Psyllid Responses to Colored Sticky Traps and the Colors of Juvenile and Adult Leaves of the Heteroblastic Host Plant *Eucalyptus globulus*

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ABSTRACT Heteroblasty describes plants with juvenile and adult vegetative shoots that differ morphologically. Virtually nothing is known about the affects of this source of within-plant variation on host-plant selection by herbivorous insects. This is the first study on the use of visual cues by insects that specialize on different shoot types of a heteroblastic plant. Two psyllid species (*Ctenarytaina eucalypti* Maskell and *C. spatulata* Taylor) specialize on glaucous juvenile and glossy adult shoots of *Eucalyptus globulus* Labill., respectively. We compared their responses to artificial colors and to the colors of expanding and expanded juvenile versus adult leaves. Sticky traps were used to compare psyllid response to seven artificial colors. A "color tube" was developed to compare psyllid responses to leaf color. Both psyllid species preferred yellow traps over all other colors (red, blue, green, white, and clear), and color preference was independent of species. Both species were also more attracted to the colors of expanding and expanded juvenile leaves than to adult leaves. Although the psyllids perceived differences between the colors of juvenile and adult leaves of *E. globulus*, their responses suggest that leaf color alone is not the basis of discrimination between foliage types.

KEY WORDS *Eucalyptus globulus*, Psyllid, colored sticky traps, heteroblastic host plant

HERBIVOROUS INSECTS USE visual and volatile chemical cues to locate suitable host plants (Degen and Stadler 1997, Prokopy et al. 1983, Bernays and Chapman 1994). Current understanding of host-plant selection is based primarily on studies of homoblastic plant species. Homoblastic species, by definition, have leaves and shoots that are morphologically similar at different developmental phases, whereas heteroblastic species exhibit juvenile leaves and shoots that differ strikingly from adult leaves and shoots (Frydman and Wareing 1974, Gould 1993, Heenan 1997, Brennan 2000).

Heteroblasty is an important source of within-plant variation because juvenile and adult shoots often occur simultaneously within the same individual. For example, *Eucalyptus globulus* Labill. has highly glaucous, horizontally oriented, sessile juvenile leaves and glossy, vertically oriented, petiolate adult leaves (Johnson 1926). In *E. globulus*, the distribution of eggs, nymphs, and adults of two psyllid species (*Ctenarytaina eucalypti* Maskell, *C. spatulata* Taylor) depends on shoot type (Brennan 2000). *Ctenarytaina eucalypti* prefers to settle and oviposit on the juvenile shoots, but *C. spatulata* prefers the adult shoots. This pattern is an example of 'developmental resistance' (Kearsley and Whitham 1989).

Virtually nothing is known about how insects perceive or distinguish different shoot types within heteroblastic plants. In this study we investigate the importance of leaf color in host-plant selection by the two species of *Ctenarytaina* psyllids that prefer different shoot types on *E. globulus.* Our objective was to determine if the psyllids differed in their response to leaf color and if such differences could explain their preferences for the juvenile versus adult shoots. To do this we compared the responses of *C. eucalypti* and *C. spatulata* to colored sticky traps, and the color of expanding and expanded juvenile and adult leaves of *E. globulus.*

Materials and Methods

Study Site and Organism. The experiments were conducted from March to May 1999 in the San Francisco East Bay area along a row of 15 *E. globulus* trees at El Cerrito High School, El Cerrito, CA. The trees were spaced 3–4 m apart and were ≈6–15 m tall. The trees had juvenile shoots in the lower canopy (0–3 m) and adult shoots in the upper canopy (>3 m) that were colonized by *C. eucalypti* and *C. spatulata*, respectively. Both of the *Ctenarytaina* species are native to Australia and have been established in the study area for several years (Brennan et al. 1999).

