Response of *Melittia oedipus* (Lepidoptera: Sesiidae) to Visual Cues Is Increased by the Presence of Food Source

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ABSTRACT Visual and olfactory cues were shown to mediate short-distance orientation to different colors in the presence and in the absence of food in *Melittia oedipus* Oberthür (Lepidoptera: Sesiidae), a biological control agent of *Coccinia grandis* (L.) Voigt (Violales: Cucurbitaceae). Yellow was the color most preferred by *M. oedipus*, and adults landed significantly more on yellow paper moistened with honey–water. The next preferred colors were gray and white with the identical food source. Colors such as red, blue, green, brown, and black were least preferred by *M. oedipus* and attracted the adults on par with each other. The *M. oedipus* landings on petri dishes which held yellow-, gray-, and white-colored papers were significantly higher than the colorless petri dishes with olfactory stimuli only. There was no significant difference in landings on different-colored papers moistened with honey–water or with water alone in the morning compared with those in the evening. The cumulative response of *M. oedipus* to different-colored papers moistened with honey–water was significantly higher than the colored papers moistened with water only. Correspondingly, the response of *M. oedipus* to yellow-colored paper moistened with honey–water was significantly higher than the yellow-colored paper moistened with water only. Therefore, yellow paper moistened with honey–water can increase the feeding rate of *M. oedipus* and can be a potential technique in developing mass cultures for field release to control the invasive weed.

KEY WORDS *Melittia oedipus*, *Coccinia grandis*, adult orientation, visual cues, olfactory cues

Ivy gourd, *Coccinia grandis* (L.) Voigt (Violales: Cucurbitaceae), is a perennial vine and a native to Africa, but it also occurs wild in the Indo-Malayan region (Singh 1990). It is naturalized in parts of Australia, the Caribbean, the southern United States, and several Pacific Islands, and it has invaded lowland areas of the Hawaiian Islands (Linney 1986, Telford 1990, Chun 2001). This vine was introduced to Guam and Saipan in the 1980s, and invasion took place in recent years (Bamba 2008). It has occupied >80 ha in different parts of Guam, and almost one third of the land area of Saipan (Raman et al. 2006). *Coccinia grandis* is also a host for most of the pests of cucurbitaceous crops such as *Diaphania indica* (Saunders) (Lepidoptera: Pyralidae); *Aulacophora foveicollis* Lucas (Coleoptera: Chrysomelidae); melon fly [*Bactrocera cucurbitae* Coquillett (Diptera: Tephritidae)]; cotton aphid [*Aphis gossypii* Glover (Hemiptera: Aphididae)]; leafminers [*Liriomyza* spp. Mik (Diptera: Agromyzidae)]; *Leptoglossus australis* (F.) (Hemiptera: Coreidae); whiteflies [*Bemisia* spp. (Gennadius) (Hemiptera: Aleyrodidae)]; and others (USDA 2003, 2004).

A biological control program has been initiated in Guam and Saipan following the success achieved in Hawaii by introducing the natural enemies, *Acythopeus cocciniae* O'Brien & Pakaluk (Coleoptera: Curculionidae), *Acythopeus burkhartorum* O'Brien & Pakaluk (Coleoptera: Curculionidae), and *Melittia oedipus* Oberthür (Lepidoptera: Sesiidae) (Muniappan et al. 2009). *A. cocciniae* causes defoliation of *C. grandis* by the larval mining of the leaves, *A. burkhartorum* larvae cause stem and petiole galling, whereas caterpillars of *M. oedipus* begin feeding in young shoots, boring down through the larger stems and into the root, eventually killing the vine (Chun 2001). According to host specificity tests, *M. oedipus* is specific to *C. grandis* (Stone 1979, Chun 2001, USDA 2007).

