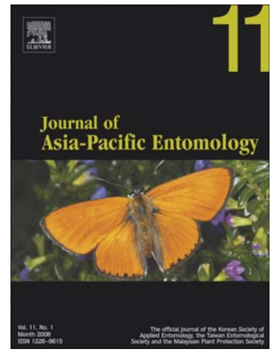

Visual and Olfactory Preference of *Harmonia axyridis* (Coleoptera: Coccinellidae) Adults to Various Companion Plants

Folukemi Adedipe, Yong-Lak Park

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Folukemi Adedipe and Yong-Lak Park

Entomology Program, Division of Plant and Soil Sciences, West Virginia University, Morgantown, WV 26506, U.S.A.

Corresponding Author: Yong-Lak Park, Entomology Program

Division of Plant and Soil Sciences

West Virginia University

Morgantown, WV 26506, U.S.A.

E-mail: yong-lak.park@mail.wvu.edu

Phone: 1-304-293-2882

Abstract: Natural enemies can be attracted to companion plants that can provide them with suitable habitat including food and shelter. This study was conducted to determine if the multicolored Asian lady beetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), could be attracted to olfactory and visual cues of various companion plant species. Ten potential plants that can attract and provide *H. axyridis* with alternate food sources (i.e. nectar and pollen) were selected for olfactory preference tests. These plants included dill (*Anethum graveolens*), yarrow (*Achillea* spp.), butterfly weed (*Asclepias tuberosa*), dandelion (*Taraxacum officinale*), bugleweed (*Ajuga reptans*), marigold (*Tagetes tenuifolia*), tansy (*Tanacetum vulgare*), goldenrod (*Solidago* spp.), morning glory (*Ipomoea* spp.), and sunflower (*Helianthus annuus*). Visual preference tests were conducted with six different colors: white, yellow, blue, red, green, and orange. The result of the olfactory preference test showed that *H. axyridis* adults significantly preferred sunflower and dill among the ten potential companion plants tested in this study. In a visual preference test, *H. axyridis* significantly preferred yellow to any other colors. The result of this study suggested that odor and color of some companion plants (olfactory and visual cues) could assist in the use of biological control by attracting *H. axyridis* adults to the cropping area.

Keywords: companion plant, farmscaping, choice test, olfactory preference, visual preference

Introduction

The multi-colored Asian lady beetle, *Harmonia axyridis* Pallas (Coleoptera: Coccinellidae), is an introduced biological control agent to the U.S.A. (Chapin and Brou 1991). *H. axyridis* is native to East Asia and multiple introductions into North America were made in 1916 and 1982 (Gordon 1985, Koch 2003, Colunga-Garcia and Gage 1998). Since the second introduction, *H. axyridis* has become a dominant lady beetle species in many parts of North America (Tedders and Schaefer 1994, LaMana and Miller 1996, Colunga-Garcia and Gage 1998). In Korea, *H. axyridis* is one of the key natural enemies controlling aphids and scales in various agricultural commodities (Park and Kim 1990, Seo and Youn 2002, Baik et al. 2009). *H. axyridis* also can be found in various habitats including meadows, semi-natural areas, nurseries, orchards, vegetable gardens and crop fields (Koch 2003, Koch and Galvan 2008). *H. axyridis* generally moves among such habitats by using olfactory and visual cues of aphid host plants (Koch 2003, Nalepa et al. 2000).

Natural enemies can be spatially relocated by providing habitat for them (Helenius 1998, Landis et al. 2000). A farm management technique used to enhance the presence and abundance of beneficial insects (e.g. natural enemies and pollinators) by manipulating the agricultural ecosystem is referred to as farmscaping, a whole-farm ecological approach to farm management (Dufour 2000). Farmscapes can provide food resources and habitats required by beneficial insects with the objective of increasing their populations on the farm (Pickett and Bugg 1998). Previous studies showed that the addition of companion plants providing nectar and pollen near crops enhanced biological control (Landis et al. 2000, Seagraves and Yeargan 2006). A few studies found that sown weed strips within cropped areas increased populations and feeding activities of natural enemies in crops (Wyss 1995, Nentwig 1998, Schoenig et al. 1998, Wratten et al. 1998). Jones and Gillett (2005) found that sunflowers attracted numerous beneficial insects and Dufour (2000) listed many flowering plants that could attract and provide supplemental food source for beneficial insects. These previous studies indicated the potential of farmscaping using companion plants to attract natural enemies.

