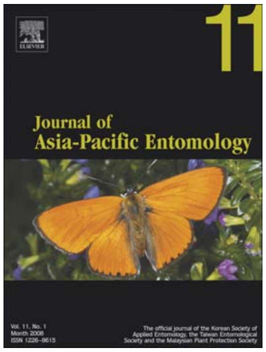

Attraction of the predator, *Harmonia axyridis* (Coleoptera: Coccinellidae), to its prey, *Myzus persicae* (Hemiptera: Aphididae), feeding on Chinese cabbage

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

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Running title: Attraction of *Harmonia axyridis* to *Myzus persicae* on host

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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 hr. 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 hr, and to 90 *M. persicae* adults after 12 hrs, suggesting that the predatory response depends on the prey density. Lady adult beetles did not preferred the volatiles induced by Diamondback moth, *Plutella xylostella*, indicates 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 may preferred by the specific insect species.

KEY WORDS: Attraction behavior; *Harmonia axyridis*; Multicolored Asian lady beetles; *Myzus persicae*

Reviewers' comments:

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 Wajnberg, 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 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 two-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 hr 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 100ml/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 two-week-old Chinese cabbage plants were rinsed of soil, bound with cotton and fixed in a glass vial (25ml). 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 hrs 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 a arm for 30 sec. 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 hrs 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, two-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 hr, respectively, as the control in this experiment. The mechanically damaged Chinese cabbage was treated in the same conditions as described above. Different densities of GPA on host plants and mechanically damaged Chinese cabbage were kept in chamber for 0, 6, 12, 24, 48, and 72 hr. And the response of lady adult beetles was monitored to the volatiles previously accumulated in chamber, in which different densities of GPA and mechanically damaged Chinese cabbage.

Attraction of lady beetle adult to host plant infested with Plutella xylostella *larva*

The diamondback moth, another herbivore of Chinese cabbage, has chewing mouth parts. Plants may produce volatiles similar in function to those produced by GPA feeding. We investigated whether diamondback moth are elicited like GPA-induced plant volatile or induced volatiles to induce the lady beetle or not.

The attraction of lady beetle adults to host plants infested with GPA was compared to the attraction of lady beetle adults to host plants infested with *P. xylostella* larva. The insects were inoculated onto two-week-old Chinese cabbage leaves 24 hr before the essay so that they emitted sufficient quantities of volatile compounds. The leaves of Chinese cabbage were infested separately with 60 GPA adults or ten 3rd instar larvae of *P. xylostella*, which are of approximately equal weight (21±1 mg). For the attraction test, 10 or 30 *P. xylostella* larvae were added to leaves and compared to uninfested plants in the same experimental conditions.

Statistical analysis

The choices of *H. axyridis* between odor sources in the olfactometer were analyzed with a two-sided binomial test for each experiment, under the null hypothesis that the distribution over the two odor sources was 50:50. Data from all T-tube assays were compared with the binomial sign test to evaluate differences from 50:50 (Zar, 1996), with $P<0.05$ and 0.01.

Results

Attraction response of predatory beetle towards the host plant and prey

There was no difference in choice when lady beetle adults and larvae were placed at the main stem of the T-tube olfactometer and the two chambers were blank (Fig. 2), indicating that there was no bias in the olfactometer. Neither uninfested cabbage nor GPA alone attracted adult or larval lady beetles (Fig. 2).

Attraction response with respect to prey density

There were no significant differences in the attraction responses of adult lady beetles to Chinese cabbage infested with 10, 30, or 90 GPA and uninfested Chinese cabbage (Fig. 3a). Whereas, Chinese cabbage infested with 60 GPA was significantly more attractive than cabbage alone, and showed an attraction response of 70.3 % ($P<0.05$). There were no significant differences in the attraction responses of larval lady beetles with respect to different densities of GPA (Fig. 3b).

Response to mechanically damaged Chinese cabbage

Asian lady beetle adults were attracted to Chinese cabbage infested with 60 GPA (Fig. 3a). Therefore, the attraction of lady beetles to Chinese cabbage infested with 60 GPA was compared to the attraction of lady beetles to cabbage alone and with mechanical damage.

There were no significant differences in the responses of the predator to Chinese cabbage with different degrees of mechanical damage (Fig. 4). Lady beetles were significantly more attracted to Chinese cabbage infested 60 GPA (69.2 %) than they were to mechanically damaged cabbage.