In the laboratory, *M. oedipus* adults emerged from pupal cases that are inside the *C. grandis* vines and females began emitting pheromones to attract males as soon as their wings were dried (Chun 2001). Mating usually took place on the day of emergence, in late morning or early afternoon on sunny days and lasted up to 2 h. Each female can lay 60–140 eggs. Eggs were laid individually on all parts of the *C. grandis* plant (G.V.P.R., unpublished data). Larvae bored into *C. grandis* stems either through young growing tips or directly into the thick stems of older vines. Pupation took place within the cut pieces of stem provided for larval development (Muniappan et al. 2009). Adults from the laboratory colonies emerged over a period of 2 to 4 mo after oviposition, with males greatly outnumbering females during the first few days of emergence. Adult life span averaged 6 d (Chun 2001, USDA 2007).

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It was observed and realized from the preliminary experiments that the adults were seen to have an orientation preference to certain colors moistened with honey–water (G.V.P.R., personal observation). Insects are known to move to a variety of sensory cues, such as odors (Vaughn et al. 1996, Reddy and Guerrero 2004), visual cues (Prokopy and Owens 1983, Reddy et al. 2004), or both (Raguso and Willis 2002, Blackmer and Cañas 2005). This has prompted us to carry out the present studies on 1) the adult's orientation/attraction to the different colors, 2) assessing the interaction between visual and olfactory cues, 3) the difference in response during different periods of the day, and 4) the effect of honey–water and water alone in attracting *M. oedipus* under free-choice and no-choice tests.

Materials and Methods

Insect Rearing. Approximately 200 *C. grandis* cuttings (≈20 cm in length by 4–5 cm in diameter) with immature stages of *M. oedipus* were brought from Kona (Hawaii) and reared in the Western Pacific Biocontrol Quarantine Laboratory (WPBQL) at the University of Guam. The controlled environmental conditions in the quarantine were maintained at a photoperiod of 12:12 (L:D) h, mean day temperature 32°C, mean night temperature 17°C, and relative humidity 65–80%. Twenty cuttings were transferred to each plastic container (45 cm in length by 30 cm in width by 15 cm in depth). Naturally grown *C. grandis* vines were cut from the field and brought to the laboratory. Vines were cut into pieces of 15 cm in length, and approximately 8 to 10 were added to the old vines with larvae/pupae. Once the vines rotted, the cuttings were gently peeled apart and pupae were taken out. Twenty to 30 pupae were placed in plastic boxes (14 cm in length by 9.5 cm in width) for adult emergence. For aeration, each plastic box had an opening (6 cm in length by 3 cm in width) on the lid, which was covered with muslin cloth and sealed with glue. Newly emerged adults (<1 d) were used for the experiments. Although adult male and female *M. oedipus* are similar in overall appearance, males have a small narrow abdomen, whereas females have a larger abdomen and a pointed ovipositor.

Color Measurements. Color characteristics were measured using a Konica Minolta CR-410 Chromameter (Minolta Instrument Systems, Ramsey, NJ). The measurements were obtained from the average of three readings for each color. Hue angle and chroma are calculated as arc tan (b^*/a^*) and $(a^{*2} + b^{*2})^{1/2}$, respectively, where a^* and b^* are the color coordinates. Chroma provides a measure of color intensity, and hue angle indicates the sample color (Wrolstad et al. 2005). Hue angle is expressed on a 360° grid where 0° is red, 90° is yellow, 180° is green, and 270° is blue.

Response of *M. oedipus* to Different Colors and Food. The effects of color alone or food alone, and thus the extent to which olfactory stimuli may interact with visual cues are quantified in this experiment. The response of *M. oedipus* to different colors of brown, black, gray, yellow, red, white, green and blue (Tree House Pad and Paper Inc., LLC, Corona, CA) or colorless petri dishes (no paper) (Falcon, Oxnard, CA) were conducted in insect-proof cages (65 by 60 by 60 cm) during the day in the quarantine room with the environmental conditions described above. To examine the diurnal response of *M. oedipus,* the experiments were run in the morning (1000–1100 hours) and late afternoon (evening) (1600–1700 hours). A potted *C. grandis* plant (≈20 cm height) was placed inside the insect-proof cage.

