Laboratory and Field Response of the Emerald Ash Borer (Coleoptera: Buprestidae), to Selected Regions of the Electromagnetic Spectrum

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ABSTRACT Retinal sensitivity of *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae) was examined with an aim to improve trap efficacy for the beetle. Electroretinogram (ERG) recordings from dark-adapted compound eyes of male and female were measured at different wavelengths across the spectrum ranging from 300 to 700 nm. The spectral sensitivity curves revealed peaks in the UV (340 nm), the violet/purple (420–430 nm), blue (460 nm), and green (540–560 nm) regions of the spectrum. Females were sensitive to red regions of the spectrum (640–670 nm), whereas males were not. A spectrophotometer was used to measure the wavelength and reflectance for ash foliage, purple corrugated plastic traps, as well as the elytra and abdomen of adult *A. planipennis*. Traps were painted using colors based on ERG and spectrophotometer measurements and compared with purple corrugated plastic traps currently used by the USDA–APHIS–PPQ–EAB National Survey. In a field assay conducted along the edges of several *A. planipennis*-infested ash stands, there were no significant differences in trap catch among green, red, or purple treatments. Dark blue traps caught significantly fewer *A. planipennis* than red, light green, or dark purple traps. In a second assay where purple and green treatments were placed in the mid canopy of ash trees (≈13 m in height), trap catch was significantly higher on green treatments. We hypothesize that when placed in the mid-canopy, green traps constitute a foliage-type stimulus that elicits food-seeking and/or host seeking behavior by *A. planipennis*.

KEY WORDS emerald ash borer, electroretinogram, spectral sensitivity, insect trap color

The emerald ash borer *Agrilus planipennis* Fairmaire (Coleoptera: Buprestidae), an invasive pest of ash trees (*Fraxinus* spp.), was first reported in North America around the cities of Detroit, MI, and Windsor, Ontario, Canada, in 2002 (Haack et al. 2002). Larvae of *A. planipennis* feed in the phloem and cambial regions forming S-shaped galleries that disrupt nutrient and water flow. This can kill trees within 2–3 yr of infestation (Liu et al. 2003). Adults emerge in May and June (in Michigan), with flight activity lasting until August. It has also been reported in Ohio and Maryland (2003), northern Indiana (2004), northern Illinois (2006), eastern Pennsylvania and West Virginia (2007), and most recently in Virginia, Wisconsin, Missouri, Minnesota, Kentucky, and New York as well as Quebec, Canada (2009; http://www.emeraldashborer.info and http://www.inspection.gc.ca/). Movement of firewood and nursery trees has been the primary means of long-range spread of the insect in North America (Marchant 2006).

There is an important need for regulatory agencies to be able to detect new populations of *A. planipennis* and delimit existing populations for management purposes (Cappaert et al. 2005). Detection of *A. planipennis* at low densities by tree inspection has been ineffective because external symptoms of damage such as crown dieback, bark splits, epicormic branching, and exit holes (throughout the tree surface) are not apparent until heavy infestation has already taken place (Cappaert et al. 2005, Francese et al. 2005). *A. planipennis* are attracted to ash trees that have been stressed by girdling or herbicide treatments (Poland et al. 2004). However, using "girdled trap trees" is destructive, labor intensive and costly. Adult *A. planipennis* are attracted to host volatiles (Rodriguez-Saona et al. 2006, Crook et al. 2008a). Oils such as Manuka oil and Phoebe oil contain high amounts of several antennally active sesquiterpenes found in ash bark and show promise as effective lures for "long-range" attraction (Crook et al. 2008a).

