Alightment of *Edovum puttleri* Grissell (Hymenoptera: Eulophidae) in Response to Colors Mimicking Three Hosts of the Colorado Potato Beetle (Coleoptera: Chrysomelidae)[1]

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J. Entomol. Sci. 32(4): 386-397 (October 1997)

ABSTRACT The effect of leaf color on alighting behavior of *Edovum puttleri* Grissell was examined in a laboratory sticky-board test. Paint models mimicking the leaf top colors of eggplant, potato, and tomato and a model mimicking average underside leaf color were tested in paired comparisons. The underside leaf color mimic was the most preferred; no significant differences in landing rates occurred among any of the three leaf top color mimics. The underside leaf color mimic also was tested against two gray shades sharing the same intensity of reflectance; no significant differences were detected. The parasitoid, therefore, preferentially alighted based on the intensity and not the hue of the models. For all assays, a significant departure from randomness was found because a higher percentage of females alighted on the paint models than that which was in the colony. This suggests that parasitoids were alighting in a search for host eggs.

Key Words *Edovum puttleri,* parasitoid, color, *Leptinotarsa decemlineata*, host searching

Color preferences of herbivorous insects have been extensively studied (Prokopy et al. 1975, Greany et al. 1978, Landolt et al. 1988, Robacker et al. 1990, Collier and Finch 1990). Several characteristics of the substrate can influence visual orientation and alighting, including predominant hue, intensity of light reflected, shape, size, and background (Moericke et al. 1975).

When selecting colors for use in creating a plant mimic, the color most representative of the host plant part being exploited by a herbivore is often most attractive. For example, the apple maggot fly, *Rhagoletis pomonella* (Walsh), is attracted to large yellow rectangles as a response to foliage (food) and to red spheres as a response to fruit (oviposition sites) (Prokopy 1968). Likewise, the

[1] Received 12 June 1996; Accepted for publication 19 April 1997.

walnut husk fly, *R. completa* Cresson, in addition to preferring yellow rectangles, shows a preference for green spheres having a reflectance pattern similar to the walnuts it utilizes for mating and oviposition sites (Riedl and Hislop 1985). Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), females ready to oviposit are attracted to red and black spheres mimicking the colors of ripening fruit (Cytrynowicz et al. 1982). Those insects orienting more often to yellow hues are thought to do so because of the "supernormal" stimulus of foliage produced by yellow, which reflects light in the same region of the spectrum as green, but to a greater extent (Prokopy 1972). Therefore, both hue and intensity of reflected light can influence attractiveness of a substrate.

Similar color preferences have been observed for entomophagous insects that orient to the food-plants of their hosts. Moore (1969) observed that parasitic tachinid flies were attracted to the same colors as their host, the apple maggot fly. Tachinid parasitoids such as *Parasetigena silvestris* (Robineau-Desvoidy), *Patelloa silvatica* (Aldrich & Webber), and *Gonia sagax* Townsend also responded more to brighter colors such as yellow and white than to duller colors, suggesting a response to foliage, which reflects most light in the same region as the attractive colors (Weseloh 1981). Yellow traps were also more attractive to citrus blackfly, *Aleurocanthus woglumi* Ashby, parasitoids *Amitus hesperidum* Silvestri and *Prospaltella opulenta* Silvestri (Dowell and Cherry 1981), and to the California red scale, *Aonidiella aurantii* (Maskell), parasitoid *Aphytis melinus* DeBach, whose host is often found on mature foliage (Moreno et al. 1984). On the other hand, bark inhabiting San Jose scale, *Quadraspidiotus perniciosus* (Comstock), is prey to several species of parasitoids that respond to black more often than to yellow or white (McClain et al. 1990).

Reflectance in the green region is also attractive to certain parasitoids. *Campoletis perdistinctus* (Viereck) responded most to wavelengths in the green region (Hollingsworth et al. 1970), while *C. sonorensis* (Cameron) oviposited more often in its host *Heliothis zea* (Boddie) when on yellow substrates and in the presence of a green light source (Schmidt et al. 1978). Arthur (1966) showed that the ichneumonid parasitoid *Itoplectis conquisitor* (Say) could be conditioned to associate colors of the oviposition site with the presence of its host and, thereby, improve its searching ability.

