Response of Apple Maggot and Cherry Fruit Fly (Diptera: Tephritidae) to Color and Contrast Cues from Small Deposits

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Abstract

Rhagoletis fruit flies are strongly attracted to relatively large yellow surfaces that act as leaf mimics. Our objective was to determine whether apple maggot, Rhagoletis pomonella (Walsh), and cherry fruit fly, R. cingulata (Loew), (Diptera: Tephritidae) are more attracted to small yellow deposits than other colors, and whether contrast with the background modifies the response to deposit color. The experimental setup consisted of Petri dish arenas with sucrose deposits on the inside surface of the cover or the bottom. Female flies were introduced individually into the arena, and flies were either observed for 5 min or until they touched the sucrose with their labellum. Initially, translucent-colored deposits were produced by drawing a colored dot outside the cover of the same size as a sucrose deposit placed inside. In later trials, sucrose deposits contained pigment and wax emulsion and were opaque. More apple maggot flies found translucent yellow deposits than black, red or clear. When opaque deposits were used, there were no differences in the numbers of apple maggot flies that found deposits of different colors, but more cherry fruit flies found yellow than white, pink or blue deposits. Contrast cues were produced by depositing colored sucrose on the bottom of the Petri dish and placing the dish on a white or black background. More apple maggot and cherry fruit flies found yellow deposits than black or white, but the number of flies of both species that found a deposit strongly contrasting with the background was not significantly different from those finding the yellow deposit. We conclude that apple maggot and cherry fruit fly find small yellow deposits, or colors strongly contrasting with the background, more often than other colors, and that the response to color cue varies in different species.

Key Words color, cue, Rhagoletis, pomonella, cingulata, apple, cherry

Vision plays a major role in diverse aspects of insect behavior, alone or in combination with other sensory systems (Prokopy and Owens 1983, Giurfa and Menzel 1997, Briscoe and Chittka 2001). Visual perception by insects depends upon the size and nature of the viewed surface, the optical background, the illuminant, and the viewer’s angle and sensitivity (Prokopy and Owens 1983). Most insects studied so far have green receptors maximally sensitive at ~530 nm wavelength, UV receptors (λmax ~350 nm) and blue receptors (λmax ~440 nm) (Briscoe and Chittka 2001). In Rhagoletis flies, the spectral response curves of the compound eyes of the apple maggot showed a broad major peak at 400 - 530 nm (blue-green to yellow) (Agee 1985). This type of response is typical of most herbivorous insects that respond positively to yellow in behavioral assays. Prokopy and Owens (1983) suggest that, for these insects, yellow constitutes a supernormal foliage-type stimulus, reflecting peak energy in the same wavelength of

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the insect-visible spectrum as foliage reflects, but at a greater intensity. Visual stimuli attractive to *Rhagoletis* fruit flies have been integrated into traps deployed in monitoring for pest management purposes (Prokopy 1972, Prokopy and Coli 1978, Liburd et al. 2001). Traps most effective in capturing *Rhagoletis* flies include yellow boards (Prokopy 1972) and red spheres (Prokopy 1968). Contrast with the background also plays a major role in the visual orientation of *Rhagoletis*, as traps creating more intense contrast tend to capture more flies (Owens and Prokopy 1984, Owens and Prokopy 1986).

The use of visual cues for finding oviposition sites and mates is well documented in *Rhagoletis* fruit flies, but the importance of visual cues in food-finding behavior is not known. These insects use vision and odor to find host plants from a distance (Aluja and Prokopy 1992, Green et al. 1994). Flies find abundant and highly-apparent host fruit solely on the basis of vision, but odor cues will interact with visual cues if fruit is scarce or less apparent (Aluja and Prokopy 1993). Females also use visual cues to choose fruit for oviposition, along with other stimuli (Prokopy and Boller 1971, Henneman and Papaj 1999). Male flies use vision to locate females on or in the vicinity of host fruit, which also serves as a landmark for flies of both sexes to meet (Prokopy and Bush 1973). With respect to feeding behavior, food resources used by *Rhagoletis* include aphid honeydew, nectar from floral and extrafloral nectaries, fruit juices from wounds and oviposition sites, insect frass and bird droppings (Boller and Prokopy 1976, Hendrichs and Prokopy 1994). Flies need carbohydrates for energy, and females must ingest protein for egg development (Webster et al. 1979). Along with feeding on easily-identifiable substances, flies also spend a large proportion of their active time feeding on substances diffused on the leaf surface that are invisible to a human observer (Bateman 1972, Smith and Prokopy 1981, Smith 1984, Hendrichs and Prokopy 1990, 1994, Yee 2002). This behavior has been described as “dabbing” or “grazing” on the leaf surface because flies touch the leaf surface repeatedly with their proboscis (Hendrichs and Prokopy 1990). Substances found in small amounts on leaf surfaces include microorganisms, pollen and plant exudates (Hendrichs et al. 1993).

