Journal of Asia-Pacific Entomology 13 (2010) 255–260

Contents lists available at ScienceDirect

Journal of Asia-Pacific Entomology

journal homepage: www.elsevier.com/locate/jape

Attraction of the predator, *Harmonia axyridis* (Coleoptera: Coccinellidae), to its prey, *Myzus persicae* (Hemiptera: Aphididae), feeding on Chinese cabbage

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ARTICLE INFO

Article history:
Received 23 June 2009
Revised 16 June 2010
Accepted 27 June 2010

Keywords:
Attraction behavior
Harmonia axyridis
Multicolored Asian lady beetles
Myzus persicae

ABSTRACT

The predatory multicolored Asian lady beetle, *Harmonia axyridis,* was attracted to volatiles released from Chinese cabbage infested by the green peach aphid, *Myzus persicae,* in T-tube olfactometer choice tests. However, lady beetle adults and larvae did not respond to clean air, Chinese cabbage alone or green peach aphid alone. Of different prey densities, *H. axyridis* adults were most attracted to Chinese cabbage infested by 60 *M. persicae* adults after 24 h. However, *H. axyridis* larvae were not attracted to Chinese cabbage infested by *M. persicae*. Mechanically damaged Chinese cabbage attracted neither lady beetle adults nor larvae. Predatory adults were attracted to 60 *M. persicae* adults after 24 and 48 h, and to 90 *M. persicae* adults after 12 h, suggesting that the predatory response depends on the prey density. Lady adult beetles did not prefer the volatiles induced by Diamondback moth, *Plutella xylostella*, indicating that specific host insect specificity attracts respective natural enemies. It can be explained that the volatile compounds emitted from the host plant as a result of herbivore attack preferred by the specific insect species.

Introduction

The green peach aphid (GPA), *Myzus persicae* (Sülzer) (Hemiptera: Aphididae), has a worldwide distribution, including the Republic of Korea. It infests hundreds of species of plants in more than 66 families. The aphid mainly exists on young plant tissues, causing reduced leaf size, delayed growth of the plant, and reduced yield (Petitt and Amilowitz, 1982). GPA is an important pest of greenhouse vegetables and horticultural plants, and reduces commercial value by transmitting plant viruses (Kim et al., 2005).

Chemical insecticides are the major tool to combat insect pests on crop plants. Extensive use of insecticides in crop systems, however, may cause resurgence of primary pests, replacement by secondary pests, environmental contamination, adverse effects on nontarget organisms, and development of pest resistance (Nauen and Denholm, 2005; Vieira et al., 2001; Frampton, 1999). Therefore, alternatives to chemically intensive pest management are necessary. In a natural community, aphid predators such as Coccinellidae, Syrphidae, and Cecidomyiidae are important and can cause high aphid mortality (Youn et al., 2003). Natural enemies of aphids can reduce the rate of population increase and the use of lady beetles in biological control of aphids has been successful (Seo and Youn, 2000).

The harlequin lady beetle or multicolored Asian lady beetle, *Harmonia axyridis* (Pallas) (Coleoptera: Coccinellidae), has been introduced as a biological control agent of aphids and other hemipteran pests. *H. axyridis* has been introduced to many European countries, including Belgium (1997), the Czech Republic (2003), France (1982), Germany (1998), and Greece (1994) (Roy and Wajenberg, 2008). *H. axyridis* is indigenous to many regions of Asia, such as Taiwan, China, South Korea, Japan, Southern Siberia, Ryukyu Islands and Bonin Island. It is a generalist predator that feeds primarily on several aphid species (Seo and Youn, 2000) and has been recognized for its potential contribution to the integrated management of various crop aphids, including *M. persicae.* It has been used successfully in greenhouses, orchards, gardens, outdoor crops for aphid management (Majerus, 1994).

Plants attacked by herbivores may emit volatile compounds to attract predators or parasitoids (Vuorinen et al., 2004a,b; Dicke et al., 2003; Shiojiri et al., 2001; Dickens, 1999). Predators may locate their hosts by chance, by using chemical cues such as plant-produced volatiles (Ozawa et al., 2000), and by using visual and olfactory cues (Bahlai et al., 2008; Zhu and Park, 2005; Ninkovic et al., 2001). In a tritrophic system with lima bean plants, two-spotted spider mites (*Tetranychus urticae*) and predatory mites (*Phytoseiulus persimilis*), plants emitted herbivore-induced plant volatile compounds (HIPVs) such as terpenoids and methyl salicylate, which attract predatory mites (Ozawa et al., 2000). Attraction of predators or parasitoids

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1226-8615/$ – see front matter
doi:10.1016/j.aspen.2010.06.009

would be beneficial to the host plant to alleviate the harmful effects inflicted by herbivores (Lou et al., 2005; Zhu and Park, 2005).

We examined the attraction of the predatory multicolored Asian lady beetle to host plants infested with the herbivore, GPA. Understanding the factors influencing the attraction of the lady beetle may provide fundamental data for controlling GPA.

Materials and methods

Test insects

The green peach aphids (GPA) and the diamondback moths (DBM), *Plutella xylostella* (Lepidoptera: Plutellidae), used in this study were collected from Chungbuk province, South Korea, in 2006. Insects were reared through several generations in plastic containers (15 × 23 × 8 cm) and provided with 2-week-old Chinese cabbage leaves (*Brassica rapae* L. cv. Chunchujeonguk) as food under the following conditions: 25 ± 2 °C, 50–60% RH and a 16L: 8D h photoperiod. The multicolored Asian lady beetle, *Harmonia axyridis,* was collected from areas near Chungbuk National University and reared by feeding them GPA for several generations under the same rearing conditions as that of GPA. Adult (10 days after eclosion) and 4th instar multicolored Asian lady beetles and 3rd instar DBM were used in this study.