Attraction response over time

Attraction response of lady adult beetles was tested to the previously accumulated volatile compounds in chamber for 0, 6, 12, 24, 48 and 72 hr, in which infested by different densities of GPA on host plant and produced by mechanically damaged Chinese cabbage. On the same hour, all attraction response to the volatiles in chamber was only compared with uninfested cabbage as the control, respectively (Fig. 5).

Compared to the uninfested cabbage, the Chinese cabbage with 10 or 30 GPA did not attract the lady beetle adults. Also, the Chinese cabbage damaged mechanically with a pin did not attract the lady beetle adults. The Chinese cabbage infested with 60 GPA did not show any attraction until 12 hr after inoculation. However, 70.3 and 77.8% attraction responses were observed at 24 hr and 48 hr after inoculation, respectively. The Chinese cabbage infested with 90 GPA showed an attraction response of 72.2%, but the attraction response of the lady beetle adults decreased under those of Chinese cabbage infested with 60 GPA after 24 hr.

Attraction response to the inoculation of P. xylostella

The attraction response of the lady beetle adults to Chinese cabbage infested with the diamondback moth was examined. The results are shown in Fig. 6.

There were no significant differences in the responses of lady beetle adults to the volatiles of 10 *P. xylostella* per leaf *vs* uninfested plants, or 30 *P. xylostella* per leaf *vs* uninfested plant (binomial sign test, *P*<0.05). However, significantly more adult lady beetles moved toward the volatiles from Chinese cabbage infested with 60 GPA than toward Chinese cabbage infested with 10 *P. xylostella* per leaf (binomial sign test, $P<0.05$).

Discussion

Lady beetles have been used widely as biological control agents for aphids and other hemipteran pests (Obrycki and Kring, 1998). Their biology and ecology have been reviewed extensively (Majerus, 1994). To control aphids and other hemipteran pests, it is important to understand the attraction behavior of coccinellids.

It is not known what chemical factors induce coccinellids to forage for prey. *H. axyridis* adults are able to detect individual prey at short distances using visual or olfactory cues (Stubbs, 1980; Obata, 1986; Bahlai *et al.*, 2008) and through physical contact (Kehat, 1968; Storch, 1976). There have been conflicting reports on the cause showing attraction behavior of *H. axyridis* on their prey, but the detailed the cause have not been fully elucidated. Lady beetle adults may respond to semiochemicals from aphids, to aphid honeydew, to related microorganisms (Majerus, 1994) and to related key component in the alarm pheromone of many aphids, (*E*)-β-farnesene against seven-spotted ladybird (Al Abassi *et al.,* 2000). *H. axyridis* is more strongly attracted to the odor of aphid-infested leaves than to the odor of uninfested leaves (Obata, 1997; Ninkovic *et al.,* 2001).

From this study, it is possible to explain the behavior showing the attraction behavior of *H. axyridis* against GPA-infested cabbage. Plant and carnivorous arthropod interactions are mediated by herbivore-induced plant volatiles (HIPVs) (Dicke *et al.*, 1999; Takabayashi *et al.,* 1994; Takabayashi and Dicke, 1993). HIPVs are important in interactions between plants, two-spotted spider mites and predatory mites (Matsushima *et al.,* 2006; De Boer *et al.,* 2005; Maeda and Takabayashi, 2001; Arimura *et al.,* 2001).

The objective of this study is to clarify the relationship between the density of GPA on plants and the production of plant volatiles over time. We focus on a tritrophic system consisting of Chinese cabbage, the herbivore *Myzus persicae* and the predator *Harmonia axyridis*. The predatory lady beetle adults are strongly attracted to the Chinese cabbage infested with 60 GPA.

As shown in Fig. 2, we found that lady beetle larvae and adults did not appear to be attracted to Chinese cabbage alone or to GPA alone. The predator may be attracted by olfactory cues produced by infested plants (Ozawa *et al.*, 2000). It is also possible to attract *H. axyridis* with other factors, such as semiochemicals or honeydew. If semiochemicals or honeydew produced by *M. persicae* act as olfactory cues, *H. axyridis* would be attracted to GPA only, but they were not. Chinese cabbage alone induced no response of *H. axyridis* adult and larva that possible olfactory cues have no relation. Therefore, we investigated the attraction conditions of *H. axyridis* to the volatiles from cabbage infested with different densities of *M. persicae*. However, it is impossible to know exactly about the volatile compounds at current status, the cause needs to explain for further study.