The apple maggot, *R. pomonella* (Walsh), and cherry fruit fly, *R. cingulata* (Loew), are major pests of apples and cherries, respectively (Reissig 2003, Teixeira et al. 2007). The development and commercial availability of safer insecticides that act on flies mostly by ingestion (e.g., spinosad) has prompted renewed interest in the use of insecticidal baits for control of fruit-infesting *Rhagoletis* pests (Barry et al. 2005, Pelz et al. 2005, Yee and Chapman 2005). However, the degree of fruit protection provided by currently available insecticidal baits does not meet the stringent quality standards imposed by the market (Pelz et al. 2005). Baits rely on volatile attractants for manipulating the flies’ food-finding behavior, and feeding stimulants to promote the ingestion of a lethal dose of the toxicant (Moreno and Mangan 2002). To increase the attraction of flies to baits and, therefore, improve interaction of flies with the toxicant, the release of ammonia by bait deposits has been increased (e.g., Pelz-Stelinski et al. 2006). Use of visual cues has yet to be explored as a potential avenue to increase performance of insecticidal baits. As part of an overall effort to improve the efficacy of fruit fly baits, the present laboratory study examined the effect of color and contrast cues on finding (*sensu* Miller and Strickler 1984) of small deposits by apple maggot and cherry fruit flies.

**Materials and Methods**

**Insects.** Apple maggot pupae were obtained from infested apples collected in August 2005 and 2006 in Fennville, MI. Cherry fruit fly pupae were obtained from
infested tart cherries, collected in August 2006 in Fennville, MI. Pupae were stored at 4°C for at least 3 months and brought to 25°C as needed. After emergence, males and females were kept together in 30 x 30 x 30 cm plastic cages. Flies were kept at 21 - 23°C in the same laboratory where experiments were conducted. Food consisted of a 1:4 mixture of yeast protein hydrolysate and sugar. Food and water were provided separately. Food was removed from the cage 18 - 24 h prior to flies being used to increase the flies' tendency to interact with sucrose deposits. Flies were assayed in the morning, and only female flies aged 5 - 8 d were used in these experiments.

**Experimental setup.** We used an experimental arena that induced flies to walk on a surface and interact with the colored deposits because flies find food substances in nature ultimately by ambulatory activity. All tests were no-choice and were conducted by exposing female flies individually to a 5 - 7 mm diam dry sucrose deposit, consisting of 25 μL of 32% sucrose in distilled water, in a 10 x 1.5 cm Petri dish with inside dimensions of 8.5 x 1.4 cm. A fly was introduced into the side of the arena with the help of a small vial. Petri dishes were placed on a white surface inside a 1 x 1 x 1 m white fabric cage that minimized external stimulation. The arena was illuminated from above by natural light supplemented with incandescent light provided by a fiber optic microscope illuminator and by 2 incandescent lamps placed on the sides of the cage. Contact with sucrose provided an objective marker for ending the trial because almost all flies that contacted the deposit with their tarsi lowered their labellum and fed. When this happened the fly had found the deposit and the assay was terminated. Flies were observed until they either touched the deposit with their labellum or 5 min had passed. Each fly was used only once and discarded. This experimental methodology was precisely replicated in all experiments described below.

**Deposit color.** Initially, we placed a 5 - 7 mm diam sucrose deposit centrally on the inner surface of the cover of the Petri dish arena. A dot of the same diameter as the deposit inside was drawn outside the cover of the arena, immediately above the sucrose deposit inside. Black and red dots were drawn using Sharpie™ permanent markers (Sandford Corporation, Oak Brook, IL). Yellow dots were painted using TRY-33 yellow pigment (DAYGLO Color Corp., Cleveland, OH) in a 70% ethanol solution. The dots caused the deposits to transmit light of the same color of the dot above, or less light in the case of a black dot. In the first experiment in this set, apple maggot females were exposed to treatments consisting of a sucrose-only deposit (clear), or a deposit paired with a black or yellow dot. In the second experiment, we exposed apple maggot females to treatments consisting of a sucrose deposit paired with black, red or yellow dots. In both experiments, we used 25 female flies for each treatment.