Responses to Colored Sticky Traps. To assess psyllid responses to artificial colors, we conducted an experiment with seven colors of sticky traps (white, red, blue, green, yellow, fluorescent yellow, and clear) during March and April. The traps were 1 mm thick rectangles (10 by 15 cm) and were coated on both sides with a thin layer of Stickem Special (Seabright, Emeryville, CA). The white, red, blue, green, and yellow traps were made of colored vinyl, and the clear

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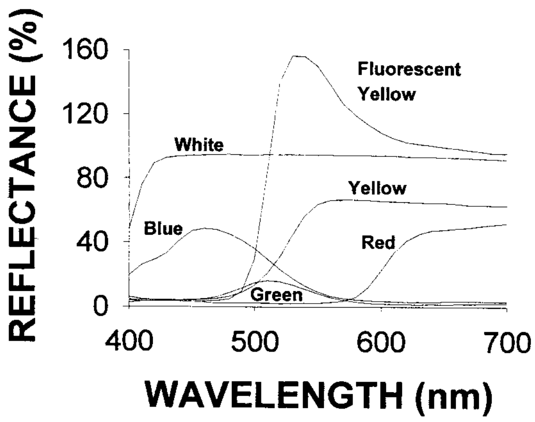

Fig. 1. Spectral reflectance of the colored sticky traps.

traps were made of polycarbonate (Tap Plastics, Dublin, CA). The fluorescent yellow traps were made of white cardboard that was painted with two coats of fluorescent yellow spray paint (#17052, Ace Hardware, Oak Brook, IL). The visible spectral reflectance of the colors (Fig. 1) was obtained with a Miniscan XE spectrophotometer (HunterLab, Reston, VA). The experiment was a randomized complete block design with three blocks. Each block included four 4-m long horizontal nylon lines to which we attached a randomly arranged set of the seven traps. Two lines were 0.5 m from the tree foliage and at two heights (1.5 and 3 m), and two lines were 1.5 m from the foliage and at both heights. The blocks were oriented end-to-end on the eastern side and parallel to the row of trees. The traps were hung by clips 50 cm apart on the lines, with one side facing the trees (west) and the other side facing east. The traps were replaced every 24 h at ≈1830 hours, and the number and sex of each species of psyllid on both sides of each trap was determined under a dissecting microscope. We summed the data over the 6-d period and performed an analysis of variance (ANOVA) for each psyllid species with the PROC GLM procedure in SAS (SAS Institute 1985). To satisfy assumptions of ANOVA, the data were transformed as $\log(1+x)$, but arithmetic means are reported in the results. Tukey's honestly significant difference (HSD), which controls for the experimentwise error rate was used for mean separation within each species.

Spectral Reflectance of Expanding and Expanded Juvenile and Adult Leaves. To characterize the spectral reflectance of the juvenile and adult leaves, we collected both types of leaves that were 1–4 m high in the canopies of four trees of *E. globulus.* The visible spectral reflectance of the abaxial and adaxial surfaces of expanding and expanded juvenile and adult leaves were measured with the spectrophotometer. For both surfaces of each leaf type, one measurement was taken from each of the four replicate trees.

Responses to the Colors of Expanding and Expanded Juvenile Versus Adult Leaves. A novel device called a "color tube" (Fig. 2) was developed to test the responses of the psyllids to the colors of expanding and expanded juvenile versus adult leaves. Each color tube was made of a 24 cm long black plastic tube (60 mm o.d., 52 mm i.d.). Psyllids were introduced to the center of the color tube via a 5 cm deep removable funnel-shaped cage. The interior surface of the color tube was lined with acid-free black mat paper to minimize reflection. To darken the cage, a black plastic skirt that attached around the circumference of the support stand was lifted over the color tube from both sides and fastened on top. Because these psyllids are negatively gravitropic and positively phototropic, they