Edovum puttleri Grissell (Hymenoptera: Eulophidae), an egg parasitoid of the Colorado potato beetle *Leptinotarsa decemlineata* (Say), was collected in Colombia and Mexico for use in integrated control programs (Grissell 1981, Logan et al. 1987). While proving effective for control of the beetle in eggplant, where parasitism levels of 71 to 91% have been reported (Lashomb et al. 1987), its control in potato remains insufficient, with average parasitism rates of 50 to 56% (Schroder and Athanas 1989a, b).

While studying host egg kairomones for *E. puttleri,* Leonard et al. (1987) observed that females were strongly attracted to white surfaces. However, no formal study of color preference by the parasitoid has been undertaken. To begin understanding why *E. puttleri's* ability to find its host's egg masses differs between the two plant species, this study has examined the effect of leaf color on *E. puttleri's* alighting behavior. We report on the parasitoid's response to leaf color of three Colorado potato beetle hosts.

Materials and Methods

Edovum puttleri reared in 4L glass jars with access to distilled water and honey were maintained at 25 ± 1 C and 50 to 60% RH, under 16 hr L:D. Additional wasps were received as adults from the New Jersey Department of Agriculture Beneficial Insects Rearing Laboratory, Trenton, NJ, and kept under the same conditions as parasitoids reared in the laboratory. All *E. puttleri* originated from a colony of the Colombian biotype (Puttler and Long 1983).

Egg masses were obtained from a colony of Colorado potato beetle reared on potato (*Solanum tuberosum* L. cv. "Superior"). The wasps were maintained on 1 to 2-day-old Colorado potato beetle egg masses (Lashcomb et al. 1987). One day before being used in tests, *E. puttleri* were kept from host eggs to prevent depletion of eggs by females. Corrigan et al. (1991) reported that female *E. puttleri* can develop a full clutch of eggs in 2 days and maintain them for 5 days in the absence of host eggs. All *E. puttleri* tested were between 7 and 15 days old, within the age of greatest parasitism (Lashomb et al. 1987).

Eggplant (*S. melongena* L. cv. "Harris Special"), potato (*S. tuberosum* cv. "Superior") and tomato (*Lycopersicon esculentum* L. cv. "Better Boy"), grown under greenhouse conditions, were the basis of leaf color analyses.

Painted models were made to mimic the colors of the upper leaf surfaces of eggplant, potato and tomato plants. The lower leaf surfaces of these plants, which were too similar in color to blend paints for separate models were represented by one color. Paints corresponding to each of the 4 leaf colors and 2 ratios of gray were applied to 20.5 × 20.5 cm cardboard squares, over which a layer of Stikem Special was applied to trap the parasitoids. All paints used were Benjamin Moore (Montvale, NJ), mixed in the following ratios: eggplant leaf mimic paint - 872 ml #5 base, 29.56 ml yellow oxide, 9.24 ml lamp black, 40.65 ml yellow, 59.12 ml tinting green, 9.24 ml red oxide, 12.93 ml white; potato leaf mimic paint - 857 ml #4 base, 44.34 ml yellow oxide, 8.31 ml lamp black, 11.09 ml red oxide, 22.17 ml yellow, 64.67 ml tinting green; tomato leaf mimic paint - 857 ml #4 base, 44.34 ml yellow oxide, 1.85 ml lamp black, 7.39 ml red oxide, 36.95 ml yellow, 44.34 ml tinting green, 11.06 ml blue; underside leaf mimic paint - 872 ml #5 base, 44.34 ml yellow, 5.54 ml blue bright, 8.31 ml orange, 11.09 ml white, 3.70 ml tinting green; gray paints - ratios of Benjamin Moore Decorators White No. 215 01 and Ox Line Black Ruberol Flat Interior No. 617 33.

To measure the accuracy of paint models in mimicking the leaf colors, reflectance spectra of both leaves and paints were obtained, using a Cary 2390 spectrophotometer with a reflectance attachment. Three sets of measurements were taken of each paint and leaf type on two different days, and percent reflectance was calculated for wavelengths between 300 and 650 nm. Additionally, a series of gray paints was mixed (5% to 50% black) and their spectra similarly measured to test if attraction to paint models was due to the hues tested or the intensity of reflectance alone.

All assays were conducted in a walk-in evironmental chamber maintained at 25 ± 1 C and 50 to 60% RH. A 65 × 83 × 87 cm cage was lined with sheer white interfacing fabric and surrounded by fluorescent lights. Six paint models as described above were suspended vertically from wooden dowels running horizontally along the middle of each side of the cage. Each position was approximately equidistant from the next.