The attraction of the moths to colored papers/colorless petri dish moistened with honey–water or water only were carried out on free-choice and no-choice tests. The papers of different colors chosen in this experiment were cut into circles to fit into each petri dish (9 cm in diameter). For the free choice test, these eight colored pieces of paper, moistened with 5-ml solution of honey–water (50:50 vol:vol) were placed in eight petri dishes and introduced inside the experimental arena, along with one additional colorless petri dish containing honey–water only (no paper). This was to compare the landings on petri dishes which held colored papers and olfactory stimuli with the ones on control (colorless petri dishes with olfactory stimuli only). Simultaneously, an additional eight petri dishes with the different-colored papers moistened with tap water were similarly introduced along with one colorless petri dish with tap water only (no paper) to serve as control. Overall, there were 18 petri dishes, placed randomly onto the cage's floor. The inter petri dish distance was 5 cm. Four males and four female moths were released 30 min before start of the experiment. No petri dishes were present in the arena at that time of release. In no-choice tests, the response of *M. oedipus* was tested using only a single color or colorless treatment in each day; unlike the free-choice test, only two dishes were used, one with honey–water and one with water alone included simultaneously. All treatments were replicated four times. The petri dish positions were rerandomized each test day. The insects, pieces of paper, and food sources used for the tests conducted in the morning were different from those used in the afternoon. Individual insects were used for only one test. Data collected included the number of adults landing on each petri dish for 1 h during the morning and in the afternoon. The artificial light intensity was measured at the surface of the test arena during the experiments, in particular for the tests involving morning versus evening responses using a Lux-Meter (Testo GmbH & Co., Lenzkirch, Germany).

Statistical Analysis. The number of adults responding in each test was converted into percentages. The number of landings evaluated was based on multiple landings made by eight moths (four males; four females). Different treatments were tested in multichoice way for preference, thus Generalized linear mixed model with binomial distribution and logit function was used to test the effects of four factors (color, treatment, choice-no-choice, and time) and their two-way interactions. Because none of the two-way interactions were statistically significant (Table 2), a re-

Table 1. Color measurements of the test papers used in the present study

Color	L*	a*	b*	Chroma	Hue angle (h°)
Yellow	96.0 ± 0.3	−8.8 ± 0.1	24.5 ± 0.4	26.1 ± 0.4	109.8 ± 0.1
Gray	56.6 ± 0.0	−1.7 ± 0.0	−23.6 ± 0.0	23.7 ± 0.0	86.0 ± 0.1
Blue	48.7 ± 0.0	16.4 ± 0.1	−36.6 ± 0.0	40.1 ± 0.1	294.1 ± 0.1
Brown	62.3 ± 0.4	5.6 ± 0.3	19.2 ± 0.3	20.0 ± 0.3	73.7 ± 0.7
Red	48.7 ± 0.1	53.9 ± 0.1	23.1 ± 0.1	58.6 ± 0.1	23.3 ± 0.1
Black	33.8 ± 0.1	7.6 ± 0.2	−5.4 ± 0.1	9.3 ± 0.1	324.6 ± 0.9
White	94.4 ± 0.0	−0.8 ± 0.0	4.9 ± 0.0	4.9 ± 0.0	99.8 ± 0.0
Green	55.8 ± 0.3	−35.2 ± 1.4	6.5 ± 2.3	35.8 ± 1.8	169.7 ± 3.4

Means were generated from three observations.

duced model with only main effects was fitted again (Table 3). Least significant difference test was used to check the significance differences between multiple means. All statistical procedures were conducted using The GLIMMIX Procedure SAS version 9.13 (SAS Institute 2004).

Results

Trap color measurement values (L*, a*, b*, chroma, and hue angle) are given in Table 1. No differences were observed in the responses of males and females to the stimuli offered; therefore, we combined the data from males and females that landed on a particular petri dish over a 1-h period.

Effect of Color and Food on the Attraction of *M. oedipus*. Adult attraction to different-colored papers was affected by the color in the petri dish (Figs. 1 and 2). In a free-choice test, yellow was the color most preferred by *M. oedipus* and was significantly higher ($P < 0.001$; least significant difference test) than the next most preferred colors gray and white. Colors such as red, blue, green, brown, and black attracted the adults on par with each other and statistically were found to be nonsignificant ($P > 0.05$; least significant difference test) compared with control. In a no-choice test (Fig. 2), the adults were significantly ($P < 0.001$; least significant difference test) more attracted to yellow than the succeeding preferred gray. All other test colors were to be significantly on par with white.

