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Behavioral Response of Western Flower Thrips to Visual and Olfactory Cues

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Abstract Behavioral responses of *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) to visual and olfactory cues were assessed in a cylindrical black box and a Y-tube olfactometer. *Frankliniella occidentalis* preferred circular shapes to other five geometrical patterns (rectangle, triangle, inverted triangle, diamond, and modified circle imitating flower petals) in multiple choice tests. In pair wise choice tests, the thrips preferred the yellow artificial flower shape to the geometrical patterns tested. *Frankliniella occidentalis* stayed on the artificial flower about four times longer than on the geometrical patterns. Higher numbers of thrips responded to a combination of *p*-anisaldehyde and artificial flower compared to the arm with only the olfactory or the visual cue. These results suggest that shape is an important cue for *F. occidentalis*.

Keywords *Frankliniella occidentalis* · sticky card · flower model · olfactometer · anisaldehyde · ethyl nicotinate

Introduction

Frankliniella occidentalis Pergande (Thysanoptera: Thripidae) is one of the most economically important pests of various crops worldwide (Morse and Hoddle 2006). Thrips damage plants by feeding on leaves, fruits, or petals (Kirk 1997). However, due to the thigmokinetic behavior (occupying narrow crevices within or between plant parts) of thrips, damage in floral buds or flowers is difficult to detect during early infestation (Childers 1997). Early detection of thrips is important for the development of timely control measures. Understanding host finding behavior of thrips would be helpful to develop monitoring tools for early thrips detection. As other insects, thrips locate plants using color, shape, size and volatiles associated

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with these plants (Frey et al. 1994; Cho et al. 1995; Childers and Brecht 1996; Terry 1997; Teulon et al. 1999; de Kogel and Koschier 2003).

Many studies have evaluated the response of *F. occidentalis* to olfactory and color cues. Thrips uses floral volatiles such as benzonoids to find their host (Teulon et al. 1993; Terry 1997). In addition, some non-floral odors like ethyl nicotinate are attractive to *F. occidentalis* (Koschier et al. 2000). Based on studies on visual attraction to colors, color sticky cards have been developed and are used primarily for monitoring thrips populations (Vernon and Gillespie 1990; Muirhead-Thomson 1991; Parrella et al. 2003; Chu et al. 2005; Seo et al. 2006; Pizzol et al. 2010) and occasionally as a form of control (Kawai and Kitamura 1987). Kogel and Koschier (2003) suggested that color is a dominant factor for thrips orientation towards flowers.

Apart from olfactory and color cues, geometrical patterns and shapes have been also tested as attractive visual cues for thrips (Moreno et al. 1984; de Jager et al. 1995). Floral petal configuration is thought to affect host plant preference of anthophilous thrips. Chrysanthemum flowers with disc florets are more attractive to *F. occidentalis* than chrysanthemum flowers with spider-type florets (de Jager et al. 1995). In our previous studies, a higher number of *F. occidentalis* was attracted to circular yellow sticky cards (Mainali and Lim 2010; Lim and Mainali 2009) or flower-shaped sticky trap (Mainali and Lim 2008) compared to ordinary rectangular yellow sticky cards. To understand thrips visual cue (including shape and color) and olfactory cue preferences, we conducted behavioral studies using a cylindrical black box and a Y-tube olfactometer in the laboratory. We focused on determining thrips preferences to various geometrical patterns, artificial flowers, and olfactory cues. The results may help in developing traps that could be implemented for efficient monitoring or for the management of the thrips.

Methods

Thrips Rearing

Adult *F. occidentalis* were collected from a strawberry greenhouse located in Songcheon, Andong, Korea in 2006 and reared on leaves of red kidney bean, *Phaseolus vulgaris* L. (Fabaceae), according to the method described in Mainali and Lim (2010).