Although many natural enemies have been used to suppress pest populations in various agroecosystem, there have been no good methods for growers to utilize generalist natural enemies (e.g. soldier bugs and lady beetles) have been developed. In fact, many generalist natural enemies that can potentially control many insect pests already reside in agricultural ecosystems during the growing season. One way to take advantage of such generalist natural enemies existing on the farm is using farmscapes to attract them into the area where target pests are aggregated and cause damage on crops. *H. axyridis* is a generalist predator of a variety of pests including aphids (Koch 2003) and European corn borer eggs (Musser and Shelton 2003). Our observation and previous studies (Hukusima and Kamei 1970, Park and Obrycki 2004) implied that *H. axyridis* resided both inside and outside of cropping areas on the farm, but populations were generally not high in the cropping area in certain periods during the growing season. *H. axyridis* are primarily carnivore but many studies showed that *H. axyridis* could feed on pollen and nectar (see Lundgren 2009 for review). Previous studies also showed that *H. axyridis* responded to plant cues (Koch 2003, Bahlai et al. 2008, Berkvens et al. 2008).

This study was conducted to test ten potential companion plants that can be used for farmscapes to attract *H. axyridis* into cropping areas based on olfactory and visual response of *H. axyridis* to the companion plants. This study involved a series of laboratory choice tests to determine whether plants were attractive to *H. axyridis* adults.

Materials and Methods

Experimental insects and plants

Adult *H. axyridis* were collected from various agricultural fields in Morgantown (West Virginia, U.S.A.) for experiments. *H. axyridis* was maintained in the laboratory (23 ± 2.1°C) and fed with aphids including *Aphis gossypii* Glover, *Aphis spiraecola* Patch, *Aphis craccivora* Koch, and *Aphis pomi* De Geer. Male and female *H. axyridis* were separated and starved for 24h before the experiments.

Ten potential plants were selected for experiments based on three criteria: (1) plants are commonly planted or found in various agroecosystems, (2) plants have been listed in the literature to attract natural enemies, and (3) blooming time of plants coincides with occurrence of *H. axyridis*. These plants included dill (*Anethum graveolens*), tansy (*Tanacetum vulgare*), goldenrod (*Solidago* spp.), sunflower (*Helianthus annuus*), yarrow (*Achillea* spp.), butterfly weed (*Asclepias tuberosa*), morning glory (*Ipomoea* spp.), dandelion (*Taraxacum officinale*), bugleweed (*Ajuga reptans*), and marigold (*Tagetes tenuifolia*). Before experiments, field-collected plants were checked for other insects (e.g. aphids) and honeydew; only insect-free and honeydew-free plants were used for experiments.

Olfactory preference test

The response of *H. axyridis* adults to different flower odors was investigated with a Y-tube olfactometer (Analytical Research Systems Inc., Gainesville, Florida, U.S.A.). The olfactometer consisted of a Y-shaped 2.5-cm-diameter glass tube and a base tube measuring 28 and 13 cm long, respectively (Fig. 1). Each arm was connected to a flow-meter which provided the same amount of air to the two arms and an odor source container made of a glass cylinder (25 cm in diameter and 40 cm in height). The olfactometer also was equipped with humidifiers to provide the same amount of moisture to the Y-tube. The air circulated in each olfactometer arm was set to 40 psi so that *H. axyridis* adults were able to walk upwind toward the arms of the tube.