Screening for attraction condition

The attraction of the lady beetles to the host plant and prey was tested in the laboratory using a manufactured T-tube olfactometer with chamber. The chamber, a cylindrical cage (9 cm dia. × 18 cm ht.), can contain a plant sample as an odor source or the control and is connected to either end of the T-tube olfactometer (ID 1.5 cm × arm 24 cm × stem 16 cm; angle between arms 180°) (Fig. 1). A piece of nylon mesh was covered either end of T-tube olfactometer to prohibit the insect pass through it and to pass the purified air. Air was pulled through the main stem of T-tube olfactometer via the chamber at a flow rate of 100 ml/min using a vacuum pump (Thomas Medi pump®). Pressurized air was filtered through activated charcoal, a molecular sieve, and silica gel blue to obtain purified air.

Attraction responses of predatory lady beetles to the host plant and prey were investigated. An empty glass vial (with cotton) was used as a blank control in this experiment. Roots of 2-week-old Chinese cabbage plants were rinsed of soil, bound with cotton and fixed in a glass vial (25 ml). Fresh cabbage not infested by herbivore was also used as a control. Approximately 60 GPA were introduced into the treatment chamber by placing them in a petri-dish at the same height as the top portion of the Chinese cabbage leaf. When necessary, herbivores were allowed to move onto the plant. Twenty-four hours after all materials were prepared, attraction responses were measured. Lady beetle adults and larvae were starved for 24 h prior to use. Each adult or larval lady beetle was placed in the stem of the olfactometer, and a decision was recorded when the lady beetle moved into and remained in the end (within 8 cm from the either end) of an arm for 30 s. If a lady beetle adult stayed before T-junction or in another ambiguous region, they were counted as "no-choice." Experiments were conducted in the room maintained at 28 ± 3 °C with 50 ± 10% RH. Insect responses were evaluated by recording the chamber in which the insect stayed after 5 min. T-tube olfactometers were switched other one after 5 repetitions to avoid the possible effects of remaining volatile compounds. Each treatment was replicated forty times. After use, the olfactometer was washed out using methanol and neutral detergent.

Attraction of lady beetle to GPA and mechanically damaged host plant

The attraction of lady beetles to GPA adults was tested using a T-tube olfactometer. Two-week-old Chinese cabbage leaves were prepared in a glass vial and 10, 30, 60, or 90 GPA adults were added per plant. For twenty-four hours, GPA fed on the Chinese cabbage in a closed chamber, allowing emission and accumulation of volatile compounds. The attraction of lady beetle larvae and adults to Chinese cabbage infested by different densities of GPA was observed and compared with the untreated control (fresh cabbage), as described above.

The attraction of lady beetles to artificially damaged Chinese cabbage was also tested. Leaf tissue was wounded by piercing with an insect pin (Shiga No. 3, Tokyo, Japan. Approx. dia. <0.4 mm) 1, 5, 10, 30, or 60 times per plant. Prior to the experiment, uninfested Chinese cabbage leaves and artificially damaged Chinese cabbage leaves were put in separate closed chambers of the olfactometer for 24 h to allow accumulation of emitted volatile compounds. The preference of adult lady beetles for cabbage with 60 holes or with 60 GPA was also examined. Insect responses were recorded as described above. Each treatment was replicated forty times.

Attraction of lady beetles to different GPA densities on host plants over time

The attraction of lady beetles to different densities of GPA on host plants and mechanically damaged Chinese cabbage over time was examined. In the T-tube olfactometer, 2-week-old Chinese cabbage leaves were infested with 10, 30, 60, or 90 GPA per plant and damaged mechanically with a pin to make 60 holes a leaf per plant was kept in one side. Each treatment was compared with uninfested cabbage placed in the other side for 0, 6, 12, 24, 48, and 72 h, respectively, as the control in this experiment. The mechanically damaged Chinese

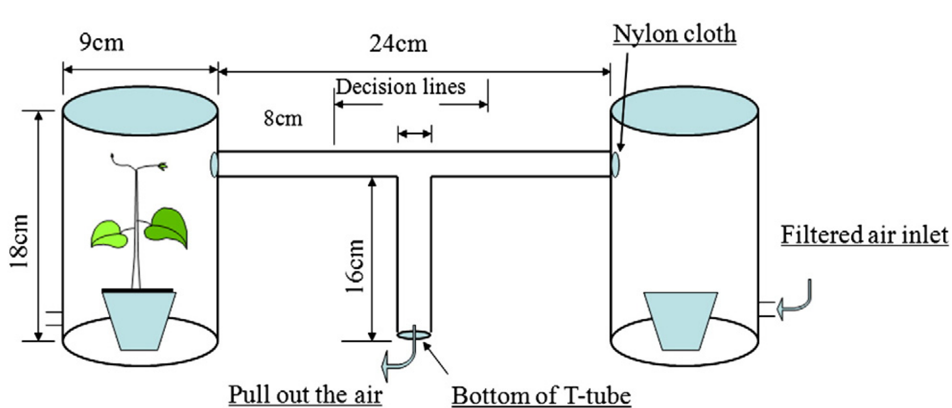

Fig. 1. A diagram of the T-tube olfactometer used in this study. The clean air was blown through the T-tube olfactometer at a flow rate of 100 ml/min using a vacuum pump. The pressurized air was filtered through activated charcoal, a molecular sieve and silica gel to obtain purified air (not shown in the figure).