Response to Visual Cues

Geometrical Patterns of Paper

Multiple choice experiments were carried out inside a cylindrical black box (ID= 16 cm, H=22 cm). The internal wall of the box was covered with black paper. Yellow paper (P09, Samwon Paper Ltd., Seoul, Korea) was cut into six different patterns: rectangle, circle, triangle, inverted triangle, diamond, and modified circle imitating flower petals. All the patterns had the same surface area (5 cm^2). Inside the black box, the six patterns were placed at equal distances (60° angles) from the center. Previously, *F. occidentalis* pupae were placed individually inside centrifugal tubes (1.5 ml) containing moistened cotton. After emerging from pupa, one adult female thrips which was less than 12 hours old and unfed was released into each box at the center of the circle. Temperature in the experiment unit was measured that ranged from 24 to 26°C (PC-7800B, Sato Keiryoki Mfg. Co. Ltd., Tokyo, Japan). Light was provided by a fluorescent tube (27W, DX-370, Samjung, Seoul, Korea) located above the box. To avoid positional effects, the box was rotated 180° after every five replications. Observations were made on the thrips preferences and residence times on each pattern for 5 minutes. If any thrips failed to choose a pattern within 3 minutes, it was discarded. The residence time was recorded using a stop watch. A total of 60 thrips was tested. Reflectance patterns (Fig. 1) of the yellow paper used for making various geometrical patterns and petals of artificial flowers were measured using a spectrum color meter (JS555, Minolta, Tokyo, Japan).

Artificial Flowers

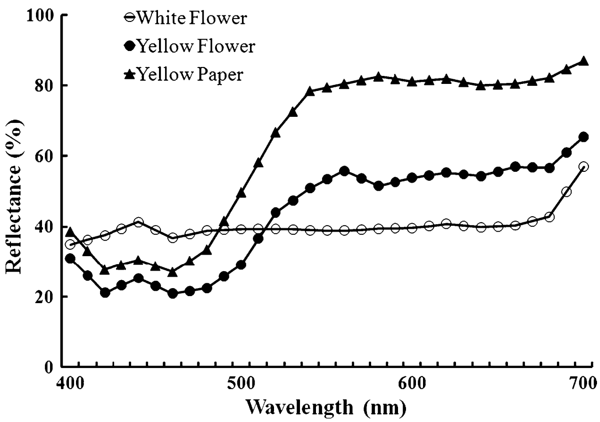
An equilateral triangle (side length: 12 cm) was drawn on the bottom of each black box. Newly emerged thrips adults were placed individually at one vertex of the triangle. Thrips responses were compared among artificial strawberry flowers (D=1.3 cm, yellow or white) and yellow papers in circular, modified circular and rectangular shapes. Two patterns were placed at the other two vertices of the triangle on the bottom of the black box so that thrips could choose the patterns freely. Patterns were illuminated by a light source located above the black box. Observations were made on choice and residence time on the patterns. A total of 60 thrips was tested for each pair of patterns.

Similarly, individual thrips were exposed to yellow artificial flowers with different number of petals (1 to 6) in multiple choice design as described in the previous section. All yellow flowers were painted with fabric paint (Dylon International, Seoul, Korea). Frequency of thrips visits and residence time on the flowers were recorded with 60 thrips. Similar observations were taken as stated above.

Response to Visual Cues with Olfactory Component

Responses of *F. occidentalis* to visual cues with/without olfactory components were examined in a Y-shaped glass tube. The Y-tube (stem 9 cm; arms 8 cm at 130° angle; ID 1.5 cm) was constructed as described by Colazza et al. (1997). Medical-grade

Fig. 1 Spectral reflectance of the artificial flowers and the yellow paper used to make various geometric patterns



compressed air flowed through both arms, creating an air stream of 300 ml/min/arm. The olfactometer was illuminated by fluorescent tubes located above the device. Experiments were conducted in a dark bioassay room, where the temperature was maintained at 23–25°C.