Five hundred *E. puttleri* were released into the cage containing the six painted models. Water and a solution of 33% honey released from a dental wick were provided on the floor of the cage. After 24 h, the paint models were removed, and the number and sex of parasitoids trapped on each square were recorded. Following this, all untrapped parasitoids were removed from the cage using an aspirator. To determine if the models were more attractive to females than males, the sex ratio of a sample of 50 *E. puttleri* from the colony jar also was taken at each test and compared to the ratio caught on the squares.

Paired comparisons were made for all color combinations, with three squares of each color being used each time the test was repeated. To minimize position effects for each replicate the six squares were randomly assigned initial positions, and the test was repeated six times, rotating the squares one position each time. Five replicates of the six rotations were completed for each paired comparison.

Additionally, two gray paints were tested against the most attractive leaf color to determine if that color was attractive due to its hue or its intensity of reflectance. A gray consisting of 50% black was chosen as having the closest overall percent reflectance to that of the leaf color, as measured by the total area under the reflectance spectra in the wavelength region measured. A gray consisting of 35% black was chosen as having the closest percent reflectance in the green part of the spectrum (490-560 nm). Differences between landing rates among colors and grays were analyzed by Student *t* test (SAS Institute 1985).

Results and Discussion

Reflectance spectra recorded for all leaves and paints between 300 and 650 nm, the range of light visible to insects (Goldsmith and Bernard 1974), are presented in Fig. 1-3. The spectral analysis of eggplant, potato and tomato leaves revealed peak reflectance between 540 and 560 nm, the usual maximum reflectance of green leaves and the most intensely green part of the spectrum (Shull 1929). Reflectance of green leaves typically ranges from 5 to 25% (Pearman 1966, Shull 1929). The maximum reflectance reported here was approximately 4 to 5%. The underside of each leaf type is lighter in color and consequently had a higher percent reflectance than corresponding leaf tops over most of the region measured. Leaf mimic paints all showed slightly higher reflectance than their corresponding leaf types. Because models were closer in overall reflectance to their corresponding leaf types than they were to each other in the region of higher leaf reflectance, they were determined suitable mimics of leaf types.

There were no significant differences in alighting response by the parasitoid among the three leaf top models for eggplant, potato and tomato (Table 1). Because the three leaf colors mimicked by the models are very similar in maximal reflectance, this result supports Moericke's (1969) conclusion that insects are not able to distinguish between green leaf colors with comparable