Two different tests were conducted to select suitable plants that attract *H. axyridis* adults based on olfactory preference: single-plant choice test and two-plant choice test. Single-plant preference test was designed to determine if each plant can attract *H. axyridis* adults. Flowers of each plant were field collected at bloom time and choice tests were carried out using the Y-tube olfactometer. One flower cut from each plant was placed in one of the odor-source containers and no plant (i.e. empty chamber) was provided in the other container. One adult *H. axyridis* was introduced at the start point in the Y-tube olfactory meter. The direction of movement toward the two arms of the olfactormeter was determined when a predator reached the far end of one of the arms. This test was repeated 50 times with 25 different male and 25 different female *H. axyridis* for each plant. Y-tube was washed thoroughly with warm water three times between tests with individual *H. axyridis*. We replaced *H. axyridis* when it did not respond to any choices 1 min after introduction to the Y-tube. If the size of flower was bigger than the containers a part of flower including petals was used.

Based on the result of the first test, we selected five plants that significantly ($P < 0.05$) attracted *H. axyridis* adults: bugleweed, dill, butterfly weed, sunflower, and marigold. With the

Y-tube choice test, the following pairs of flowers were compared depending on their synchrony of flowering time in the fields of West Virginia: dill vs. butterfly weed, sunflower vs. bugleweed, marigold vs. sunflower, and sunflower vs. dill. One *H. axyridis* adult was introduced at the start point in the Y-tube olfactometer and its choice to a plant was recorded. This test was repeated 25 times for each sex of *H. axyridis* adult.

Preference of *H. axyridis* adults to a plant for each trial was tested for statistical differences with a chi-square test using SAS (SAS Institute 2008) to determine if the distribution of *H. axyridis* choices was deviated from random at $\alpha = 0.05$.

Visual preference test

To test color preference of *H. axyridis* adults, six different colored cardboard papers were chosen. These colors included yellow (RGB values = 189, 166, and 44), blue (RGB values = 89, 123, and 178), green (RGB values = 50, 132, and 88), red (RGB values = 206, 50, and 71), orange (RGB values = 219, 76, and 40) and white (RGB values = 183, 188, and 196). The cardboard papers (22 cm by 21 cm) were randomly arranged in a rectangle form on a plexiglass frame on an experimental box (Fig. 2). Tanglefoot® (Tanglefoot Company, Grand Rapids, MI) was applied to the inner part of the plexiglass. White balance card (PortaBrace, North Bennington, Vermont, U.S.A.) was used to obtain correct RGB values of colors by removing unrealistic color casts.

A total of ten *H. axyridis* adults (five males and five females) were introduced into the box to evaluate which color would attract them. The number of adults caught on each color was recorded after 30 min. from the release. This experiment was replicated ten times with naïve *H.*

axyridis adults during the experiment. Preference of *H. axyridis* adults to different colors was determined with ANOVA and Tukey's standardized range test at 5% error rate was used to compare means of color preference (SAS Institute 2008).

Results

Olfactory preference

When single plants were tested against no plant for preference, 85 – 95% of *H. axyridis* adults successfully made choices. *H. axyridis* adults exhibited a significant ($P < 0.05$) preference to bugleweed, marigold, butterfly weed, dill, and sunflower (Table 1); dandelion flowers significantly ($P < 0.05$) repelled *H. axyridis* adults. This study also showed that males and females of *H. axyridis* exhibited different preferences to certain plants; males were significantly ($P < 0.05$) repelled by yarrow and tansy, but females were significantly attracted to the plants (Table 1).

From the two-plant choice tests, 95-100% of *H. axyridis* adults used in the experiment successfully made choices. We found that the only significant preference was found from the dill vs. butterfly weed comparison; female *H. axyridis* prefered dill over butterly weed (Table 2). Also, female *H. axyridis* preferred sunflower to marigold. Although more *H. axyridis* adults preferred sunflower compared with bugleweed, marigold, and dill, the preferences were statistically not significant ($P > 0.05$).

Visual preference test

Among the six colors tested in the experiment (yellow, blue, red, orange, green, and white), *H. axyridis* adults exhibited a significant preference for yellow over other colors (F = 62.25; df = 5, 59; $P < 0.01$) (Table 3). It was also found that blue and red were the least attractive colors to *H. axyridis* adults.