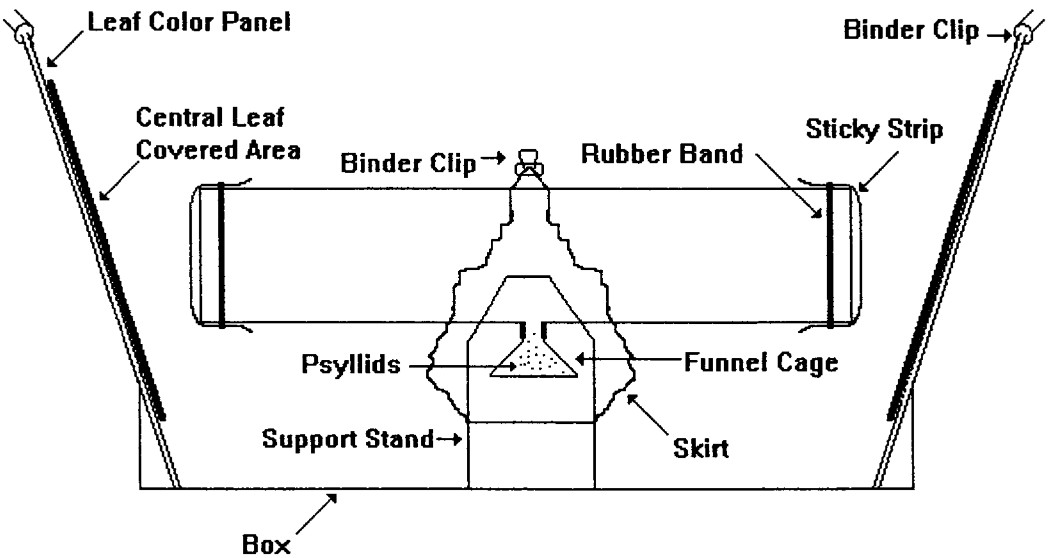

Fig. 2. Two dimensional diagram of a color tube. See text for actual dimensions.

moved from the funnel cage into the color tube and to either end of the color tube depending on their color preference. A vertically oriented 14 by 3-cm strip of clear transparency sheet coated with a thin layer of Stickem trapped approximately half of the psyllids exiting the ends of the color tube. The strip was coated only where it faced into the color tube. The areas on each side of the strip were open and allowed an unobstructed view of the leaf color panels from inside the color tube. The ends of the color tube were ≈2.5 cm from the leaf color panels. Each 20 by 30-cm leaf color panel was made of white vinyl to which approximately rectangular pieces of freshly collected leaves were attached by their adaxial surface with double stick tape to form a contiguous central 10 cm wide by 15-cm high leaf-covered area. Because the leaf color panels were made of pieces of leaves we assume that leaf shape was not apparent to the psyllids. Other than the inside wall of the color tube, the leaf-covered areas of the leaf color panels were apparently the only visual cues available to psyllids within the color tubes. To illuminate equally the leaf color panels at both ends of the color tube, the box holding the color tube was oriented with its long dimension perpendicular to the direction of the sun's rays. Preliminary experiments indicated that the psyllids did not perceive possible leaf volatile cues from the leaf color panels (Brennan 2000), and that the response of the psyllids to various colors at the ends of the color tube was the same as their response to those colors as sticky traps in the above experiment (Brennan, unpublished data).

The color preferences of both psyllid species were evaluated simultaneously in separate yet parallel color tubes and in separate experiments with the expanding and expanded leaves. The expanding and expanded leaf experiments occurred between 0900 and 1700 hours over 3 d during late April and early May, and included four replicates each. In each replicate ≈125 psyllids of each species (males and females combined) were evaluated. The approximate proportion of males in each replicate was 0.5 for *C. eucalypti* and 0.7 for *C. spatulata*, reflecting the sex ratio of psyllids on the foliage. Psyllids were collected from juvenile shoots (*C. eucalypti*) and adult shoots (*C. spatulata*) by shaking branches into large plastic bags 1–2 h before the psyllids were used. Approximately 30–60 min before each replicate, the psyllids were aspirated from the bags into the funnel cages. During each replicate, the cage was attached to the color tube, and the psyllids were allowed ≈35 min to respond. The orientation of each box (relative to the sun) was adjusted every 10–15 min to ensure equal lighting at each leaf color panel. After each replicate the sticky strips were removed from the ends of the color tube, and the number of psyllids was counted.

The experiments were conducted in an open area on the western side of the row of trees described above. For each replicate, we randomized the two species of psyllid among two color tubes, and randomized the end at which each leaf color panel was placed. Fresh leaf color panels were used every other replication. Within each species the data were analyzed with paired *t*-tests to compare the preference of the psyllids for the expanding juvenile versus adult leaves, and for the expanded juvenile versus adult leaves.

Table 1. ANOVA of *C. eucalypti* and *C. spatulata* caught on the seven colored sticky traps at two heights and distances from the row of *E. globulus* trees

Source	MS	*F*	df	*P*
Block	7.4	18.7	2	***
Color	33.3	83.9	6	***
Species	8.0	20.2	1	***
Sex	17.6	44.1	1	***
Trap height	11.2	28.0	1	***
Trap distance	0.7	1.8	1	0.18
Color × Spp.	0.7	1.7	6	0.12
Height × Spp.	12.3	30.8	1	***
Distance × Spp.	3.1	7.8	1	**
Sex × Spp.	0.3	0.7	1	0.41
Sex × Color	0.1	0.3	6	0.96
Error			308	

All values are based on transformed data (log $[x+1]$) of mean 6-d catch/trap. ***, Significant at the $P \leq 0.001$ level.