In the free-choice tests, the *M. oedipus* landings on petri dishes that held yellow-, gray-, and white-colored papers were significantly higher ($P < 0.001$; least significant difference test; Fig. 1) than the colorless petri dishes (colorless petri dishes with olfactory stimuli only). However, in the no-choice tests, the landings on petri dishes which held only yellow- and gray-colored papers were significantly higher ($P < 0.001$; least significant difference test; Fig. 2) than the colorless petri dishes.

Difference in Diurnal Response of *M. oedipus* to Different Test Colors. Overall, in both the free-choice and no-choice tests, there were no significant differences in landings on different-colored papers moistened with honey–water or with water alone in the morning compared with those in the evening ($P > 0.05$; least significant difference test; Tables 2 and 3). The average light intensity prevailing during the study was 1,550 lux in the morning and 1,552 lux in the evening.

Differentiation in Response of *M. oedipus* to Food Source. In the free-choice and no-choice tests, the cumulative response of *M. oedipus* to different-colored papers moistened with honey–water were significantly higher ($P < 0.05$; least significant difference test; Fig. 3) than the colored papers moistened with tap water only.

Discussion

Numerous studies have been conducted on responses of herbivorous insects to resource-associated visual and chemical stimuli. Most studies on color preference published so far with species of Sesiidae have involved use of pheromone traps in the context of mating disruption of species that are pests of crops such as *Melittia cucurbitae* Harris (Lepidoptera: Sesiidae) (Klun et al. 1990, Jackson et al. 2005) and *Synanthedon tipuliformis* Clerck (Lepidoptera: Sesiidae) (Karalius and Bûda 1990, Bûda and Karalius 1993, Suckling et al. 2005). The current study is

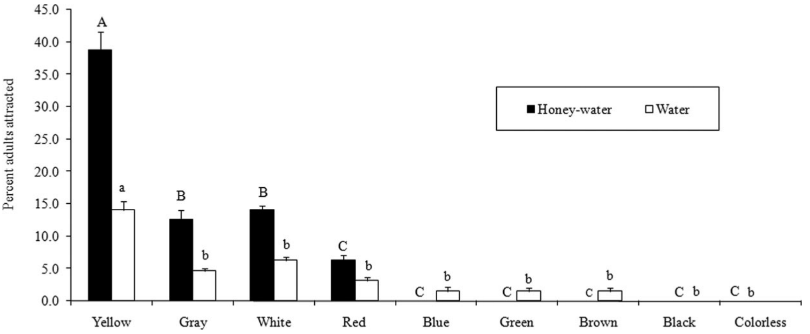

Fig. 1. Mean percentage ± SE of *M. oedipus* attracted to different-colored papers encumbered in a petri dish or colorless petri dish damped with honey–water or water alone in free-choice test. Means in each column followed by different letters are significantly different among different colors according to least square means. Means were generated from four tests each using eight insects (four males and four females). Colorless, petri dish without a paper.

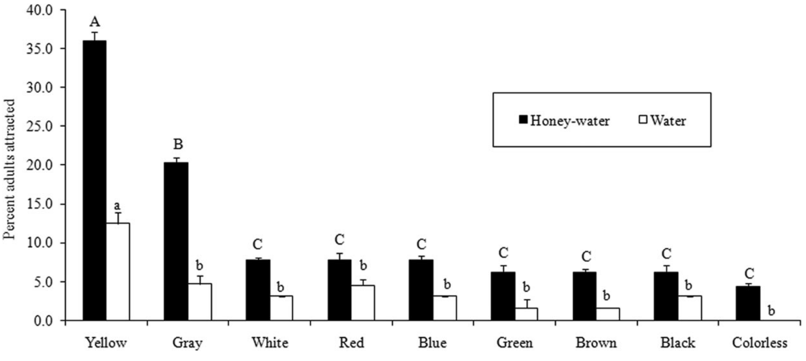

Fig. 2. Mean percentage ± SE of *M. oedipus* attracted to different-colored papers encumbered in a petri dish or colorless petri dish damped with honey–water or water alone in no-choice test. Means in each column followed by different letters are significantly different among different colors according to least significant difference test. Means were generated from four tests each using eight insects (four males and four females). Colorless, petri dish without a paper.