As shown in Fig. 3, the amounts of volatile compounds affect the degree to which natural enemies are attracted to infested Chinese cabbage. This has been reported in the *Lotus japonicus* with similar result of ours. Using a Y-tube olfactometer, the authors found that 60 infested *L. japonicus* shoots attracted *P. persimilis*, while 30 infested shoots did not (Ozawa *et al.,* 2000). Moreover, lady beetle larvae were not attracted to the volatiles that attracted adults (Seo and Youn, 2000). Predators may be attracted to the volatile compounds such as kinds, amount, and releasing time from hosts when infested with the various range of herbivore's densities. It could be explained why lady beetle was differently attracted to the volatiles of different GPA densities with respect to over time.

As shown in Fig. 4, plant generally receives an attack physically and chemically by herbivores. When herbivores penetrate the plants to suck the sap, they make small holes in the leaf. Although it is impossible to make artificial holes identical to an herbivore penetration event, we mechanically damaged leaves in a similar manner. Volatile compounds may differently emit by various induction, it remains unchanged that mechanical damage do not attract a natural enemy. Shiojiri *et al.* (2000) reported that the volatiles emitted from plants infested by the herbivores can strongly attract natural enemies such as parasitic wasps, and that plant volatiles produced by mechanical damage do not. Obata (1986) reported that *H. axyridis* was more strongly attracted to the odor of aphid (*Aphis citricola*) - infested leaves than to the odor of uninfested leaves and implicated possible olfactory cues. *H. axyridis* showed attractiveness with similar results on these reports with herbivore and mechanical damage. Therefore, it is assumed that the emission of volatile compounds from the Chinese cabbage that were chemically stimulated by the feeding of GPA may attract the adult of lady beetle but not volatiles emitted in response to mechanical damage. Because the importance of the volatile compounds that produced by infesting herbivores and/or mechanical damages, we tried many times to detect the volatiles, however, we failed to analyze the volatile compounds from the host plant in this time.

As shown in Fig. 5, volatiles from higher GPA numbers are more attracted the lady adult beetles in short time, but decreased their attractiveness over time. The attractiveness of which it peak and decline is related to number of GPA as passage of time. Attractiveness represented as peaks recorded 1st in 90 then 60, still increasing in 30 GPA until 72 hr after treatment. The Chinese cabbage infested with 90 GPA showed an attraction response of 72.2%, but the attraction ratios of the lady beetle adults decreased after 24 hr. Chinese cabbage infested with many GPA may emit too many volatiles which may lead to confusion of the predator in detecting the odor source. Lou *et al.* (2005) reported that the attraction of the parasitic wasp to the brown planthopper was closely related to infestation time and host density. Moreover, attraction was only observed 6 h to 24 h after infestation. Densities that were too high or too low host did not attract the parasitic wasp (Lou *et al.*, 2005). Takabayashi *et al.* (1994) also reported that an old cucumber leaf infested with the two-spotted mite was not attractive to the spider mite predator, *P. persimilis*, whereas, young cucumber leaves attract the spider mite predator. These results also suggest that the attraction of lady beetle may differently respond to the volatile compounds produced when herbivores infests plants in different age, amounts of emitted volatiles and herbivore densities. For instance, the volatile compounds emitted from cabbage infested with a cabbage butterfly, *Pieris rapae*, varied significantly with the compounds emitted from cabbage, which was infested with white butterfly, *P. brassicae* (Geervliet *et al.*, 1997). That is different volatile compounds emitted from host plant by different kinds of herbivore and mode of feeding such as phloem feeder and tissue feeder. In the case of physically damaged plants, the volatiles emitted are significantly different from those derived from plants infested with herbivores (Ozawa *et al.*, 2000).

In the present investigation, DBM feeding did not stimulate volatiles to attract the lady beetle adults. Since we tried to get the volatiles from the *P. xylostella* infested plants but failed. We hypothesize that the volatiles were emitted only by the feeding stimulus of the green peach aphid. The findings of the present investigation concurred with the findings of earlier researchers (Similar reports were documented already (Fujiwara *et al.*, 2000; De Moraes *et al.*, 1998; Takabayashi and Dicke, 1993). Thus, *P. xylostella* infestation did not alter the plant volatiles, which is in contrast to the substantial induction caused by the tissue feeders (Lou *et al.*, 2005). Turlings *et al.* (1998) found that the aphid *Rhopalosiphum maidis* induced no measurable emissions of volatiles in maize, even after heavy infestation, whereas feeding by caterpillars caused major increases in volatile emissions. Therefore, the attraction response of lady beetle adults to different plants infested with GPA should be examined further. The compositions of volatile compounds differ considerably depending on the herbivore species and crop variety (Loughrin *et al.,* 1995; Takabayashi *et al.,* 1991).

