants damaged by aphids (Figure 3-A).

The attraction of naïve DBM females to plants damaged by *C. binotalis* larvae was also greater than that to plants damaged by DBM larvae, but the difference was not significant (t = -1.49, P = 0.141, df = 93; Figure 3-B).

## DISCUSSION

Results of the present study demonstrated and confirmed that the orientation response of DBM females to their host plants depends on the information sent by the host or by the herbivores infesting the plant [19, 3]. Different types of damage could cause a variation or differences in the VOCs emitted by the same host [20]. Producing and sprinkling of these VOCs could induce typical response in DBM females which varied with how their hosts were damaged. Interestingly, in this study, regardless of the type of damages, the DBM females responded according to the VOCs information they got from both damaged and undamaged plants. It has been suggested that DBM females will prefer undamaged plants over damaged ones because of the attractive VOCs emanated by undamaged plants and that they avoid plants with any type of damage (including its own larvae)

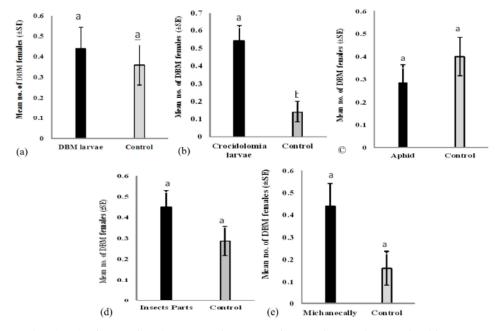


Fig. 2: Mean number (±SE) of DBM females attracted to VOCs from undamaged (control) Chinese mustard plants (B. juncea) and damaged by; (A) DBM (P. xylostella) larvae, (B) Cabbage leaf webber (C. binotalis) larvae, (C) aphid (L. erysimi), (D) detached insect body parts, and (E) mechanically damaged plants, as tested using Y-olfactometer.

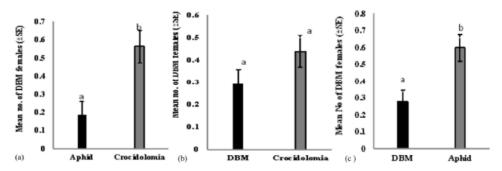


Fig. 3: Mean number (±SE) of DBM females attracted to VOCs from Chinese mustard plants (B. juncea) damaged by; (A) aphids (L. erysimi) and to plants damaged by cabbage leaf webber (C. binotalis) larvae, (B) DBM larvae and to plants damaged by C. binotalis larvae, and (C) by aphids (L. erysimi) and to plants damaged by DBM larvae tested using Y-olfactometer.

or the VOCs emitted might be repellents. However, we found no significant difference between the number of DBM females responded to the undamaged plants and those attracted to plants infested/injured by their own larvae. In addition, DBM females appear to prefer plants damaged by *C. binotalis* larvae over undamaged plants and over plants damaged/infested by other herbivores (aphids) where the difference was highly significant. The plants in all three cases probably emitted different VOCs that attracted or repelled the tested insects to different extents. These differences in the DBM female's behaviour have three probable explanations:

- The mustard plants infested by *C. binotalis* larvae produce VOCs that are attractive to DBM females.
- The mustard plants that harbour a number of DBM larvae or the actual larvae themselves produce repellent VOCs that are not present when the plants are attacked by *C. binotalis*, which encourages the DBM females to choose the plants attacked by *C. binotalis*.
- The volatiles emitted by the plants attacked by DBM larvae are not that attractive to the tested DBM females.

The results presented in Figures (2-B) and (3-A) support the first explanation, where the greater attractive effect of the VOCs emitted by plants damaged by *C. binotalis.* The difference was significantly high (P < 0.05) between the mean numbers of DBM females attracted by plants damaged by *C. binotalis* larvae and intact plants, as well as plants damaged by aphids.

Secondly, although the majority of tested insects went to plants damaged by *C. binotalis* larvae compared to DBM-damaged plants, the difference was not significant (P > 0.05). So it is most probably attributed to

the volatiles emitted by DBM-damaged plants are not attractive to DBM females as the females reacted evenly when they were offered DBM-damaged plants and an undamaged plant. This suggests that undamaged and DBM-damaged plants provide somewhat equivalent olfactory stimuli to DBM females (Figure 2-A). However, this finding is not in agreement with the results of Kaori Shiojiri [21], who found that in the case of volatiles, from cabbage plants with differential damage, there was a significant increase in VOCs from DBM-damaged plants over the undamaged plants.