Yellow artificial flowers were used as visual cues without odor cues and tested against clean air and real flower odor. The artificial flowers were also conjugated with different volatile sources and tested against clean air or the artificial flower. The artificial flower was placed 3 cm from the junction of Y-tube arms. Newly emerged adult female thrips were introduced into the Y-tube at the entrance of the stem and its preference was recorded for the first 5 minutes. Each thrips was allowed a maximum of 3 minutes to choose one arm of the Y-tube. If a thrips failed to choose within 3 minutes, it was discarded. A choice was considered to have occurred when a thrips went down one arm, touched the pattern and stayed for more than 1 minute in the arm. Y-tube glass was changed for each thrips tested. After each experimental trial, the olfactometer was cleaned with dish washing detergent and rinsed with water and 99.9% acetone thoroughly before oven drying.

p-anisaldehyde (benzenoid, 10 μl, 99%, Acros, New Jersey, USA), ethyl nicotinate (non-floral odor, 10 μl, 99%, Aldrich, St. Louis, USA), and a fully opened strawberry flower (genuine source of floral odor) were tested. Preference of the thrips to *p*-anisaldehyde versus clean air and to ethyl nicotinate versus clean air was recorded. Two jars with odor sources were connected to the arms of the Y-tube by rubber tubes. Air humidity was equalized by passing air into a jar containing distilled water before introducing the air to the odor jars. Thrips preference to odors compared to clean air was recorded. Also, thrips' preference between *p*-anisaldehyde and ethyl nicotinate was tested. A choice was considered to have occurred when a thrips reach one of the arms and stayed for more than 1 minutes. All tests were replicated 60 times.

Further tests were carried out using visual cues coupled with the olfactory cues in Y-tube olfactometer. Yellow artificial flowers in the Y-tube arm with a test odor of *p*-anisaldehyde and ethyl nicotinate were examined against the arm with clean air or with yellow artificial flowers. Various pairs of odor and visual cue were tested to determine the relative attractiveness to visual cues compared to odor cues.

Statistical Analyses

The ratios of thrips attracted to geometrical patterns, the ratios of the thrips attracted to artificial flowers with different numbers of petals (1–6), and the ratios of thrips in pairwise tests were compared using the normal approximation of the chi-square test. Residence time of the thrips was analyzed using *t*-test.

Results

Response to Visual Cues

Geometrical Patterns

In the multiple choice tests carried out inside the black cylindrical box, among the six geometrical patterns, 2.5 to 3.5 times more thrips chose the circular shape first compared to the diamond, the rectangular, or the inverted triangular shapes (χ^2= 13.320, *d.f.*=5, *P*=0.021; Fig. 2a). However, the residence time of the thrips on all the geometrical patterns did not differ statistically (Fig. 2b). Almost 33% of the thrips made a second choice after leaving the pattern they chose first, and 17% of the thrips made third choice. However, the frequency of second choice and residence time during second visit was not statistically different, though the trend of second visit occurred frequently in the circular shape followed by the triangular or the rectangular shapes.

Artificial Flowers

In pair wise tests in the cylindrical box, significantly more thrips made their first visit to yellow artificial flower compared to all other geometrical patterns and the white artificial flower. Thrips chose the yellow artificial flower 3 times (Z_c=5.287, $P<0.001$), 1.7 times (Z_c=2.743, P=0.006) and 2.3 times (Z_c=4.193, $P<0.001$) more than the rectangular, circular and modified circular shapes, respectively (Fig. 3a). The residence time of the thrips on the yellow artificial flower was almost 3 times more than that on the rectangular (t=7.696, *d.f.*=58, $P<0.001$), circular (t=13.488, *d.f.*=58, $P<0.001$) and modified circular (t=14.362, *d.f.*=58, $P<0.001$, Fig. 4a) shapes.

There was no significant difference in the choice between the white artificial flower and the yellow rectangular shape. However, residence time of the thrips during their first visit was significantly higher on the white artificial flower (t=12.206, *d.f.*=58, $P<0.001$). The thrips chose the yellow artificial flower first