It is impossible to know the exact volatile compounds from GPA-infested cabbage to attract *H. axyridis* at current status, the volatile compounds will need to characterize for further study, by considering individual and combined factors.

From this study, it could be concluded that the attraction of the predatory carnivore depends on prey density and on the type of the herbivore present on the host plant. Since the volatile compounds from the host plant species may be variable with respect to the herbivore species, urther studies on the emission of volatile compounds from the Chinese cabbage when infested with different herbivore insect species is warranted.

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Figure captions

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 100ml/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 Fig.).

Fig. 2. Olfactory responses of *H. axyridis* a) adults and b) larvae to *M. persicae*. Clean air, cabbage and 60 *M. persicae* alone were used as the odor sources. Binomial sign test (Zar, 1996), ns=not significantly different.

Fig. 3. Olfactory responses of *H. axyridis* a) adults and b) larvae to 10, 30, 60 or 90 *M. persicae* on Chinese cabbage. Binomial sign test (Zar, 1996), * $P<0.05$, ns=not significantly different.

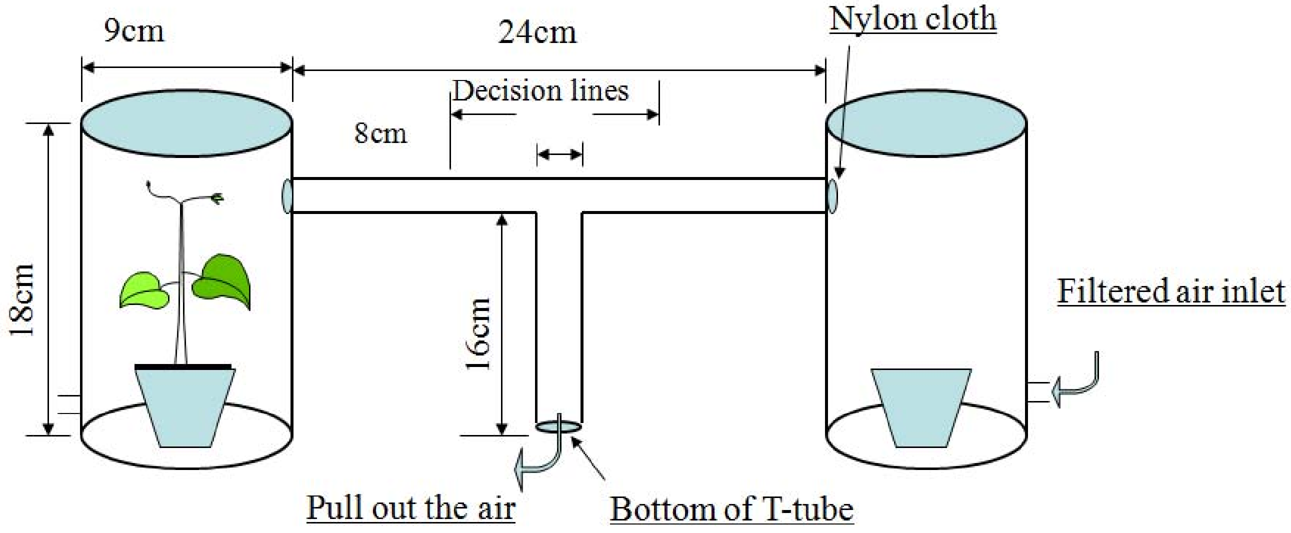
Fig. 4. Olfactory responses of *H. axyridis* adults to mechanically damaged Chinese cabbage leaves and 60 *M. persicae* on Chinese cabbage leaves. Binomial sign test (Zar, 1996), * $P<0.05$, ns=not significantly different.

Fig. 5. Olfactory responses of *H. axyridis* adults to various inoculation densities and to 60 holes as a function of time. All treatments at every time were compared to the uninfested cabbage control. An asterisk over the mark denotes a significant difference as determined by sign test at $P<0.05$ and 0.01.

Fig. 6. Olfactory responses of *H. axyridis* adults to *P. xylostella* and to 60 *M. persicae* on Chinese cabbage leaves. Binomial sign test (Zar, 1996), * $P<0.05$, ns=not significantly different.