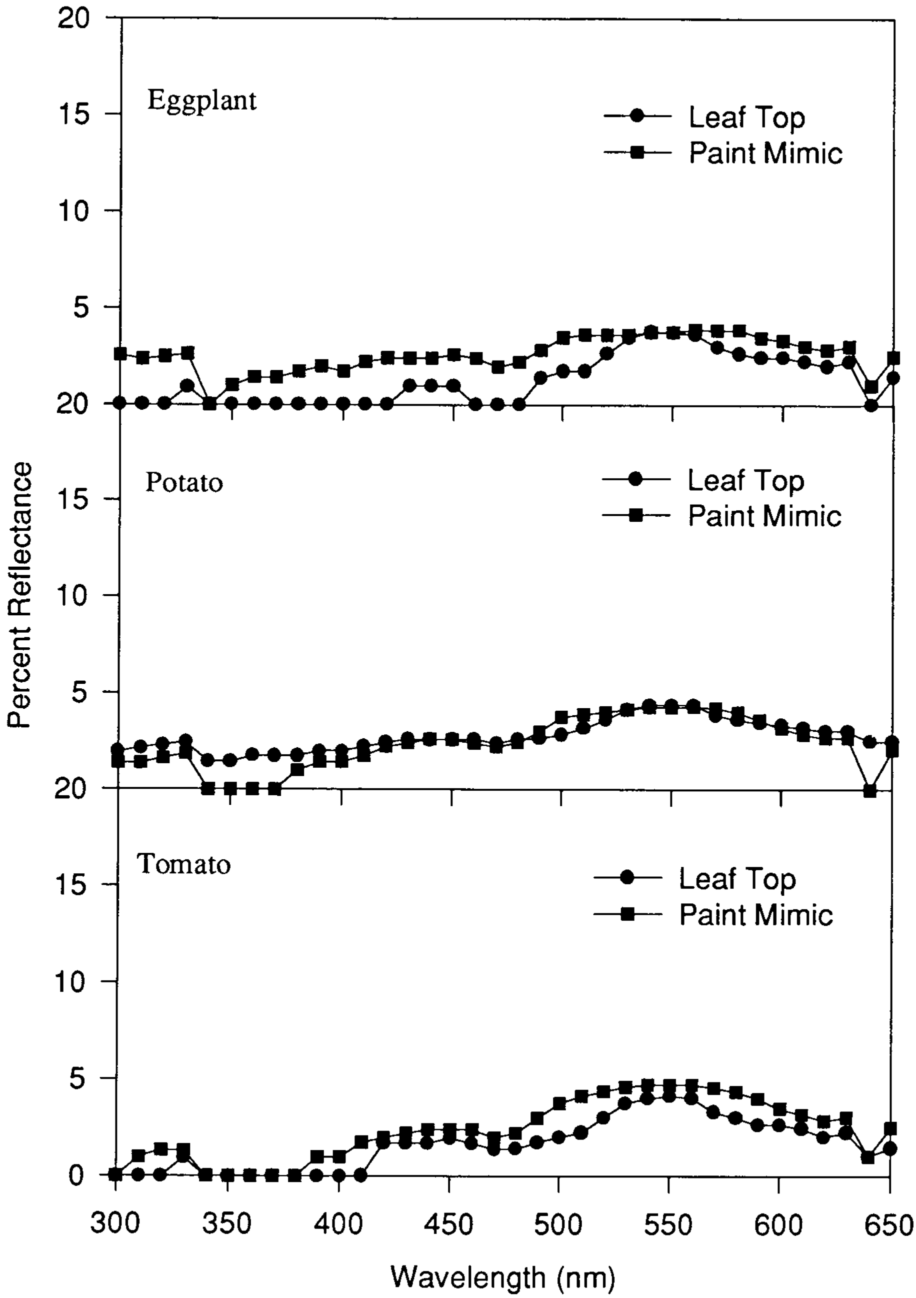

Fig. 1. Reflectance spectra of leaf surfaces of eggplant, potato, and tomato and their respective paint minics between 300-650 nm.

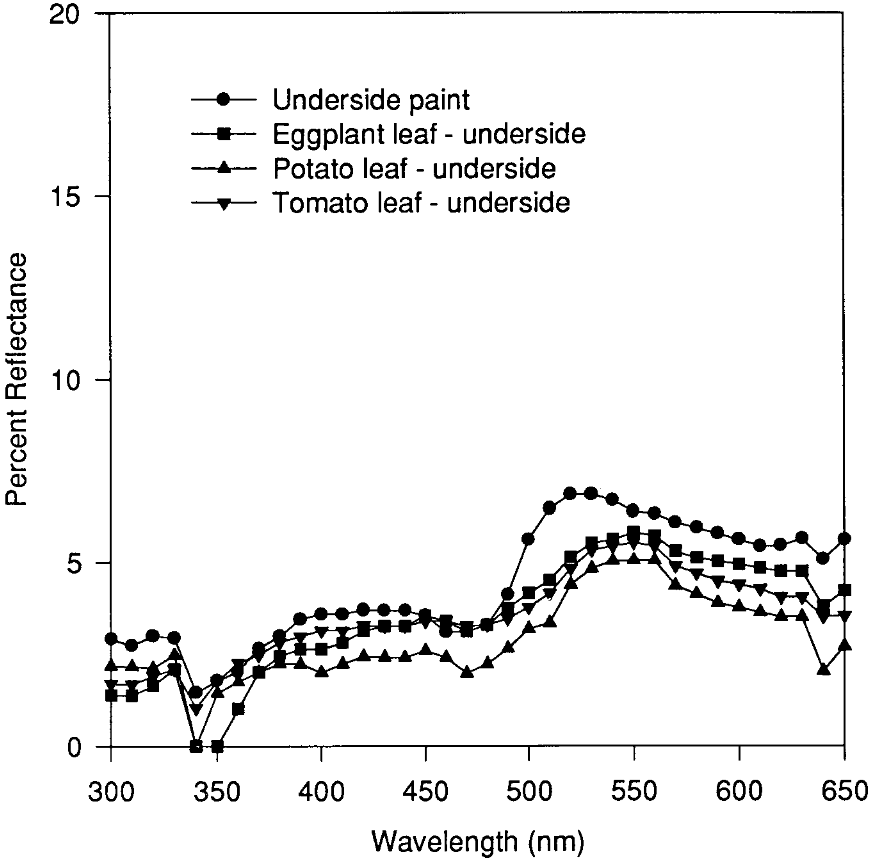

Fig. 2. Reflectance spectra of leaf undersides (eggplant, potato, and tomato) and underside paint mimic between 300-650 nm.