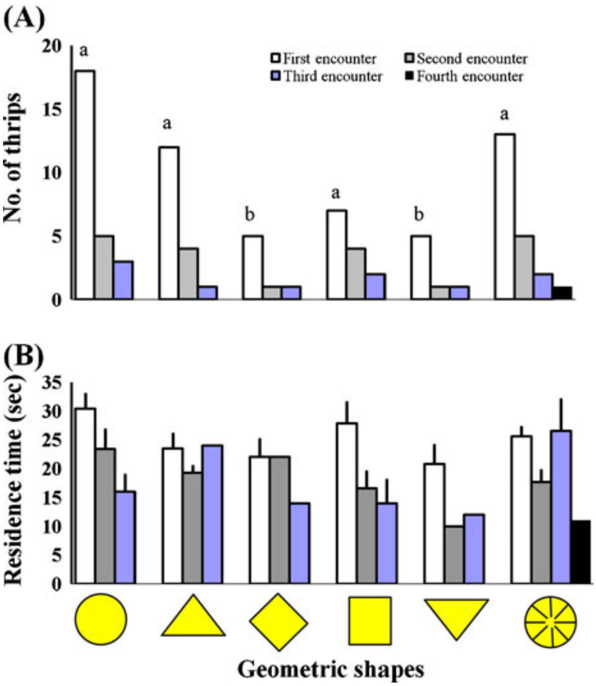


Fig. 2 Frequency of *F. occidentalis* visits to various geometrical patterns (**a**) and residence time on the patterns (**b**) tested in a black cylindrical box. Values with the same letter are not significantly different at $P>0.05$

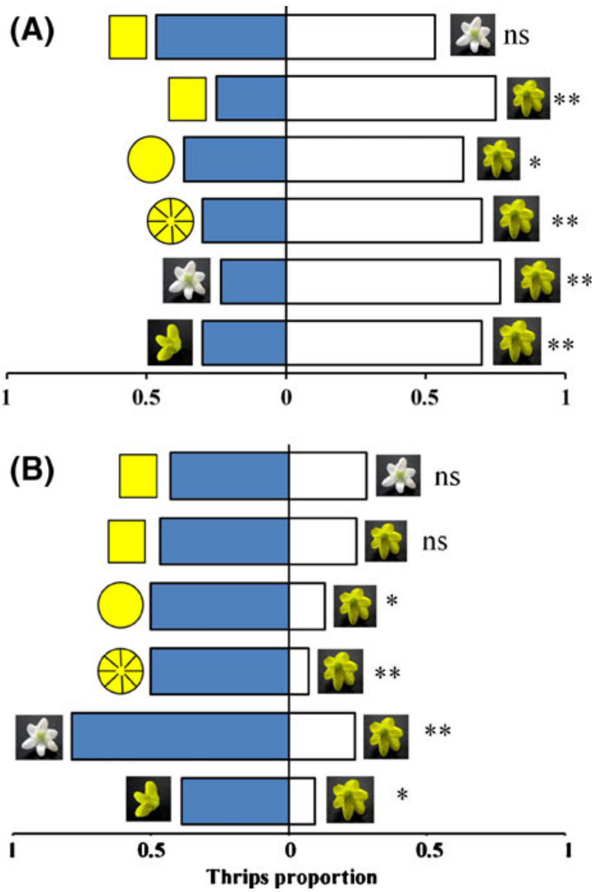


Fig. 3 Proportion of *F. occidentalis* first visits to artificial flowers and geometrical patterns (**a**) and proportion of abandonment of first visited cue (**b**). *0.01< $P \leq 0.05$ and **0.001< $P \leq 0.01$; ns=not significant ($P>0.05$) from a paired *t*-test. Pairwise choice tests were conducted in a black cylindrical box

significantly more than the white artificial flowers (Z_c=5.654, $P<0.001$) or asymmetrical yellow artificial flowers (Z_c=4.193, $P<0.001$). Residence time of the thrips on the yellow artificial flowers was 2.3 times (t=7.363, $d.f.$=58, $P<0.001$) and 1.6 times longer (t=6.402, $d.f.$=58, $P<0.001$) than on the white artificial flower and yellow artificial asymmetric flower, respectively.

Approx. 24, 13, and 7% of the thrips which first visited the yellow artificial flower made a second visit to the yellow rectangular shape, the circular shape and the modified circular shape, respectively, while 46.6, 50 and 50% of the thrips which first visited the yellow rectangular shape made a second visit to the circular shape and the modified circular shape and the yellow artificial flower, respectively (Fig. 3b). The residence time during the second visit (Fig. 4b) was also higher on the yellow artificial flower compared to the rectangular (t=7.998, $d.f.$=16, $P<0.001$), the circular (t=7.666, $d.f.$=14, $P<0.001$) and the modified circular (t=5.569, $d.f.$=14, $P<0.001$) shapes.