(Fig. 1) (Author: Yoon, C., Seo, D.K., Yang, J.O., Kang, S.H. and Kim, G.H.*)

(Title: Attraction of *Harmonia axyridis* to *Myzus persicae* on host)



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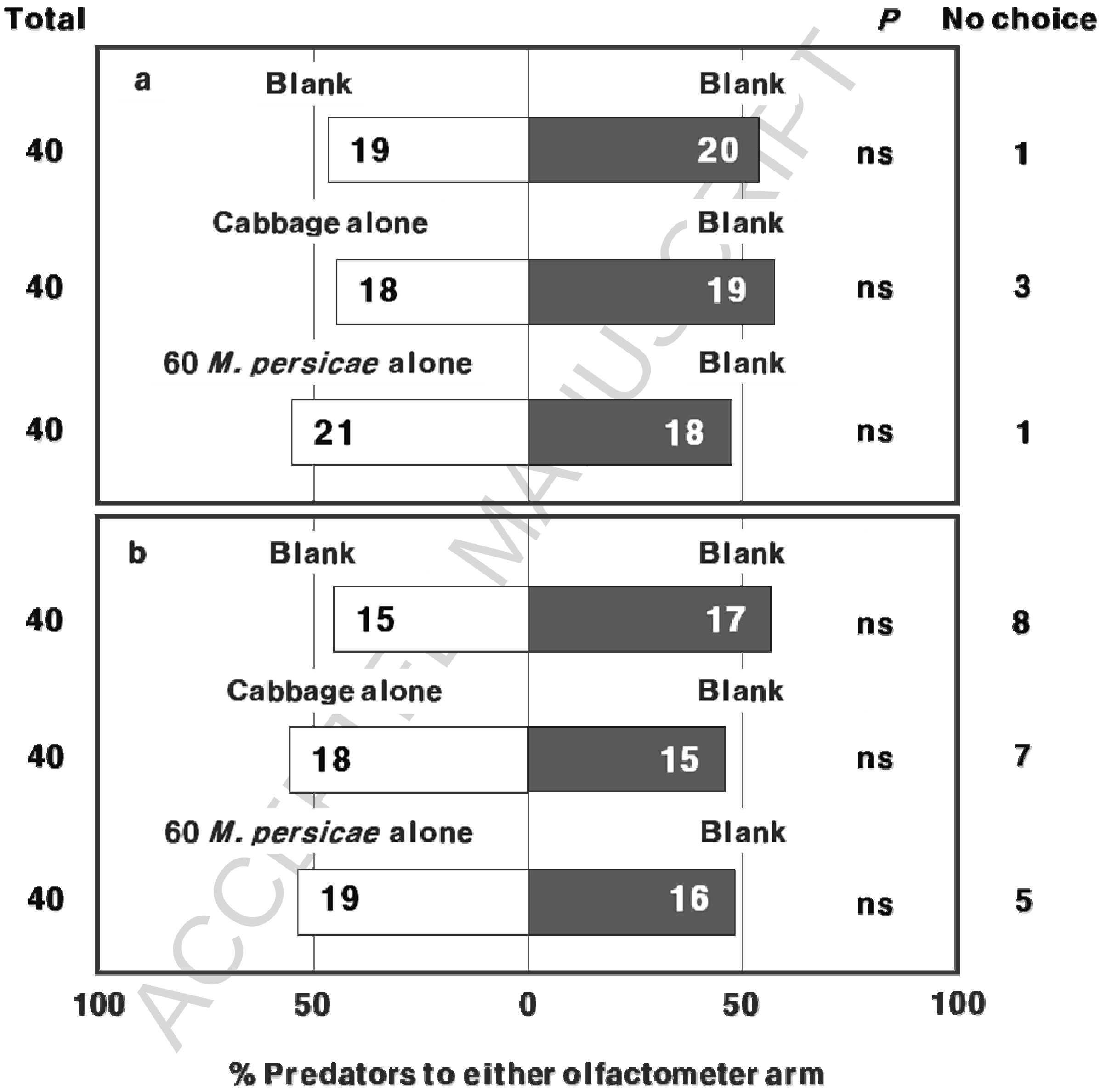

