Olfactory Response of *Plutella xylostella* (Lepidoptera: Yponomeutidae) Adults to Refugia Plant

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**Abstract.** Selection of refugia plants is an important step when engineering the agroecological system. The refugia plants can be repellent or attractant to insect pests and in many species the role of each plant can be studied using the olfactory responses of the insect to volatile chemical cues emitted by the plant. The objective of this study was to determine the olfactory response of *Plutella xylostella* (Lepidoptera: Yponomeutidae) adults to several refugia plant candidates. Y-tube olfactometer tests were used to determine responses of *P. xylostella* to candidate plant species including: *Ageratum conyzoides*, *Galinsoga parviflora*, *Synedrella nodiflora*, *Sphagneticola trilobata*, *Brassica rapa*, *Rorippa indica*, *Arachis pintoi*, *Ocimum basilicum*, *Apium graveolens*, and *Portulaca oleracea*. Olfactory choice tests were conducted to compare preferences of insect. The results showed that *R. indica*, *G. parviflora*, and *S. trilobata* flowers attracted *P. xylostella*. *R. indica* and *B. rapa* leaves had high attractiveness for *P. xylostella* adults. At the same time, the *A. graveolens*, *A. conyzoides*, and *O. basilicum* leaves showed potential repellency. The conclusions of this study are *R. indica* and *B. rapa* have potential for use as trap crops, while *A. graveolens*, *O. basilicum*, and *A. conyzoides* have potential as repellent plants. Further study is necessary to test the olfactory response of *P. xylostella*’s natural enemies to these refugia plants.

**Key words:** Ecological engineering, choice test, insect olfactory, repellent plant, trap crop.

1. Introduction

Diamondback moth *Plutella xylostella* (Lepidoptera: Yponomeutidae) is one of the important pests in cabbage cultivation [1, 2] and caused high yield losses in cabbage, which ranged from 87.5-100% [3-5]. *P. xylostella* are often controlled by farmers using synthetic insecticides [6]. Synthetic insecticides are more widely used by farmers because they are considered faster, more effective, and efficient. However, the use of insecticides at high doses and intensity can have negative impacts on the environment, human health, natural enemies and insect pests themselves [7,8]. One of the negative effects of intensive use of insecticides is the emergence of resistance [9-13].

An alternative approach to the control of this pest is agroecosystem engineering. One approach that has been proved successful as a component of integrated pest management (IPM) systems for the control of other pests involves habitat engineering, in many cases planting refugia plants around cultivated plants. These refugia plants can act as a source of nutrition for natural enemies of pests, trap crops, and pest repellents. The presence of of such flowering refugia plants can have a positive effect on
natural enemy populations while simultaneously suppressing pest numbers [14]. Several plants such as Allium sativum, Mentha spicata, Ocimum gratissimum, have been reported to be repellent to P. xylostella [15,16].

The technique relies on a broad understanding of both the agro-ecosystem involved and the biology and behaviour of the pest and its natural enemies. Careful investigation and informed choice of refugia plants to be used is often the earliest stage of the development of practical habitat engineering methods, and requires a detailed understanding of the olfactory response of pest and natural enemies to a plant [17]. Olfactory ability is often central to early stages of host location, by insects for both food sources or egg laying [18]. Olfactory responses to a plant provide an overview of the relationship between the two. Insect olfactory responses that tend to be negative towards a plant or part of a plant can indicate the presence of volatile compounds that have resistance activity, whereas, if insects show a positive olfactory response, it frequently indicates that the plant has the potential to be a host or a source of food (attractants) [19,20]. Y-tube olfactometer tests have been used successfully in many studies, and are considered a reliable method for testing such responses [21]. Few studies have been published from South East Asia which identify refugia plants which have potential as repellent or trap plants for P. xylostella. Therefore, research is needed to determine the types of refugia plants that act as parasitoid attractants, as well as trap plants and repellents for pests. The results of this study provide information that can be used when selecting the types of refugia plants suitable for manipulating the cabbage planting habitat. Research objective of this study was to determine the olfactory responses of P. xylostella adults to flowers and leaves of refugia plants. The olfactory response will be used to predict the potential of a refugia plant as either a repellent or trap crop.

2. Methods
2.1 Plutella xylostella Rearing
P. xylostella used in this study were descendants of the adult population taken from Pacet District, Cianjur Regency. The imagos obtained were cultured at the Physiology and Insect Toxicology Laboratory, Department of Plant Protection, Faculty of Agriculture, IPB. Insect adults were fed with a 10% honey solution applied to a cotton ball. Pesticide-free cabbage leaves are used for larvae feed and for laying adult eggs.

2.2 Propagation of Refugia Plants
The refugia plants used have several criteria, i.e., 1) can grow well in a cabbage growing environment, 2) plant height is around 10-40 cm, 3) does not compete with the main plant, and 4) is known or has been reported to be repellent or attractant for pests (adapted from [22]). Ten refugia plants used in this study include the Asteraceae family: Ageratum conyzoides (L.) L., Galinsoga parviflora Cav., Sphagnetica triloba (L.) Pruski, Synedrella nodiflora (L.) Gaertn., Portulacaceae family: Portulaca oleracea L., Brassicaceae family: Rorippa indica (L.) Hiern, Brassica rapa L., Apiaceae family: Apium graveolens L., Lamiaceae family: Ocimum basilicum L., and Fabaceae family: Arachis pintoi Krapov. & W.C.Gregory. The refugia plants obtained are then planted in polybags containing soil: compost (1:1). Plant maintenance is done by watering it sufficiently.