apparently the first attempt to investigate responses of adults of *M. oedipus*, a candidate biological control agent of ivy gourd (*C. grandis*), to color and food with the aim of "enhancing the mass rearing technique, enhanced biocontrol and monitoring of this species."

In the majority of herbivorous insects, visual cues, olfactory cues, or both play a key role in finding a host location. This has actually been used, with certain accomplishment, in insect pest control programs. Nectar-feeding insects generally use vision and olfaction to find rewarding flowers and different species may give different weight to the two sensory modalities (Balkenius et al. 2006). The authors reported that the nocturnal *Deilephila elpenor* L. (Lepidoptera: Sphingidae) responded preferably to the odor, whereas the diurnal *Macroglossum stellatarum* L. (Lepidoptera: Sphingidae) strongly favored the visual stimulus. We hypothesize that during bright daylight visual cues may have become more important than odor.

In the current study, the response of *M. oedipus* to the yellow-colored paper moistened with honey–water was most attractive. However, it is not known why *M. oedipus* is attracted to the color yellow. Similar results were obtained in the case of the currant moth, *S. tipuliformis,* which is highly attracted to patches of yellow (Suckling et al. 2005). Similarly, the squash vine borer, *M. cucurbitae,* also has been reported to be attracted to yellow (traps) (Jackson et al. 2005). Bûda and Karalius (1993) found the effectiveness of pheromone stimuli was modified by the color of the traps. They reported that among pheromone traps with eight different colors, yellow traps seemed to be most effective in trapping the *S. tipuliformis,* immediately followed by green. However, dark colors such as black and brown were known to have inhibitory effect on the attractivity. It is well known that certain insect species, including lepidopterans, have orientation preference to some specific colors (Prokopy and Owens 1983). To quote such preferences on other species, *Spodoptera frugiperda* (J.E. Smith) (Lepidoptera: Noctuidae) was highly attracted to green-yellow-white (Meagher 2001), *Theresimima ampellophaga* Bayle-Barelle (Lepidoptera: Procridinae) to yellow (Subchev et al. 2004), and *Euproctis pseudoconspersa* (Strand) (Lepidoptera: Lymantriidae) to white (Wang et al. 2005), just to name a few. However, there is one contradictory report in the case of *Plutella xylostella* (L.) (Lepidoptera: Plutellidae). Although traps with yellow color are widely recommended for monitoring the population of *P. xylostella* (Sivapragasam and Saito 1986, Walsh et al. 2001), Calyecac-Cortero et al. (2002) reported that the color of the trap did not have any influence on the capture of the moths.

Table 2. Statistical parameters of the fixed effects

Class	Num df	Den df	*F* value	*P* value
Color (black, blue, brown, gray, green, red, white, and yellow)	7	29	2.6	0.0337
Treatment (honey and water)	1	29	1.5	0.2342
Color × treatment	7	29	0.3	0.9446
Choice (free-choice and no-choice)	1	29	0.0	0.9687
Color × choice	7	29	0.4	0.8767
Treatment × choice	1	29	0.0	0.8949
Time (morning and evening)	1	29	1.0	0.3094
Color × time	7	29	0.1	0.9985
Choice × time	1	29	0.65	0.4281
Treatment × time	1	29	0.2	0.6772

Num, numerator; Den, denominator.

Table 3. Results of the reduced model with only main effects fitted

Effect	Num df	Den df	*F* value	*P* value
Color (black, blue, brown, gray, green, red, white, and yellow)	7	53	4.1	0.0011
Treatment (honey and water)	1	53	5.6	0.0213
Choice (free-choice and no-choice)	1	53	1.6	0.2161
Time (morning and evening)	1	53	1.5	0.2161

Num, numerator; Den, denominator.