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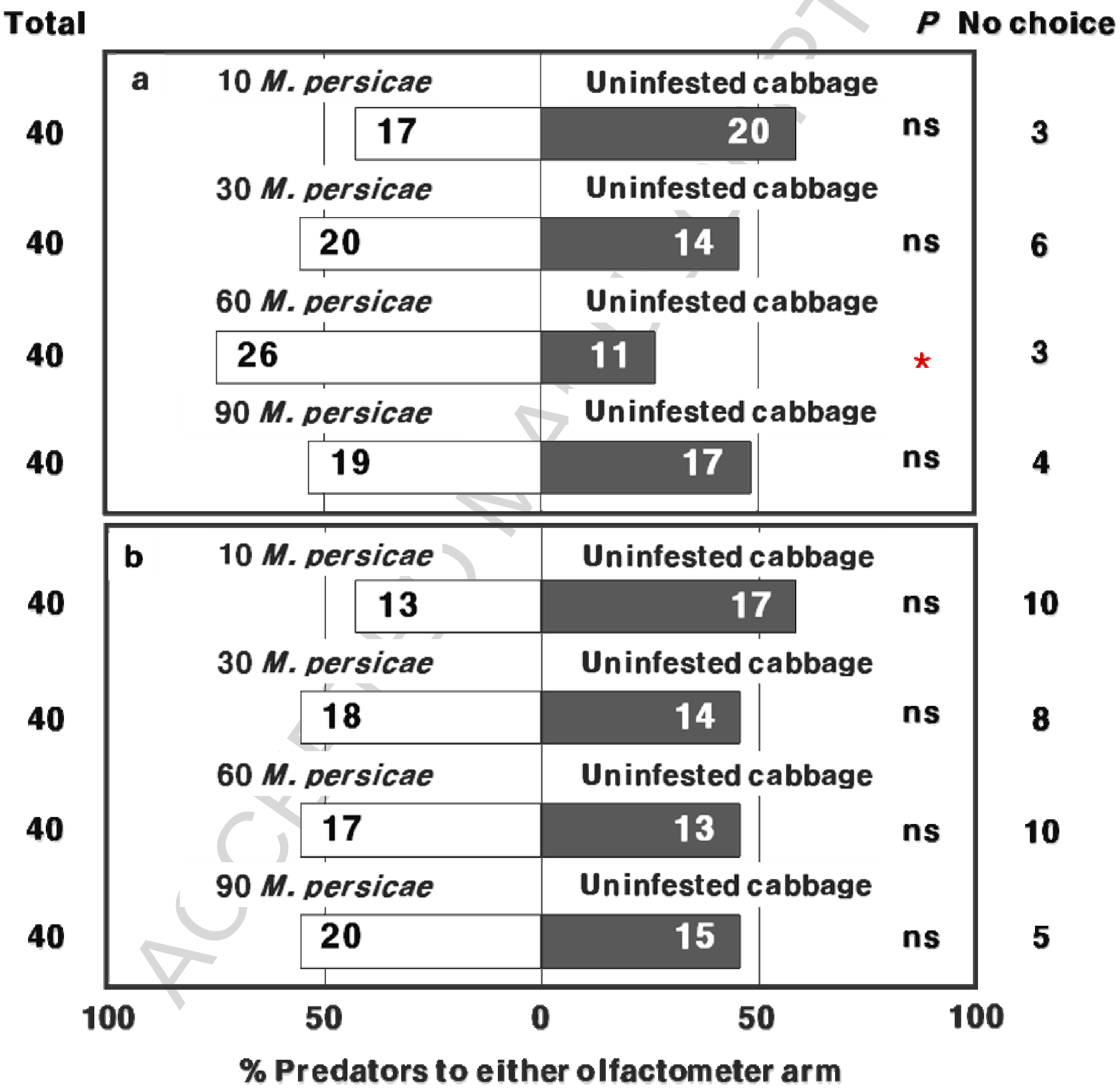