For the second set of experiments included in this section, we added pigments to the sucrose deposits because adding pigment is how insecticidal bait can potentially be modified. Also, we included 10% paraffin wax emulsion in the deposit because these experiments were conducted as part of a project dedicated to increasing bait efficacy and rainfastness (Teixeira et al. 2009). These modified deposits were almost opaque, differing mostly in reflected light color. Black, blue and pink pigments were extracted from Sharpie™ permanent markers. For yellow, we used TRY-33 pigment. We conducted one trial with apple maggot and another with cherry fruit fly. Flies were exposed to black (blue with cherry fruit fly), pink, white and yellow deposits. In both experiments, we used 30 female flies for each treatment.

**Contrast with background.** In this set of experiments we also used opaque deposits containing sucrose, pigment and wax emulsion. In contrast with all the previous experiments, the sucrose deposits were placed on the inner surface of bottom of the
arena. This change allowed for altering the visual characteristics of the background. The deposits consisted of the same mixture of sucrose, wax, and pigment as in the previous experiments. To determine how female apple maggot and cherry fruit flies responded to colored deposits placed against a white background, the arena was placed on a white surface, and flies were exposed to treatments consisting of a black, white or yellow deposit. In each experiment, we used 47 and 30 female apple maggot and cherry fruit fly, respectively, for each treatment.

The response of female apple maggot and cherry fruit fly to colored deposits placed against a black background was determined by placing the arena on a white background with an ellipse drawn in black. The longer axis of the ellipse was of the same length as the diameter of the dish, and the smaller axis was 2.5 cm long. We did not use a completely black surface because flies had previously shown little tendency to walk on this type of surface. The dishes were placed so that the deposits were located centrally on the ellipse. Flies were exposed to treatments consisting of black, white or yellow deposits. In each experiment, we used 36 and 20 female apple maggot and cherry fruit fly, respectively, for each treatment.

**Data analyses.** For each experiment, the proportions of flies finding a deposit or not were analyzed using chi-square tests. When more than half of the cells had expected counts less than 5, we used Fisher’s Exact Test. These analyses were performed using PROC FREQ of SAS (SAS Institute 2001). Multiple comparisons for proportions were conducted using the macro %COMPPROP of SAS using a Tukey-type multiple comparison test (Zar 1999). We evaluated assumptions of normality and equality of variance for the data on time to find the deposit using PROC UNIVARIATE of SAS. Differences among treatments were analyzed using one-way ANOVA after performing a square-root transformation to achieve homogeneity of variance, using PROC ANOVA of SAS.

**Results**

Fly behavior was characterized by extensive walking along the perimeter of the arena, combined with forays to the center. Periods of walking were usually interspersed with periods of grooming when flies remained immobile. Only a few of the hundreds of flies tested flew in the arena and alighted directly on the sucrose deposit. These flies were also counted as having found the deposit because immediately after alighting on it they touched it with their labellum. Flies found the sucrose deposit after bouts of walking of varying duration that started anywhere from the edge of the arena to just a few millimeters from the deposit. Flies contacted the sucrose by walking straight toward it, or by rotating and turning toward the deposit, if located nearby. There were no significant differences among treatments in the time flies took to find the deposit in any of the experiments. Therefore, we do not mention this result for the experiments below. The overall mean time (± SD) to find the deposits was 135.0 ± 41.3 sec.

**Deposit color.** In the first set of experiments, when apple maggot flies were exposed to translucent sucrose deposits, flies found yellow deposits more often than clear (sucrose only) or black deposits (\( \chi^2 = 10.3; \text{df} = 2; \ P = 0.006 \)) (Table 1). When apple maggot flies were exposed to black, red or yellow deposits, flies also found yellow deposits more often than black or red (Fisher’s Exact Test; \( n = 75; \ P = 0.008 \)).

In the second set of experiments, apple maggot and cherry fruit fly responded differently to opaque pigmented deposits (Table 2). With apple maggot, there were no differences in the number of flies finding black, pink, white or yellow deposits (\( \chi^2 = 1.4; \))
df = 3; \( P = 0.71 \)). With cherry fruit fly, flies found yellow deposits more often than blue, pink or white deposits (\( \chi^2 = 11.7; \ df = 3; \ P = 0.009 \)). Overall, a greater proportion of cherry fruit flies found deposits of any color than apple maggot when comparing the experiments in this set (\( \chi^2 = 44.59; \ df = 1; \ P < 0.001 \)). The proportion of apple maggot flies that found deposits was 0.20 whereas that of cherry fruit fly was 0.57.