hues except in cases where leaf's color intensity or tint differs. There was a significant difference ($P \leq 0.01$), however, in response to the underside color as compared to each of the three leaf top colors. *Edovum puttleri* preferred the underside leaf mimic. Typically, female Colorado potato beetles deposit egg masses on the lower leaf surfaces. Based on this behavior, a preference by *E. puttleri* females for underside leaf colors would allow females to limit their search to areas most likely to contain egg masses. There were no significant interactions between color and replicate in any of the test combinations.

Once the underside color was determined to be the most preferred, it was tested against two grays (35% black and 50% black) most similar to it in their

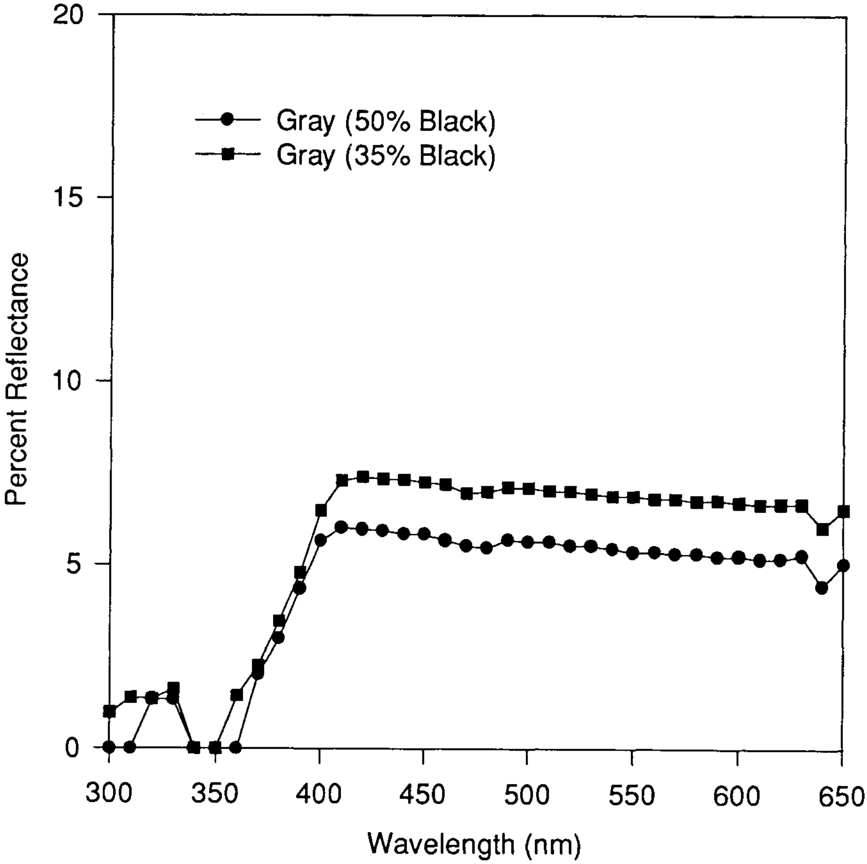

Fig. 3. Reflectance spectra of gray paints (35% black and 50% black) between 300-650 nm.

reflectance patterns. No significant differences in response by the parasitoid were revealed (Table 2), indicating that the more important factor in the attraction of the underside color is not its hue, but rather its intensity of reflectance.

Mean percent of females trapped on the models in all test combinations was significantly greater than mean percent of females in the colony from which parasitoids were taken at the time of each test ($P \leq 0.01$) (Table 3). This predominately female response suggests that the individuals landing on the models were involved in a search process and landed on what they perceived as their host's food-plant. However, there was no significant difference between

Table 1. Number of *E. puttleri* alighting on colored paint mimics (mean ± SD), by test pairs.