Oliver et al. (2002) found that buprestids of several genera (including *Agrilus*) showed a preference for colors in the violet range (400–430 nm). In addition to this, Oliver et al. (2004) and Francese et al. (2005, 2008) found that purple-violet colored traps with large

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silhouettes were most attractive to adult *A. planipennis.* Purple plastic sticky panel traps have since been used for *A. planipennis* trap placement studies (Francese et al. 2008). They are also used to test the efficiency of ash-based semiochemical lures in the field (Poland and McCullough 2007, Crook et al. 2008a). In 2008, the Emerald Ash Borer National Survey program implemented the use of purple prism traps baited with Manuka oil for monitoring purposes. Baited purple traps detected 10 new infestations in 2008, including a new state record in Missouri (http://www.emeraldashborer.info). Although the current trapping system has proven effective, a continuing goal of our research is to further improve trap efficacy.

Recent studies on *A. planipennis* (Lance et al. 2007; Lelito et al. 2007, 2008, 2009; Crook et al. 2008b; Silk et al. 2009) have shown that vision and a female produced contact pheromone have an important role in "short-range" mate selection. It is therefore important to study *A. planipennis* vision further with respect to developing a better trap for this insect. The role of sex pheromones and other olfactory stimuli for monitoring herbivorous pests has received far more research attention than the role of visual stimuli. Visual traps, however, could be of great value in trapping programs as they usually attract both sexes (Prokopy and Owens 1978, 1983).

There have been relatively few electroretinogram (ERG) studies on coleopterans (Groberman and Borden 1980). We used the ERG technique to measure the spectral sensitivity of *A. planipennis* vision, focusing on differences that may exist between the sexes. It was hoped that these measurements would better define the visual sensitivity of *A. planipennis* adults to specific wavelengths and help identify a more efficient color trap for detecting new infestations of this important, invasive pest species.

The main objectives of this research were to 1) measure reflectance and wavelengths of ash foliage as well as the elytra and abdomens of adult *A. planipennis;* 2) measure retinal responses of *A. planipennis* to wavelengths between 300–700 nm by using electroretinogram methods; and 3) Select several trap colors based on the above measurements and evaluate *A. planipennis* preferences to them in field trapping tests.

Materials and Methods

Insects. Adult *A. planipennis* were reared from infested ash material collected in Howell, MI, and shipped to the USDA insect containment facility at Otis ANGB, Buzzards Bay, MA. Insects were held in groups of 10, separated by sex in plastic 473-ml (16-oz) drinking cups (Solo, Urbana, IL), and provided with fresh foliage of *Fraxinus uhdei* (Wenzig) Lingelsh and water in 30-ml (1-oz) plastic cups fitted with a cotton wick. Insects were allowed to feed for at least seven days before they were used for electrophysiological experiments.

ERG Recordings. The ERG system consisted of a lamp that delivered light stimuli to the compound eye of a beetle through a monochromator that was connected to a liquid light guide cable and focusing lens. Monochromatic light output between 300 and 700 nm was obtained by passing light from a 75-W Xenon short arc lamp through a model 101 monochromotor with 1,200 line/mm and 300-nm grating (Photon Technology International, Birmingham, NJ). Light settings for the monochromotor were controlled by a MD1000 controller/shutter system connected to a portable computer running Felix32 (version 1.1 Win 2000/XP, Photon Technology International, Birmingham, NJ). The monochromotor bilater slit was set to a 1.25-mm open setting (giving a 5-nm reciprocal dispersion). Light from the monochromotor passed into a liquid light guide, which terminated in a symmetric-convex lens (precision figured for 1:1 imaging) that focused a columnar 0.5 cm-wide beam directly onto the insect eye preparation at a distance of 5 cm. Stimulating flashes lasted one second, and the interval between flashes was 90 s. Flash stimulation wavelengths between 300 and 700 nm in increments of 10 nm were presented randomly to the insect. Every four stimulations a reference light of 350 nm was flashed on the insect preparation so that data could be normalized against it later (i.e., taking into consideration possible reduction in responses over time as insect life decayed). The entire insect preparation ($n = 4$ for each sex) was allowed to adapt to total darkness for 10 min before a spectral sensitivity run was started. The insect remained in the dark between stimulations of light. The room was darkened during operation of recording equipment to minimize light interference from external sources.