Discussion

Compared to specialist natural enemies, generalist natural enemies can feed on a broad range of prey species in multiple genera, which is a major advantage of using them to control multiple pest insects in cropping systems. For example, soldier bugs feed on more than 75 insect pests including Colorado potato beetles, Mexican bean beetles, earworms, cutworms, cucumber beetles, armyworms, flea beetles, weevils, and stink bugs (McPherson 1980). Lady beetles can feed on aphids, mealybugs, scale insects, and eggs of moths (Fisher et al. 1999, Dufour 2000, Park and Obrycki 2004); feeding activity of these pests sustains during flowering period. Another advantage of using generalist natural enemies is that they commonly reside on the farm throughout the growing season and can utilize plants as energy sources for reproduction and survival (Gillett 2005, Landis et al. 2005, Spellman et al. 2006). Lady beetles can inhabit a farm even during scarcity of prey insects by feeding on pollen, nectar, or plant sap (Landis et al. 2005, Spellman et al. 2006, Fiedler and Landis 2007). Lady beetles are primarily insectivore but they can survive by feeding on pollen and nectar (Lundgren 2009); *H. axyridis* larvae were much more likely to consume pollen than adults (Weber and Lundgren 2009). Companion plants can provide such food and shelter for natural enemies as well as stimulate relocations of natural enemies into the cropping areas where they can control insect pests (Johanowicz and Mitchell 2000, Mensah 1999, Nentwig 1998, Schoenig et al. 1998, Wratten et al. 1998, Rodenhouse et al. 1992).

Olfactory cues of companion plants could play an important role in attracting natural enemies into cropping areas. Some natural enemies use plant volatiles to search for prey (Schaller and Nentwig 2000) and such chemicals can provide an adaptive advantage to natural enemies by shortening search time required to find prey (Hattingh and Samways 1995). In this study we found that *H. axyridis* were highly attracted to olfactory cues of sunflower, which was consistent with field observations by Jones and Gillett (2005) who showed that sunflower plants attracted natural enemies when sunflowers reached a minimum height of 2.4 cm. The results of our study provide evidence that *H. axyridis* adults were highly attracted to volatiles from the followers of bugleweed, dill, marigold, sunflower, and goldenrod; volatiles associated with the plants were able to attract *H. axyridis* adults even in the absence of prey although many of these plants are acceptable hosts for aphids. Although other plants (i.e. yarrow, morning glory, goldenrod and tansy) were listed as potential attractant plants in the literature (Dufour 2000), the result of this study showed that there were no overall (i.e. male and female combined) significant ($P > 0.05$) olfactory preferences by *H. axyridis* adults; dandelion flowers significantly ($P < 0.05$) repelled *H. axyridis* adults.

Visual cues also play important roles in host-finding activity of *H. axyridis*. The result of our study suggested that adult *H. axyridis* could be attracted by color even when scent cues are eliminated. *H. axyridis* exhibited a significant preference for yellow over other colors, which was consistent with field observation by Mondor and Warren (2000) who showed that both male and female *H. axyridis* were attracted to yellow color. This could be related to aphids that are commonly found on plants with yellow flowers; stressed plants tend to be yellowish and thus yellow color could indicates the presence of aphids (Seagraves 2009). The result of our study showed that blue and red were the least preferred color to *H. axyridis* adults. We also found that both male and female *H. axyridis* showed preferences to different colors; some *H. axyridis* males were attracted to orange and red while females were not. Additionally, when *H. axyridis* search for overwintering sites, they are attracted to and land on white or bright, reflective surfaces (Koch 2003, Obata et al. 1986).