Turlings and co-workers [22] have shown that insectinfested corn plants emit volatiles but no volatiles were emitted when the same plants were damaged artificially. In the present study, DBM females always preferred the odour released by *C. binotalis*-damaged plants or by aphid-damaged plants when tested against DBMdamaged plants and interestingly, the difference was significant in both cases (P < 0.05). Therefore, we suggest the DBM females were attracted to the specific VOCs released from the plants damaged by *C. binotalis* or by aphids which are absence in the VOCs from plants damaged by DBM larvae.

In all cases where the DBM females were tested for their reaction to VOCs emanated by the Chinese mustard plants infested by aphids, the DBM females did not show any interest in the plants damaged by aphids. However, when they were offered DBM-damaged plants as a second choice, they were more attracted to the plants damaged by aphids. The purpose of this part of our research was to study if the changes in herbivore feeding mode could induce different plant volatiles that would affect the olfactory response of DBM females. This result prompts a few new questions: first, how do different herbivore species induce plant volatiles that differ depending on their feeding type? Second, how that could

# affect the DBM female's reaction when given choice such as in the nature/field? Third, Do the VOCs emanated by DBM-damaged plant induce Aphid-damaged plants to produced extra VOCs that seemingly attract DBM females?

It is known that the feeding habit of aphids, like all sap-feeding insects, is very accurate and produces little mechanical damage compared to that of chewing insects. However, plants can still detect aphid damage and emit VOCs. [23]. This feeding behaviour minimizes plant host tissue injury and loss so in essence there is little competition for food between DBM larvae and aphids. The elimination of the competition (for available food resources and oviposition) is one of the female's herbivore priorities. The presence of plants other than those occupied by same larval species may not only provide available resources for offspring, but could also minimize internal competition for nutrition. This could explain the preference of DBM females for aphid-damaged plant over DBM-damaged ones. Also, avoiding plants damaged by DBM larvae and instead laving eggs on plants damaged by other insects may reduce the chances of natural enemies finding their offspring. This explanation tends to agree with that of Reddy and Guerrero [3], who revealed that the responses to the effects induced by host plants on herbivore insect seem to be part of insect strategies to gain access to new feeding and oviposition sites. Although the chemicals emitted by plants upon contact with herbivores can equally and quickly attract insects, parasitoids and predators, it is unlikely that this is the only reason for the leaking of chemical compounds [24].

We also tried to simulate the damage that could made by the mouth parts of insects but avoiding the effect of the VOCs that come from the insect's body (pheromones) or from their saliva, frass, etc. by using the thorns on the hind legs of grasshoppers to make wounds on the cabbage plant leaves. The effect of VOCs produced by plants damaged by detached insect body parts seems to be similar to that of VOCs induced by mechanically damaged plants. In both cases the DBM female seemed to prefer the damaged plant over the undamaged plant. In the case of plant damaged by detached insect body parts, it would appear that the damage caused by hard insect mouth parts could be similar to the damage caused by manmade tools. Although the VOCs emanated by plants damaged by DBM larvae could be similar to that induced by plants damaged mechanically, the plants damaged mechanically were attractive to DBM females. This finding is in agreement with results of Bruinsma [25] and Girling [26].

#### CONCLUSION

In this study we have focused on the effect of the VOCs emitted by plants in providing information about the plant host and on the capability of herbivorous insects to react to this information. We found that the DBM female responded differently according to types of plant damaged. The DBM female was more attracted to plants damaged by Cabbage Leaf Webber (C. binotalis) larvae than to plants damaged by aphids or by larvae of DBM. This need further study to answer as to why and what are the extra things in the VOCs emanated by plants damaged by C. binotalis. If those particular components of VOCs characterized and isolated and tested against DBM, then we might be able to get a compound that could be used as attractant to DBM or as a trap like a pheromone traps. This will further improve the integrated DBM management. Interestingly, DBM-damaged plants seemed to induce extra VOCs emanated by aphiddamaged plants that attracted more DBM to previously less attractive aphid-damaged plant to DBM females. These plant to plant chemical communications which affect the herbivores host oviposition behaviour needs further study. This was also seen when the females were given a choice between plants damaged by aphids or C. binotalis larvae. DBM females showed strong attraction to the plants damaged by C. binotalis larvae.

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