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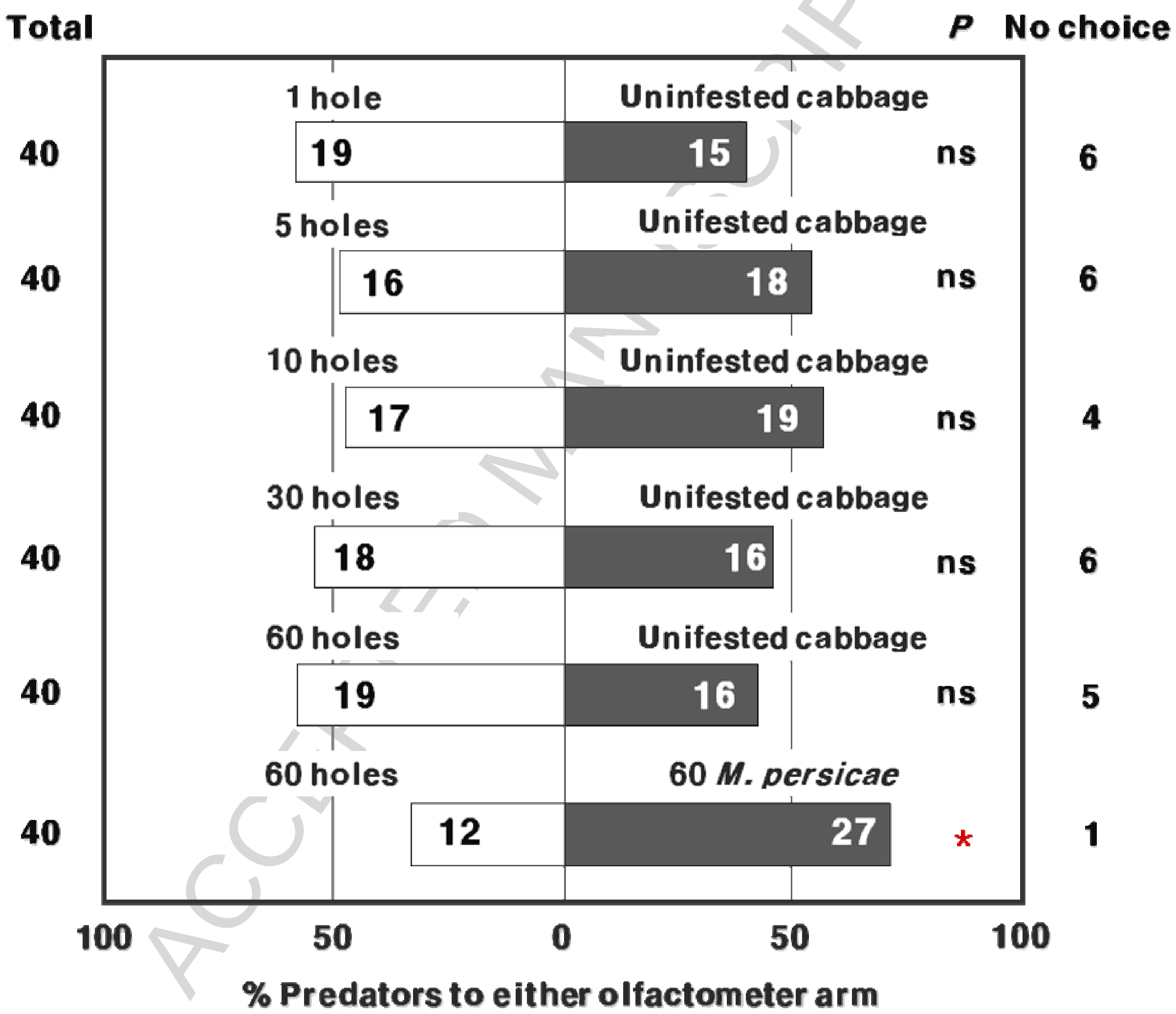