Knowledge about how natural enemies utilize both visual and olfactory cues may lead to development of strategies to attract *H. axyridis* populations for aphid biological control (Bahlai et al. 2008). The result of this study provides two important implications for biological control with *H. axyridis*. First, *H. axyridis* pontentially can be spatially relocated to cropping areas by using some companion plants. *H. axyridis* could be attracted by olfactory and visual cues of a few companion plants and yellow color, respectively. In this study, we found that volatiles of bugleweed, marigold, butterfly weed, dill, and sunflower attracted *H. axyridis* and these plants with the exception of bugleweed have yellow flowers, indicating that *H. axyridis* adults can be attracted to both visual and chemical cues of the plants. The companion plants can also be used to keep *H. axyridis* in the cropping area even when there are no aphids, because they can feed on pollen and nectar of the companion plants.

Second, depending on crop species, selective planting of attractant plants can be done to attract *H. axyridis* season long. In this study, we found that five species of flowering plants attracted *H. axyridis* with olfactory cues and those plants can be systematically planted for targeting a series of bloom throughout the season; because the blooming time of each plant is different throughout the year, suitable companion plants can be selected based on when *H.*

axyridis adults are needed for aphid control. For example, in addition to sunflower blooming during the middle of the growing season, bugleweed and goldenrod can be used early and late in the growing season, respectively, to attract *H. axyridis* adults.

Future work should examine the response of *H. axyridis* to specific volatiles associated with potential companion plants as well as chemical analyses of the volatiles. Important issues related to aphid control with *H. axyridis* include timing when aphid and *H. axyridis* populations generally first appear, when food sources (nectar and pollen from companion plants) are available and how long they last, and number of plantings and plant species mixes to enhance attraction of *H. axyridis*.

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Table 1. Chi-square statistics and associated p-values of *H. axyridis* preferences to single plant. Note that significant preference with < 50% preference to plant indicates that plants repelled *H. axyridis*

Plant species	Male (n = 25)		Female (n = 25)		Both sexes combined (n = 50)	
	% preference to plant	χ^2 statistics (p value)	% preference to plant	χ^2 statistics (p value)	% preference to plant	χ^2 statistics (p value)
bugleweed	80%	9.00 (< 0.01)	72%	4.84 (0.03)	76%	13.52 (< 0.01)
butterfly weed	76%	6.76 (< 0.01)	72%	4.84 (0.03)	74%	11.52 (< 0.01)
dandelion	28%	4.84 (0.03)	24%	6.76 (< 0.01)	26%	11.52 (< 0.01)
dill	68%	3.24 (0.07)	64%	1.96 (0.16)	66%	5.12 (0.02)
goldenrod	72%	4.84 (0.03)	40%	1.00 (0.32)	56%	0.72 (0.40)
marigold	76%	6.76 (< 0.01)	52%	0.04 (0.84)	64%	3.92 (0.05)
morning glory	48%	0.04 (0.84)	48%	0.04 (0.84)	48%	0.08 (0.78)
sunflower	84%	7.00 (< 0.01)	64%	1.96 (0.16)	74%	11.52 (< 0.01)
tansy	20%	0.69 (0.41)	100%	4.57 (0.03)	60%	2.00 (0.16)
yarrow	28%	4.84 (0.03)	72%	4.84 (0.03)	50%	0.00 (1.00)

Table 2. Chi-square statistics and associated p-values of *H. axyridis* preferences to two plants

Two plant species compared	Male (n = 25)		Female (n = 25)		Both sexes combined (n = 50)	
	preferred plant (% choice)	χ^2 statistics (p value)	preferred plant (% choice)	χ^2 statistics (p value)	preferred plant (% choice)	χ^2 statistics (p value)
dill vs. butterfly weed	dill (56%)	1.96 (0.16)	dill (76%)	6.76 (< 0.01)	dill (66%)	5.12 (0.02)
sunflower vs. bugleweed	sunflower (60%)	1.00 (0.32)	sunflower (68%)	3.24 (0.07)	sunflower (64%)	1.96 (0.16)
marigold vs. sunflower	sunflower (64%)	1.96 (0.16)	sunflower (72%)	4.83 (0.03)	sunflower (68%)	3.24 (0.07)
sunflower vs. dill	sunflower (72%)	4.83 (0.03)	sunflower (64%)	1.96 (0.16)	sunflower (68%)	3.24 (0.07)