2.3 Tests of Adult Plutella xylostella Olfactory Responses against Refugia Plants
Testing the response of insects to flowering plants was carried out using an olfactometer which refers to the method of Lu et al. [23]. The open end of one arm of the Y-tube olfactometer shaft was covered with gauze then connected by a hose to the pump (Dynapump Model 3). Between the olfactometer and the pump a flow meter ( Gilmont GF-6541-1215) was installed to monitor the air flow rate. Air was drawn into the olfactometer through intake chambers at each of the two remaining arms of the Y-tube, and plant material used in experiments was placed in these chambers. After all parts of the olfactometer had been assembled, the insects were placed in the arm with the gauze and air was drawn through the system at a rate of 100 ml/minute. The test was carried out at room temperature (±26 °C) and RH (±70%; Figure 1).

The test consisted of five experiments, namely: 1) refugia flowers vs. clean air, 2) refugia flowers vs. refugia leaves (same plant), 3) refugia flowers vs. other refugia flowers, 4) refugia leaves vs. clean air, and 5) refugia leaves + cabbage vs. cabbage leaves. Each experiment was replicated 15 times.
2.4 Data analysis
The analysis of the significant difference in the selection of odor sources in the olfactory test used the chi-square test. Data analysis used Minitab17 software.

3. Results and Discussions
The results of Experiment 1 showed that when *G. parviflora*, *R. indica*, and *S. trilobata* were tested, the *P. xylostella* adults were more attracted to air passed over the flowers than to clean air (p < 0.05) (Figure 2A). In contrast, at *A. graveolens* and *O. basilicum* flower, *P. xylostella* were more attracted to clean air and avoid floral odor. The three flowers which elicited the strongest response, *G. parviflora*, *R. indica* and *S. trilobata*, were used in Experiment 2 to compare the olfactory preferences of *P. xylostella* adults between flower and leaf scents of the same plant species. The results of experiment 2 showed that in general *P. xylostella* adults were significantly more attracted to floral scent, except in the test with *R. indica* in which no significant difference was detected (Figure 2B). This is because *R. indica* is from the Brassicaceae family that is included in the host range of *P. xylostella*, so that *P. xylostella* adults are not only attracted to its flowers, but also its leaves. Of the three types of flowers used in experiment 2 were reused in Experiment 3. The purpose of Experiment 3 was to compare the olfactory preferences between two different types of refugia flowers. The results of this test showed that in general, *R. indica* flowers had a higher attractiveness than the other two types of flowers (Figure 2C).

In Experiments 4 and 5, the tests aimed to determine the olfactory response of *P. xylostella* adults to refugia leaves. The results of experiment 4 showed that there were two refugia plants whose leaves were highly attractive to *P. xylostella* adults, namely *R. indica* and *B. rapa*, while the other eight plants had low attractiveness, even the leaves of *A. conyzoides*, *O. basilicum*, and *A. graveolens* were not attractive to the adults tested (Figure 2D). The leaves of the refugia plant which attracted *P. xylostella* have the potential to act as a trap plant, while the low attractiveness of the other plants might be explained in one of two different ways. The first possibility is that the plant is not a host of *P. xylostella*. Alternatively the plant may give out repellent volatiles. The eight plants which had low attractiveness for *P. xylostella* adults were used for the next test (Experiment 5).

Experiment 5 was used to compare the preferences of *P. xylostella* adults when leaves from cabbage plants were compared with those from refugia plants. The results of experiment 5 showed that *P. xylostella* adults were not attracted to the cabbage plants placed together with *O. basilicum*, *A. graveolens*, and *A. conyzoides* (p < 0.01) compared to five other refugia plants (Figure 2E). The test results indicated that *O. basilicum*, *A. graveolens*, and *A. conyzoides* had the potential to become repellents plants for *P. xylostella* adult, while the other five types of refugia had no potential because they did not show any repellency activity.