Results

Responses to Colored Sticky Traps. Trap color significantly affected psyllid capture, but the color preferences were the same for both psyllid species (Table 1). Yellow and fluorescent yellow were preferred over other colors (Fig. 3). Across all traps, approximately twice as many *C. spatulata* (4,570) as *C. eucalypti* (2,117) were captured, and capture was male-biased for both species (Table 2). The majority of both species were captured on the more sun-lit eastern side of the traps that faced away from the tree foliage. The effect of trap height and distance from the tree foliage depended on species, with more *C. eucalypti* captured at the traps closer to the foliage, and more *C. spatulata* captured on the higher traps (Tables 1 and 2).

Spectral Reflectance of Expanding and Expanded Juvenile and Adult Leaves. Spectral reflectance differed among the abaxial and adaxial surfaces of the expanding and expanded juvenile and adult leaves (Fig. 4). The reflectance of the juvenile leaves was greater than that of the adult leaves. The abaxial surfaces of both leaf types reflected more light at all wavelengths than adaxial surfaces especially with the expanded juvenile leaves. The maximum reflectances of both the juvenile and adult leaves were at 550 and 700 nm. The 550-nm peak reflectance was closest to that of the yellow and fluorescent yellow sticky traps, although the magnitude of the reflectance of the traps was several fold greater than that of the leaves (Figs. 1 and 4). Reflectance intensity was the main difference in the curves of the juvenile and adult leaves. The peak of the expanding adult leaves was also considerably broader between 525 and 625 nm than expanded adult leaves and the expanded and expanding juvenile leaves.

Responses to the Colors of Expanding and Expanded Juvenile Versus Adult Leaves. Significantly more psyllids of both species were captured on the

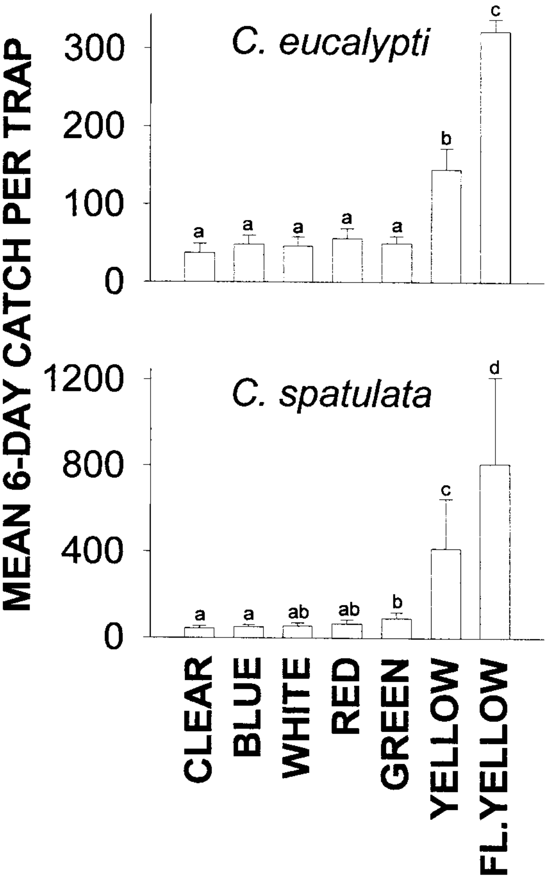

Fig. 3. Responses of *C. eucalypti* and *C. spatulata* to colored sticky traps. Each bar is the pooled mean ± 1 SE for traps at both heights and distances from the foliage. Within each species bars topped with the same letter are not significantly different at $P < 0.05$ based on Tukey's HSD (experiment-wise error rate).

ends of the color tubes facing the expanding (*C. eucalypti* $t = 5.2$, df = 3, $P < 0.01$; *C. spatulata* $t = 3.9$, df = 3, $P < 0.05$) and expanded juvenile leaves than adult leaves (*C. eucalypti* $t = 5.5$, df = 3, $P < 0.01$; *C. spatulata* $t = 16.9$, df = 3, $P < 0.001$) (Fig. 5). In both experiments, ≈80% of the psyllids placed in the color tubes responded to visual cues.