### Table 1. Total number of apple maggot flies tested, number of flies finding translucent sucrose deposits of different color (no pigment), and proportion of flies finding the sucrose deposit.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total (n)</th>
<th>Flies finding deposit (n)</th>
<th>Proportion of flies finding deposit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment 1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>25</td>
<td>4</td>
<td>0.16b</td>
</tr>
<tr>
<td>Clear</td>
<td>25</td>
<td>3</td>
<td>0.12b</td>
</tr>
<tr>
<td>Yellow</td>
<td>25</td>
<td>12</td>
<td>0.48a</td>
</tr>
<tr>
<td>Experiment 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>25</td>
<td>1</td>
<td>0.04b</td>
</tr>
<tr>
<td>Red</td>
<td>25</td>
<td>2</td>
<td>0.08b</td>
</tr>
<tr>
<td>Yellow</td>
<td>25</td>
<td>9</td>
<td>0.36a</td>
</tr>
</tbody>
</table>

In each experiment, proportions followed by the same letter are not significantly different (Corrected multiple comparisons test, \( \alpha = 0.05 \)).

### Table 2. Total number of apple maggot and cherry fruit flies tested, number of flies finding opaque pigmented sucrose deposits, and proportion of flies finding the sucrose deposit.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Total (n)</th>
<th>Flies finding deposit (n)</th>
<th>Proportion of flies finding deposit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple maggot</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>47</td>
<td>7</td>
<td>0.15</td>
</tr>
<tr>
<td>Pink</td>
<td>47</td>
<td>10</td>
<td>0.21</td>
</tr>
<tr>
<td>White</td>
<td>47</td>
<td>11</td>
<td>0.23</td>
</tr>
<tr>
<td>Yellow</td>
<td>47</td>
<td>9</td>
<td>0.19</td>
</tr>
<tr>
<td>Cherry fruit fly</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blue</td>
<td>30</td>
<td>15</td>
<td>0.50b</td>
</tr>
<tr>
<td>Pink</td>
<td>30</td>
<td>14</td>
<td>0.47b</td>
</tr>
<tr>
<td>White</td>
<td>30</td>
<td>14</td>
<td>0.47b</td>
</tr>
<tr>
<td>Yellow</td>
<td>30</td>
<td>25</td>
<td>0.83a</td>
</tr>
</tbody>
</table>

In each experiment, proportions followed by the same letter are not significantly different (Corrected multiple comparisons test, \( \alpha = 0.05 \)).
Deposit contrast with background. Against a white background, apple maggot flies exposed to opaque-pigmented deposits found yellow deposits more often than white, but not black deposits ($\chi^2 = 7.8; \text{df} = 2; P = 0.03$) (Fig. 1A). The number of flies that found black, white or yellow sucrose deposits was 12, 8, and 20, respectively. Cherry fruit flies exposed to similar deposits against a white background also found yellow deposits more often than white, but not black deposits ($\chi^2 = 8.2; \text{df} = 2; P = 0.02$) (Fig. 1B). The number of flies that found black, white or yellow sucrose deposits was 11, 10 and 20, respectively. A greater proportion of cherry fruit flies found deposits of any color against a white background than apple maggot, when all flies that found the deposits were pooled ($\chi^2 = 7.12; \text{df} = 1; P = 0.008$). The proportion of apple maggot flies that found deposits was 0.28 whereas that of cherry fruit fly was 0.46.

Against a black background, apple maggot flies found yellow deposits more often than black deposits, but not white deposits ($\chi^2 = 7.4; \text{df} = 2; P = 0.02$) (Fig. 1C). The number of flies that found black, white or yellow sucrose deposits was 1, 5, and 9, respectively. Cherry fruit flies also found yellow deposits more often than black deposits, but not white deposits (Fisher’s Exact Test; $n = 60; P = 0.03$) (Fig. 1D). The number of cherry fruit flies that found black, white or yellow sucrose deposits was 2, 3, and 9, respectively. There was no difference in the proportion of apple or cherry fruit flies that

Fig. 1. Proportion of apple maggot and cherry fruit fly finding sucrose deposits of different color placed on the inner surface of the bottom of the arena against a white background (A and B, respectively) or a black background (C and D, respectively). In each experiment, proportions marked with the same letter are not significantly different (Corrected multiple comparisons test, $\alpha = 0.05$).
found deposits of any color against a black background ($\chi^2 = 2.41$; df = 1; $P = 0.12$). The proportion of apple maggot flies that found deposits was 0.14 whereas that of cherry fruit fly was 0.23. More apple maggot and cherry fruit flies found deposits against a white background than against a black background ($\chi^2 = 7.45$; df = 1; $P = 0.006$ and chi-square = 7.66; df = 1; $P = 0.006$, respectively).