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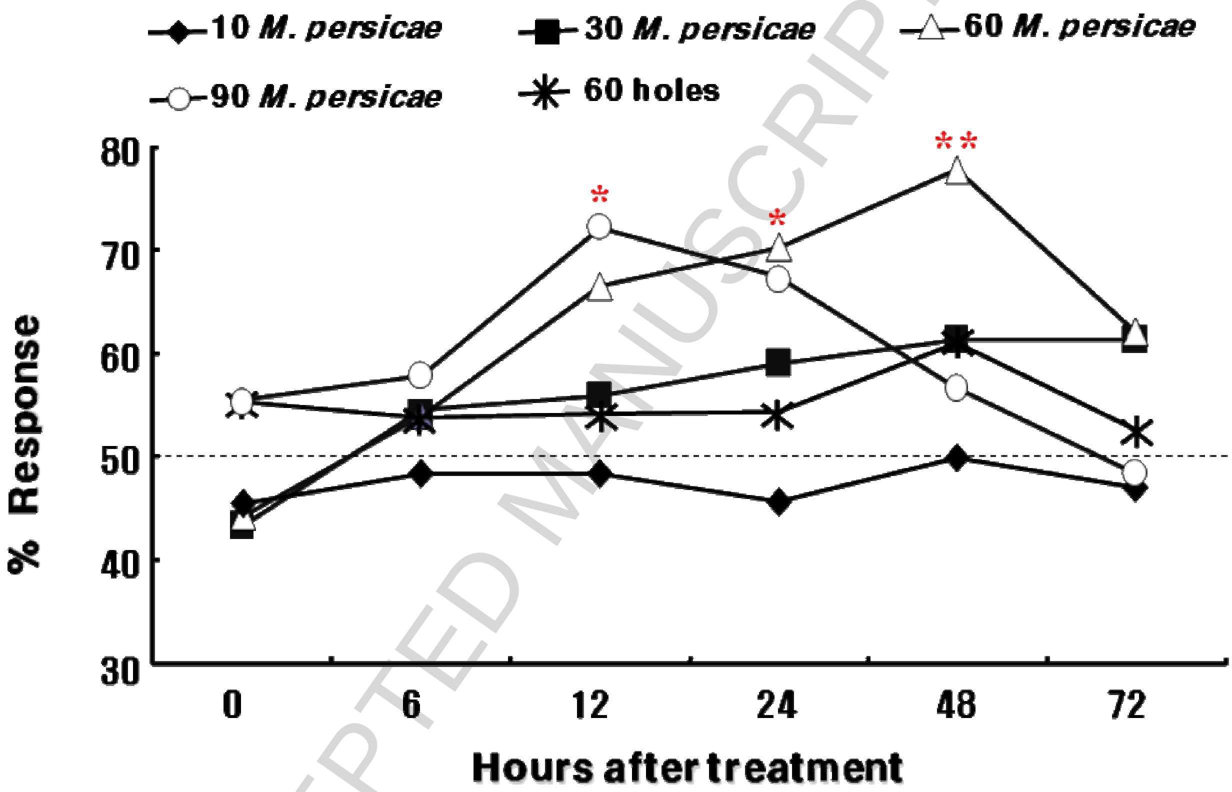

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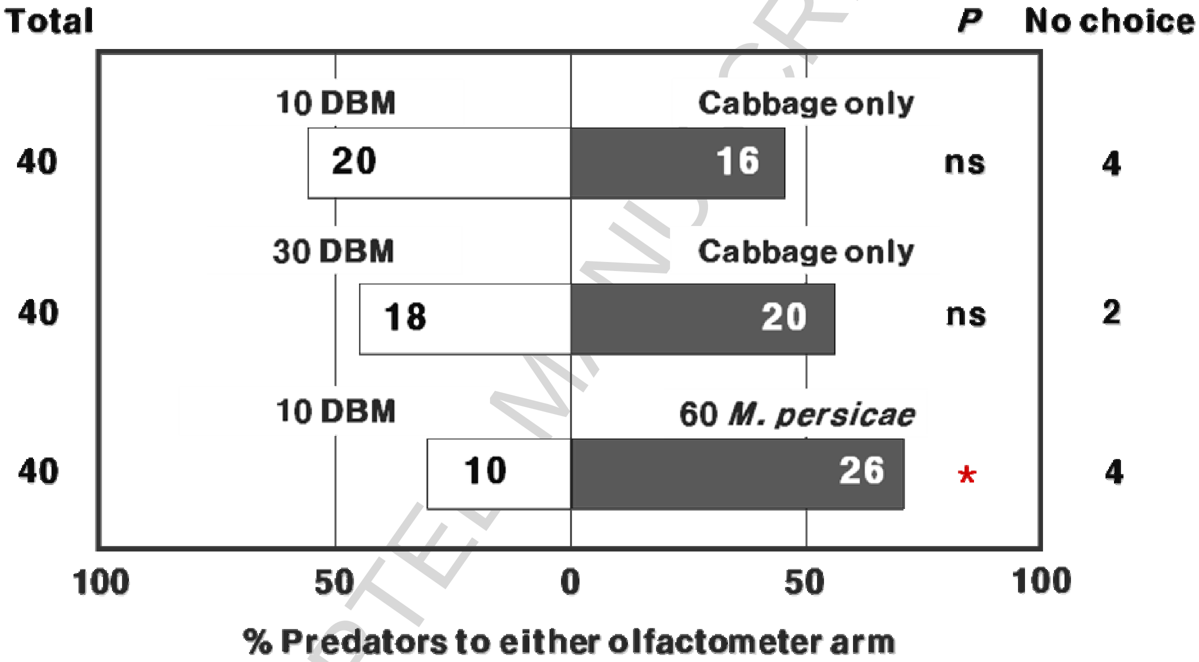

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