Table 2. Summary of psyllids caught across all artificially colored sticky traps including the ratio of males:females, ratio on the east:west (foliage facing) side of the traps, ratio of the traps at two heights, and ratio on the traps at two distances from the tree foliage

Ratio	*C. eucalypti*	*C. spatulata*
Males:females	1.7:1	1.6:1
East:west facing side of trap	5.6:1	2.3:1
3 m:1.5 m high	1.1:1	2.9:1
0.5 m:1.5 m from the foliage	1.8:1	1.0:1

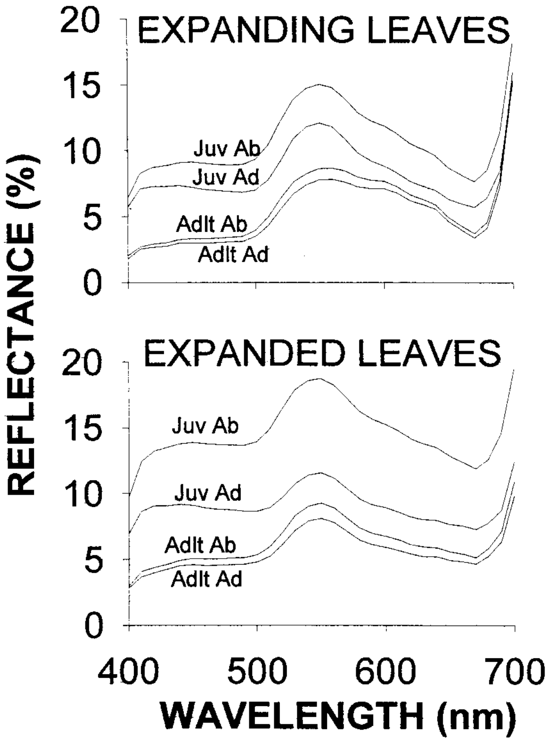

Fig. 4. Spectral reflectances of the abaxial (Ab) and adaxial (Ad) surfaces of expanding and expanded juvenile (Juv) and adult (Adlt) leaves of *E. globulus*. Each line is the mean of measurements from four replicate trees.

Discussion

Response to Host-Shoot Visual Cues. This study compared the responses of insects to differently colored leaves of a heteroblastic host plant. Our experiments demonstrated that both psyllid species perceived differences among the colored sticky traps, and between the juvenile and adult leaves. However, the responses of the psyllids suggest that color alone is not the basis of discrimination between foliage types, because both species were attracted to the same visual cues (i.e., bright yellow sticky traps, and juvenile leaf color panels). The juvenile leaves may have been attractive to both species due to their higher reflectance across all visible wavelengths. This is consistent with the greater attraction of both species to the more intense reflectance of the fluorescent yellow sticky traps versus the regular yellow traps, and the more sun-lit versus shaded surface of the sticky traps. Alternatively, both psyllids may have responded similarly to leaf color because of the absence of other cues such as leaf shape and leaf volatiles. Some insects change their response to color in the absences of volatile cues (Vaidya 1969), and others are known to use leaf shape in host plant selection (Degen and Stadler 1997). Preliminary comparisons of the juvenile and adult shoots of *E. globulus* suggest qualitative and quantitative differences in head space volatiles (E.B.B., unpublished data), but the role of volatile