Interestingly, 42.9% of the thrips which first visited the yellow rectangular shape visited the white artificial flower, whereas 28.1% of the thrips those first visited the white artificial flower visited the yellow rectangular shape. The residence time of the thrips on second visit was higher on the white artificial flower than the yellow rectangular shape (t=5.784, $d.f.$=19, $P<0.001$). Thrips abandoned the white

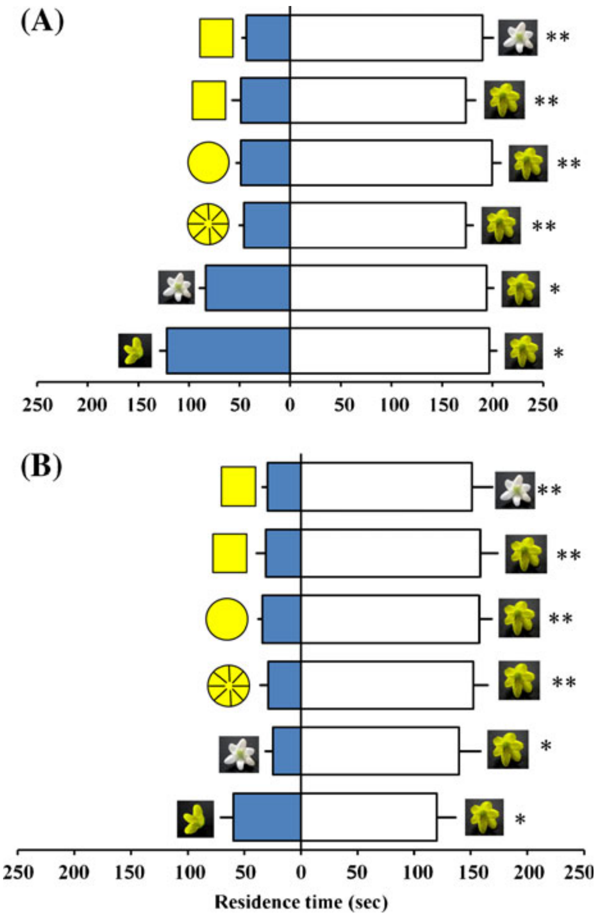


Fig. 4 Residence time of *F. occidentalis* during the first (**a**) and second visits (**b**) to artificial flowers and other geometrical patterns tested in a black cylindrical box. *0.01<P≤0.05 and **0.001<P≤0.01; ns=not significant (P>0.05) from a paired t-test

artificial flower more than yellow artificial flower (Z_c=3.399, P<0.001) and stayed longer on the yellow artificial flowers (t=3.511, *d.f.*=10, P=0.005).

Only 9.5% of the thrips which first visited the symmetrical flower visited the asymmetrical flower, whereas almost 39% of the thrips which first visited the asymmetrical flower visited the symmetrical flower. Thrips had more residence time on the symmetrical flower than on the asymmetrical flower in the second visit (t=2.594, *d.f.*=9, P=0.029).

When the thrips were exposed to yellow artificial flowers with various numbers of petals, a higher number of thrips (χ^2=35.160, *d.f.*=5, P<0.001, Fig. 5a) chose flowers with complete petals (6-petals), followed by 5 and 4 petals. Residence time of the thrips was also higher (F=7.796, *d.f.*=5, 53, P<0.001, Fig. 5b) on those flowers compared to the flowers with 3, 2 and 1 petals.

Response to Visual Cues with Olfactory Component

The arm of the Y-tube containing p-anisaldehyde (Z_c=2.738, P=0.006) was preferred by thrips. However, there was no statistical difference (Z_c=1.643, P=0.100) when