**Discussion**

This study showed that color and contrast cues influence the likelihood that females of apple maggot and cherry fruit fly will find small deposits. Color had a significant effect on the number of flies finding sucrose deposits, as yellow deposits were generally found more often than other colors. Contrast with the background also influenced the number of flies finding sucrose deposits, as the number of flies that found a deposit strongly contrasting with the background was not significantly different from the number of flies that found yellow deposits, whereas weakly contrasting deposits were found less often. Moreover, there were differences between apple maggot and cherry fruit fly in the proportion of flies that found deposits.

The effect of yellow color and contrast cues is well documented in the context of *Rhagoletis* flies finding relatively large objects, such as traps (e.g., Prokopy 1968). This study showed that apple maggot and cherry fruit fly also are sensitive to yellow color and contrast cues from 5 - 7 mm diam circular deposits. The area of a standard 22.8 × 14 cm Pherocon AM yellow sticky trap (Trece, Adair, OK) is ~1600 times larger than the area of a 5 mm diam deposit. Given that the detection of an object at any distance by the insect’s visual system is a function of the object’s dimensions (Prokopy and Owens 1983), flies may only be able to visually detect small, ~0.2 cm$^2$ deposits from a short distance. This is likely why the overall time to find a deposit was 135.0 ± 41.3 sec, despite the flies actively walking inside the 8.5 cm diam arena. However, even if the visual stimuli are only perceived from a small distance, color and contrast cues had a significant effect on how often flies found deposits.

Previous behavioral studies indicated that the response of apple maggot to different colors is based on hue (dominant wavelength of reflected light), brightness (intensity of perceived reflected light), saturation (spectral purity of reflected light) and contrast with the background (Prokopy 1972, Prokopy and Owens 1983). Contrast may, in turn, arise from hue, brightness and saturation differences, or from pattern or motion differences between items and the background, all of which may constitute stimuli to the insect (Prokopy 1986). In this study, differences in brightness were likely the most important factor with respect to detection of white and black deposits against different backgrounds. More apple maggot and cherry fruit flies found deposits on a white background than on a black background, which may have been caused by the flies spending less time on bottom surface of the arena when placed against a black background (L.T., pers. obs.).

Previous studies have shown that the response of flies to color is strongly influenced by the manner in which the color is presented. For example, Prokopy (1972) found a strong preference for yellow using 15 × 20 cm panels versus green, blue, orange, red, white, gray, or black panels, whereas Stanton (1971) found a strong preference for high brightness regardless of hue, using light reflected through a small orifice on the side of a cage. In this study, apple maggot flies always found yellow deposits more often than other colors, except when opaque deposits were placed on cover of the Petri dish. It is possible that a shift in the dominant visual stimulus from color to
contrast with the background caused the flies to find deposits in similar numbers. Nevertheless, with the same deposits, cherry fruit fly found yellow more often than other colors, suggesting that there are differences in the perception of, or response to, visual cues between the species that we assayed. Moreover, cherry fruit flies always found deposits of any color more often than apple maggot. From our related research, this appears to depend on movement behavior and will be reported elsewhere (L.T., unpubl. data). Differences between species may be related to their phylogeny, as apple maggot and cherry fruit fly belong to distinct species groups (McPheron and Han 1997, Smith and Bush 1997).

Cues used to find insecticidal bait deposits are likely to be similar to those used to find food sources such as bird droppings or wounded fruit. Considering that questions remain on the attractiveness of baits (Moreno and Mangan 2002), this study suggests that short-distance interaction between Rhagoletis fruit flies and bait deposits may be improved by modifying bait color characteristics to match the visual preferences of the insects. Future work shall include evaluating whether the addition of yellow pigment increases the attraction and overall efficacy of insecticidal bait for control of apple maggot or cherry fruit fly.

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