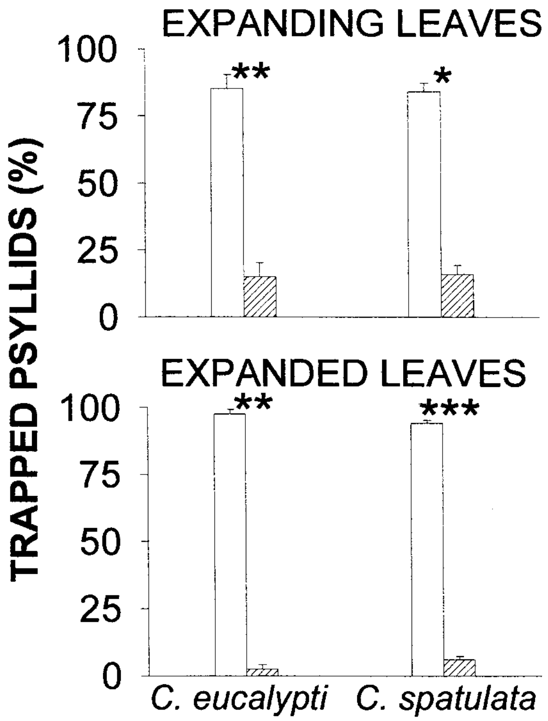

Fig. 5. Relative percentage of psyllids trapped at the ends of the color tube facing the leaf color panels with either juvenile (Juv) or adult (Adlt) leaves of *E. globulus.* Within each species and stage of leaf expansion, *, **, and ***, Significant differences at the $P < 0.05$, 0.01, and 0.001 levels, respectively, based on paired *t*-tests.

cues in host shoot selection by these psyllids has not been studied. The role of leaf shape in host plant selection by these psyllids has not been studied.

Host plant selection by herbivorous insects may involve visual and chemical cues perceived from a distance, nearby, and within the canopy (Prokopy and Owens 1983, Bernays and Chapman 1994). Because juvenile shoots are often hidden within the large adult shoot-dominated canopy of *E. globulus* trees, both *C. eucalypti* and *C. spatulata* may use similar cues from a distance, and each species may subsequently use different cues within the canopy to locate appropriate host shoots. Our experiments do not indicate whether the responses of the psyllids in the color tubes reflect their natural response to cues perceived from a distance or within the canopy.

Response to Colored Traps. The preferences of both *Ctenarytaina* species for yellow traps agree with previous studies on psyllids (Mensah and Madden 1992, Lapis and Borden 1995, Hoback et al. 1999) and indicate that yellow traps are optimal for monitoring populations of *C. eucalypti and C. spatulata.* We speculate that more psyllids of both species were captured on the eastern-facing side of the each trap because it was illuminated by the sun for a longer time each day than the western side that faced the trees. The cardinal placement of sticky traps around trees and exposure are known to affect capture of other insects (Robacker et al. 1990, Hoback et al. 1999). The majority of trapped psyllids apparently originated from the row of host trees rather than from other host areas, because we frequently observed psyllids fly toward the traps from the row of trees.

Trap height affects capture of some insects (Gillespie and Vernon 1990, Robacker et al. 1990). We speculate that more *C. spatulata* were captured on the higher traps because the majority of its preferred adult shoots were higher (>3 m) in the canopy. In contrast, the reason that trap height did not affect *C. eucalypti* may be because the preferred juvenile shoots occurred in the first 3 m of the canopy where the traps were placed. *C. eucalypti* alone was affected by the distance of the traps from the foliage, perhaps because it searches less extensively in the canopy than *C. spatulata.*

The Color Tube as a Novel Tool to Evaluate Insect Response to Leaf Color. The unique spectral reflectance of chlorophyll and other pigments in leaves makes it difficult to accurately mimic leaf color (Prokopy et al. 1983). Thus, it is preferable to use real leaves to monitor insect response to leaf color. However, this is challenging because leaves may wilt rapidly if detached, produce volatile cues, and transpire and thus cause condensation inside clear containers that are used to eliminate the effects of leaf volatiles; such condensation in turn may distort the spectral reflectance of the leaves. These problems may explain why so few studies (Vaishampayan et al. 1975, Prokopy et al. 1983) have assessed the response of insects to the color of real leaves. Color tubes are a novel tool to study the response of psyllids and possibly other insects to sunlight-illuminated leaf color without enclosing the leaves in airtight, clear containers.

In conclusion, heteroblasty is an important source of within-plant variation. Little is known about how insects, such as *C. eucalypti* and *C. spatulata* that specialize exclusively on different types of shoots, perceive this variation and select suitable shoots. Both species of psyllids responded similarly to artificial colors and preferred yellow. Although the psyllids perceived differences between the color of juvenile and adult leaves of *E. globulus,* their responses do not explain the role of leaf color in selection of the glaucous juvenile versus glossy adult leaves of *E. globulus,* because both species were attracted to the same colors (i.e., bright yellow stick traps, and juvenile leaf color).

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