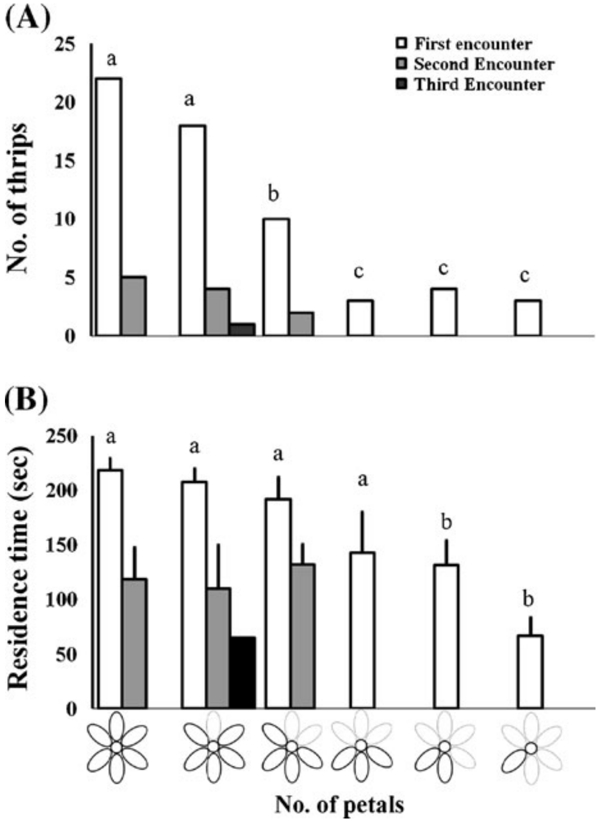


Fig. 5 Frequency of visits (**a**) and residence time (**b**) of *F. occidentalis* on artificial flowers with different numbers of petals tested in a black cylindrical box. Values with the same letter are not significantly different at $P<0.05$

a choice was given between ethyl nicotinate and clean air. There was no statistical difference when a choice was given between *p*-anisaldehyde and ethyl nicotinate ($Z_c=0.913$, $P=0.361$) (Fig. 6).

The Y-tube arm containing both *p*-anisaldehyde ($Z_c=4.199$, $P<0.001$) and the yellow artificial flower ($Z_c=3.834$, $P<0.001$) was chosen significantly more by the thrips than the arm with clean air. Similarly, ethyl nicotinate attracted more thrips when was conjugated with the yellow artificial flower ($Z_c=2.738$, $P=0.006$). The natural strawberry flower as a cue having both visual and olfactory component was also preferred by thrips compared to the arm with just clean air ($Z_c=3.873$, $P<0.001$). When the choice was given between yellow artificial flower conjugated with *p*-anisaldehyde and the yellow artificial flower, the arm with both the odor and the visual cue was preferred by the thrips ($Z_c=2.373$, $P=0.017$). However, no significant difference was found in the number of thrips choosing the arm containing both ethyl nicotinate and the yellow artificial flower compared to the arm containing only the yellow artificial flower ($Z_c=1.643$, $P=0.100$). The natural strawberry flower odor coupled with the yellow artificial flower was chosen by a higher number of thrips compared to the yellow artificial flower alone ($Z_c=2.840$, $P=0.004$, Fig. 6). When the yellow artificial flower was tested against real flower odor, we did not find any significant preference.

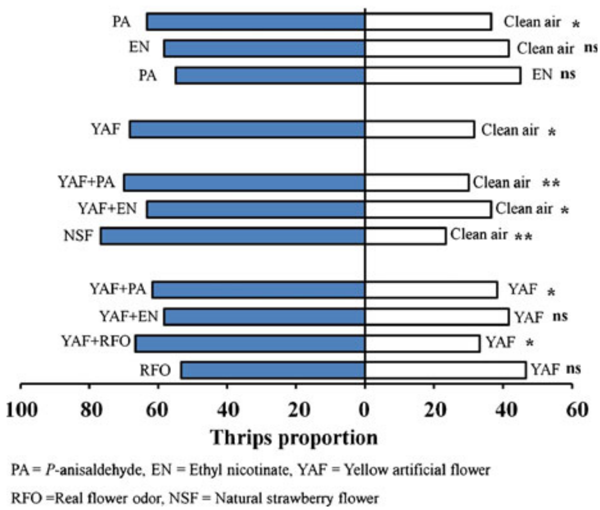


Fig. 6 Percentage of the thrips choice to the arm of the Y-tube olfactometer with visual and/or odor cues. PA, P-anisaldehyde; EN, Ethyl nicotinate; YAF, Yellow artificial flower; RFO, Real flower odor; NSF, Natural strawberry flower. *$0.01<P\leq 0.05$ and **$0.001<P\leq 0.01$; ns=not significant ($P>0.05$) from a paired t-test