Table 3. Numbers (average ± SE) of attracted *H. axyridis* to six different colors

Color	Male	Female	Both sexes combined
yellow	2.4±1.26a*	2.8±1.23a	5.2±1.23a
green	1.3±1.16b	1.5±1.27b	2.8±1.19b
white	0.7±1.25bc	0.4±0.52c	1.1±0.94c
orange	0.4±0.52c	0.0±0.00c	0.4±0.41cd
blue	0.1±0.32c	0.2±0.63c	0.3±0.49d
red	0.2±0.42c	0.0±0.00c	0.2±0.31d

*Means within each variable in each column followed by the same letter are not significantly different ($P > 0.05$; Tukey's standardized range test at 5% error rate).

Figure Legend

Fig. 1. Olfactometer used for testing olfatory preference of *H. axyridis* adults. Humidifier and flow meter were added to the Y-tube to provide the same amount of humidity and air inflow to the two chambers, respectively.

Fig. 2. A rectangle cardboard box used for testing visual preference of *H. axyridis* in response to six different colors: yellow, green, blue, orange, red and white. A total of 12 papers of the six colors (two pieces per color) were randomly assigned and attached to the outer part of the front panel made of transparent plexiglass. Tanglefoot was applied to the inner part of plexiglass and then *H. axyridis* adults were introduced into the box for the choice test.

Fig. 1.

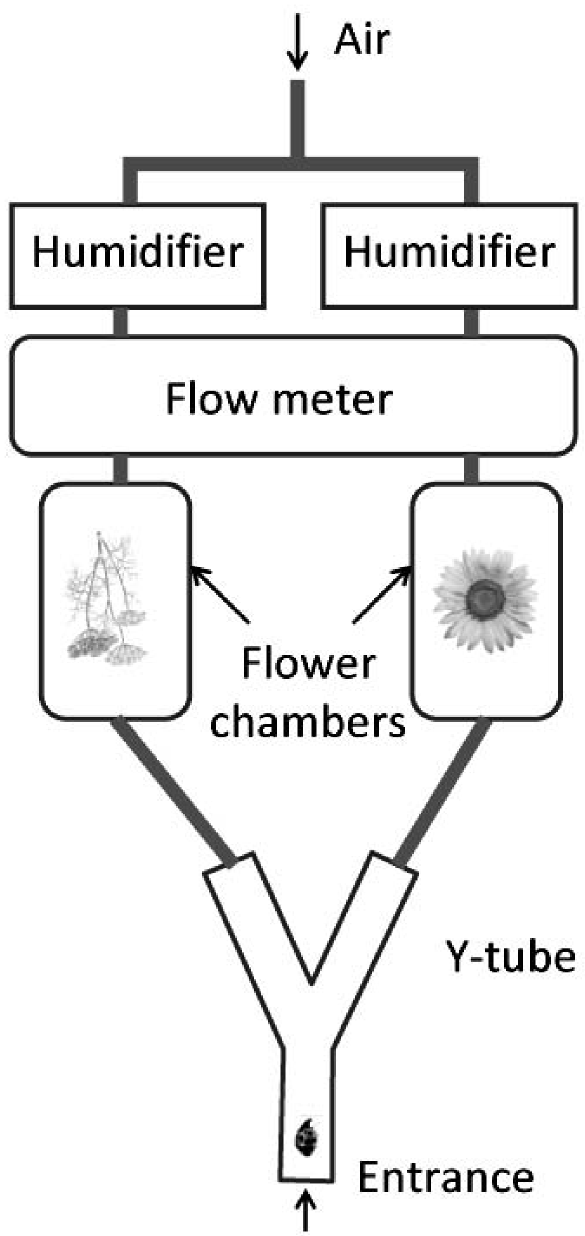
Air
Humidifier
Humidifier
Flow meter
Flower
chambers
Y-tube
Entrance

Fig. 2.