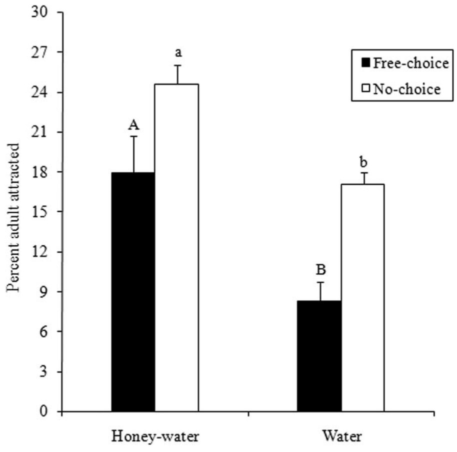

Fig. 3. Mean percentage ± SE of *M. oedipus* landings to different-colored papers held in a petri dish damped with honey–water and water. Means in each column followed by different letters are significantly different among two different food sources according to least significant difference test. Means were generated from four tests each using eight insects (four males and four females).

Our results indicated that the response of *M. oedipus* adults to different colors did not have any difference during the morning or evening in both free-choice and no-choice tests. Although the response varies depending upon the species, the peak activity of *Phyllonorycter junoniella* Zeller (Lepidoptera: Gracillariidae) was observed in the morning (Mozûraitis and Bûda, 2006). In the same way, Marchiori and Romanowski (2006) reported that although there were variations in the daily times of *Eunica eburnea* Fruhstorfer (Lepidoptera: Nymphalidae) activity throughout the seasons, there was a general trend for higher activity during the morning, between 0900 and 1145 hours. To our knowledge, there have been no reports on the difference in diurnal response of *M. oedipus* to different colors. Therefore, it is the first publication documented where there is no difference in diurnal response of a moth to different colors. However, it was observed from the current study that *M. oedipus* moths mate more during the second half of the day.

In addition, our results indicated that, the adult *M. oedipus* response to the colored papers moistened with honey–water was higher than the water alone. Our results corroborate with Shotter (1993) who reported honey–water as an attractant for Lepidoptera. The author had collected 26 species belonging to eight families, including several species of economic importance in traps baited with honey–water in a suburban garden in the United Kingdom. Similarly, Ni and Holbrook (2006) found that the honey solution and pheromone trap in container caught the greatest number of *Ephestia* (*Cadra*) *cautella* (Walker) (Lepidoptera: Pyralidae). They also demonstrated that *E. cautella* adults preferred a 10% honey solution over water, 10% beer, and 10% sucrose solutions as attractants. However, additional studies on the attraction of *M. oedipus* to the amino acid based nectar/honey would be useful and can contribute to pollinator attraction and/or feeding. Alm (1990) reported the importance of amino acids in attracting *Pieris rapae* (L.) (Lepidoptera: Pieridae) and bees to flowers.

First, the hypothesis that yellow-colored paper moistened with honey–water captures larger numbers of *M. oedipus* adults than other colored papers was supported by the results. Second, an "increase" in adult response to food source (olfactory stimuli) in the presence of visual stimuli was remarkable. This highlights the importance of visual and olfactory cues in finding a food source. The accessibility of food-based attractants will give a new breadth in exploring the interactions among visual cues and chemical cues for developing the monitoring tools. However, further studies are required to observe the response of *M. oedipus* to the many different shades of yellow, as suggested by Suckling et al. (2005) for *S. tipuliformis.* This will enhance the attraction toward food source and may help in developing mass rearing techniques for the field release to control invasive weeds. It has been reported in some cases pheromone traps are not reliable and effective for monitoring the sesiid borers (Capinera 2001, Jackson et al. 2005). The current study suggests that yellow-based visual stimuli (possibly trap color or dispenser) could provide an improvement in monitoring and controlling these borers. The prospects of developing attractants in traps as an aid for monitoring populations of weed biological control agents are tremendous.

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