Discussion

In the multiple choice tests, the significant preference of thrips for the circular shape indicates that *F. occidentalis* perceives shapes. Visual responses of thrips to geometrical patterns have been reported in other studies. Cho et al. (1995) reported cylindrical traps are better to trap *Frankliniella tritici* Lindeman compared to flat traps. *Scirtothrips citri* Moulton prefers triangular, elliptical, and rectangular shapes over circular and square ones (Moreno et al. 1984). The authors suggest that for *S. citri* the triangle, eclipse, and rectangle represent tender leaves preferred by the thrips.

The recognition of geometrical patterns by thrips is further supported by the result that the residence time on artificial flowers was longer than on the geometrical patterns tested (Fig. 4). *Frankliniella occidentalis* showed active searching behavior in the artificial flowers. Thrips wandered around the edges of the petals and spent more time at the crevices on the center of the artificial flower giving an impression that they recognize the flower (unrecorded observation). Alhough there was no pollen or any floral odor in the artificial flower, the thrips probably tried to locate the food resources on the artificial flowers. It seems that thrips were genetically tuned to perceive shapes because the thrips used for this study were reared on leaves without any flowers from egg stage.

The selection of the symmetrical yellow artificial flower at higher rates than other geometric patterns tested and higher second visit to the symmetrical yellow flower from the circle, modified circle, white artificial flower, and asymmetrical yellow artificial flower (Fig. 3) suggests that thrips use not only color but also shapes as cues for host selection. Terry (1997) reviewed studies that describe the recognition of flower morphology by the thrips. *Thrips palmi* Karny preferred the Phalaenanthe morphotype of *Dendrobium* orchids to other colors and morphotypes (Hata et al. 1991). Vernon and Gillespie (1995) found highest number of *F. occidentalis* caught per square centimeter in cylinder traps compared to any other shapes they used.

Frankliniella occidentalis caused more damage to tall chrysanthemum cultivars with many and large flowers (de Jager et al. 1995). Discrimination of flowers with different numbers of petals in this study also supports the hypothesis that thrips recognize flower morphology. *Frankliniella occidentalis* best perceived flowers with complete petals. Discrimination between different floral forms is also known in insect pollinators. *Meligethes maurus* Sturm, a coleopteran pollinator of *Erysimum mediohispanicum* Polatschek, a member of the Brassicaceae, visited Zygomorphic flowers more frequently than actinomorphic flowers (Gomez et al. 2006 cited in Glover 2007).

In Y-tube olfactometer tests, thrips chose the arm with visual cues more than the arm without any visual cue. When *p*-anisaldehyde was present in one of the arms either in combination with visual cues (e.g. yellow artificial flower) or without any visual cue, it elicited positive responses of *F. occidentalis* (Fig. 6). The preference to *p*-anisaldehyde is in agreement with the results of previous studies (Teulon et al. 1993; Koschier et al. 2000), although it is not attractive for *Thrips tabaci* (Kogel and Koschier 2003). Chemokinesis probably best describes the behavioral response because the main response of thrips to odor is flight inhibition (Teulon et al. 1999). We found that ethyl nicotinate did not affect the thrips behavior as described in the previous study by Davidson et al. (2007) who found that ethyl nicotinate did not increase trap catches of *F. occidentalis* in capsicum greenhouse. However, it can be controversial since a positive reaction of *F. occidentalis* to ethyl nicotinate was found at concentrations ranging from 10 to 0.01% in a Y-tube olfactometer (Koschier et al. 2000).

When one of the arms contained real floral odor and the other arm contained the yellow artificial flower, no significant response of the thrips was found. This result suggests that shape is as important as color. Although visual cues have been found to provide a strong stimulus to *F. occidentalis* (Davidson et al. 2006; Teulon et al. 1999), most studies have tested color and have underestimated thrips response to shape. Thrips respond to both shape and color, and, if the flower shaped sticky trap designed by Mainali and Lim (2008) which include both shape and color cues are baited with odor like *p*-anisaldehyde, their attractiveness would increase.

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