Understanding the olfactory attractiveness of insects to flowers or other parts of plants is an important step when considering the role of that plant in IPM systems. The olfactory attraction of a parasitoid or pest to a flower will indicate whether the flower might be used as a source of nutrition for the insect [24]. In addition, the level of pest repellency against a part of a plant, such as a stem or leaf, will indicate its potential as a pest repellent. Many pest and natural enemy species respond to volatile chemical cues when location sources of nutrition, as well as plant or insect hosts. Sensory structures on the insect body used to detect volatile compounds emitted by plants, such volatile compounds can be divided between two types, those that attract (positive influence), or repel insects (negative effect).
Figure 2. Response of *P. xylostella* adult olfactory on flowers & leaves of refugia plants. The percentage of interest in adult are: (A) refugia flowers with empty air, (B) flowers and leaves from the same refugia plant, (C) one type of refugia flower with another flower, (A) refugia leaves with empty air, (B) leaves cabbage and cabbage leaves + refugia. Description: Api (*A. pintoi*), Pol (*P. oleracea*), Sno (*S. nodiflora*), Str (*S. trilobata*), Agr (*A. graveolens*), Obs (*O. basilicum*), Gpa (*G. parviflora*), Aco (*A. conyzoides*), Rid (*R. indica*), Brp (*B. rapa*). Boc (*B. oleracea* var. *capitata*). ** = p <0.01, * = p <0.05.
P. xylostella's unattractiveness to several species of flowers in the current study may have been the result of test flowers being presented when still attached to their stems or leaves, which in turn may have secreted a repellent compound, thus deterring the moths. Such repellency can be seen in Experiment 4 and 5 which showed that leaves from several types of plants such as A. conyzoides, O. basilicum, and A. graveolens display high levels of repellency. Both visual cues and plant volatile compounds are used by many insects to locate host plants [25-27]. However, when used in IPM systems utilizing habitat engineering to reduce the incidence of pests, insect can also be repelled by volatile chemicals from nearby non-host plants [28, 29] without physical contact with those plant [30]. Thus, in IPM systems, such “odor-masking agents” can be used to disrupt the host-seeking behavior of insect pests reducing the resultant damage to crops [30].

Many species of moths are attracted by the odor emitted by flowers [31-33]. One of the important compounds that attract the presence of moth adult is phenylacetyldehyde (PAA) [34-36]. Meagher and Landolt [37] stated that some moths such as Argyrogramma verruca (F.), Mocis disseverans (Walker), Heliothis virescens (F.), Spodoptera eridania (Stoll) and Diaphanus hyalinata (L.) are attracted to PAA compounds. Dai et al [38] stated that P. xylostella adult are also attracted to 13 compounds in Brassica plants, one of which is PAA.

The presence of the three refugia plants (O. basicilcum, A. graveolens, and A. conyzoides) has the potential to reduce the attractiveness of some host plants (including crop plants) to P. xylostella adults. Other plants (mostly aromatic plants) have also been reported to mask the odor of the host plant, thereby changing the orientation of an insect's olfactory attraction [39, 40]. Basedow et al. [41] stated that the presence of O. basilicum in the vicinity of Vicia faba and Pyrus communis plants showed repellency activity and reduced pest attraction to the host for aphids (Aphis fabae and A. citrionala). The aphids Neotoxoptera formosana were not attracted to the aroma of Allium fistulosum and A. tuberosum in olfactory tests when combined with α-pinen from rosemary flower oil (Rosmarinus officinalis), which masked the aroma of the host as well as being repellent [41].

Zhang et al. [43] reported that α-terpinene, limonene, and linalool showed significant repellent activity and oviposition inhibition of P. xylostella, whereas verbenon and β-caryophyllene showed no significant effects. These volatile compounds are reported to be present in extracts or essential oils of three of the refugia plants that showed repellency effects in the current study, namely linalool in O. basilicum [44, 45], limonene and β-caryophyllene in A. graveolens [46] and β-caryophyllene in A. conyzoides [47]. These compounds are thought to cause the three refugia plants to have repellent activity and can reduce the number of eggs laid on the cabbage plants in the crop.

Several previous studies have reported the insecticidal or resistance activity of A. conyzoides, A. graveolens, and O. basicilcum against several types of insects. These compounds are thought to play a role in repellency of insect adult. Oil from the leaves and flowers of A. conyzoides has been reported to exhibit resistance against several insects [48]. The volatile compounds of A. conyzoides oil are insecticidal against Callosobruchus maculatus [49]. Leaf extracts of A. conyzoides showed insecticidal activity on P. xylostella [50, 51]. An essential oil from another species, A. houstonianum, has shown repellency activity against third instar larvae of P. xylostella [52]. Leaf powder of A. conyzoides and several plants of the Asteraceae family showed repellency activity against Tribolium castaneum, (Coleoptera: Tenebrionidae) [53]. Ocimum oil contains bioactive compounds that are insecticidal and repellent [54,55,56]. The essential oil of O. basilicum is reported to inhibit the oviposition activity and repellency to Callosobruchus maculatus (Coleoptera: Bruchidae), with matil chavicol and linalool as active compounds [57]. The main compounds identified in O. basilicum leaves are linalool, (Z)-cinnamic acid Me ester [58]. The essential oils of free Ocimum sinum and O. basilicum have a resistance activity against Tribolium castaneum [59,60]. The methanol extract of Apium graveolens seeds has mosquitocidal, nematicidal and antifungal activity, with active compounds sedanolid, senkyunolid-N, and senkyunolid-J [61]. N-hexane extract of A. graveolens have effective repellency activity against several genera of mosquitoes such as Aedes, Anopheles, Armigera, Culex, and Mansonia [62].

4. Conclusion

From the olfactory response of P. xylostella, there are two plants that have potential as trap plants, namely R. indica and B. rapa. In addition, there are three plants that have the potential to become repellents, namely A. graveolens, A. conyzoides, and O. basilicum.
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