Pairs tested	Mean (± SD)	% Response
Underside	70.50 (8.57)*	23.41
Eggplant	46.57 (5.57)	
Underside	82.03 (8.41)**	27.58
Potato	55.87 (6.25)	
Underside	71.07 (4.80)*	25.47
Tomato	56.27 (4.62)	
Eggplant	48.60 (4.93)	19.39
Potato	48.37 (4.81)	
Eggplant	57.23 (5.71)	21.74
Tomato	51.47 (4.95)	
Potato	58.53 (6.79)	22.94
Tomato	56.17 (6.39)	

*Means are significantly different at $P \leq 0.01$, Student's *t*.
**Means are significantly different at $P \leq 0.05$, Student's *t*.

the percent of females trapped on each color in any of the test combinations, as measured by the mean difference in percent females alighting on each color and the percentage of females present in the colony ($P \geq 0.3$ in all cases). The lack of significant difference in female response between the paired colors confirms that the paint colors reflected light the same as the host plant leaves. There was also no significant difference between underside color and either of the grays with respect to difference between percent females on the paint models and in the colony. However, the overall mean percent of females caught on all squares was significantly greater ($P \leq 0.01$) than the percent of females in the colony.

Table 2. Number of *E. puttleri* alighting on paint models of eggplant leaf underside color versus gray colors.

Pair Tested	Paint color	% Landing on Paint Models (± SD)
Underside vs Gray (35% Black)	Underside	62.00 (5.30)
	Gray	66.27 (6.16)
Underside vs. Gray (50% Black)	Underside	61.43 (6.90)
	Gray	67.93 (7.20)

Table 3. Percentage of female *E. puttleri* (mean ± SD) alighting on paint models color compared to percent females in colony, by test pairs.

Pairs tested	Mean % females in colony (± SD)	Mean % females on models (± SD)
Underside, Eggplant	89.19 (1.20)	93.38 (0.95)*
Underside, Potato	84.15 (1.73)	95.59 (0.74)*
Underside, Tomato	84.85 (2.45)	92.03 (1.75)*
Eggplant, Potato	85.80 (1.84)	95.22 (0.82)*
Eggplant, Tomato	85.94 (2.23)	92.17 (1.38)*
Potato, Tomato	89.82 (1.56)	93.78 (1.05)**
Overall mean	86.63 (0.77)	93.70 (0.48)*
Underside, Gray (35% black)	87.99 (1.66)	96.95 (0.43)*
Underside, Gray (50% black)	83.57 (1.98)	92.34 (0.81)*

* Means are significantly different at $P \leq 0.01$, Student's *t*.
** Means are significantly different at $P \leq 0.05$, Student's *t*.

Leonard et al. (1987) observed an attraction by the parasitoid to white surfaces, which represent maximum reflectance intensities. Kennedy et al. (1961) found that low-flying aphids, *Aphis fabae* Scopoli, were attracted to yellow as well as white surfaces. Distinguishing light reflected from yellow surfaces allow them to alight on plants, while attraction to white and light reflecting at the lower end of the spectrum visible to insects (blue, ultraviolet) allowed them to fly through spaces between plants by orienting toward the sky. *Edovum puttleri* may at times utilize this mechanism, explaining its attraction to white and surfaces with higher reflectance.

Response to the colored models, however, required leaving the white surface with which the cage was lined and flying to the painted models. Throughout the tests, most of the parasitoids were observed to remain on the floor of the cage (also lined with white) when not entrapped on the models. Relatively few were seen on the walls of the cage around the models, as would be expected if they were responding principally to the white surface. Therefore, the parasitoid was as likely attracted to the reflectance pattern of the models as it would be attracted to the leaves of its host's food-plant. *Edovum puttleri* may orient to the three plants tested based primarily on their pattern of intensity of reflectance.

This study has shown that while *E. puttleri* prefers the color of underside leaf surfaces where its host is most likely to be found, it shows no preference for the leaf color of three of its host's food-plants. Therefore, leaf color is probably not a viable explanation of the difference in host-finding efficiency of the parasitoid among these plant species. Color, however, may contribute to these differences when combined with other physical or chemical leaf characteristics and/or microclimate differences between upper and lower leaf surfaces.

Acknowledgments

We thank E. Vasquez and K. Probst for technical assistance. This study was supported in part from federal funds from the U. S. Department of Agriculture under agreement 58-32U4-6-13, IPM Pilot Tests, USDA 86-CRSR-2-2773. New Jersey Agricultural Experiment Station Publication Number D-08149-10-91 supported by state funds and by the U. S. Hatch Act.

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