The insect preparation for ERG involved cutting the head and prothorax from the rest of the body. The forelegs and antennae were then removed. An insect pin (size 000) was used to make a small hole on the frontal dorsal surface of the head, directly between both compound eyes. The head-prothorax preparation was then attached to electrodes of an EAG probe (Syntech, Hilversum, The Netherlands) using conductive gel (Spectra 360, Parker Laboratories, Fairfield, NJ). The recording probe tip was connected to the punctured head, whereas the indifferent (ground) probe was attached firmly to the base of the cut prothorax. Enough conductive gel was used on the recording probe so that it could enter into the punctured hole, thereby forming a good connection for electrophysiological recording. If any gel obscured either of the compound eyes, the beetle was discarded. Insect "head-probe" preparations were then connected to an IDAC-232 serial-data acquisition controller (Syntech). Signals were stored and analyzed on a PC equipped with the program EAG version 2.6 (Syntech). A Student's *t*-test was used to compare differences in sensitivity between the two sexes at each wavelength tested (JMP version 5.1, SAS Institute 2003).

Wavelength and Reflectance Measurements. An FieldSpec Pro full range spectrophotometer (Analytical Spectral Devices Inc., Boulder, CO) was used to measure spectral reflectance curves. The FieldSpec Pro FR uses a 1-m fiber optic bundle for light collec-

tion and covers the range from 350 nm to 2500 nm. The visible and near-infrared (VNIR) portion of the spectrum (350–1000 nm) used in this study was measured by a 512-channel silicon photodiode array. The spectral resolution of this array is ≈3 nm in the VNIR region based on the full-width-half-maximum response of the spectrophotometer. A contact plant probe was used on the end of the fiber optic cable to collect the readings. The probe contained a halogen light source for illumination at a fixed angle and had a spot size of 10 mm. Spectral readings were recorded as relative reflectance. To calculate the reflectance, a white reference reading was taken on a spectralon panel before each sample was scanned.

For insect measurements, a square area of ≈2 by 2 cm was required to fill the contact probes' recording surface. For full body measurements, 14 females were carefully placed together (two rows of 7, facing away from each other) on a black background. Double-sided tape was used to keep the insects in place. This gave an insect surface area of ≈2 by 2.5 cm that could be hand scanned by the spectrophotometer. The same technique was used for males (18 adults, two rows of nine). For abdomen recordings the elytra were removed first, after which the red/purple abdomen was cut below the prothorax and removed. Cut abdomens were then arranged in four rows, on a black background using double-sided tape. Abdomens were arranged as close together as possible. Twenty-eight male and 30 female abdomens were prepared. The measurable surface for both male and female abdomens was ≈2 by 2.5 cm. Measurements were taken from the dorsal surface of adult bodies and abdomens by placing the probe of the spectrophotometer directly above the insect "panels," at a distance of 1 mm. Wavelength and reflectance were recorded for one second before being analyzed on an IBM PC laptop using RS^3 software version 2.3 (Analytical Spectral Devices Inc.). Wavelength and reflectance also were measured for all primed and painted trap treatments.

Fresh green ash, *Fraxinus pennsylvanica* Marsh., leaf foliage from four trees was sampled and scanned during June, July, and August 2005. Several branches from each tree were cut using a 10-m pole pruner, and 10 leaves were collected from each tree on each date ($n = 40$). The leaves were placed in plastic bags in a cooler and transported back to the lab. Leaves were scanned the same day for reflectance and wavelength measurements using the plant contact probe on the spectrophotometer. The average reflectance and wavelength were then calculated for each group of four trees. Data were also collected from the same four trees the following May 2006.

Color Selection and Trap Design. Based on spectrophotometer and ERG measurements, eight paint colors were selected for lab and field testing. Unless specified, colors were produced using Sherwin Williams (SW) Company (Richardson, TX) stock colors: light purple (SW6554, Lite Lavender), dark purple (SW6983, Fully Purple), light green (SW6920, Center Stage Green), dark green (Benjamin Moore 'Color Preview' 2036-20, Irish Moss; Benjamin Moore & Co., Montvale, NJ), light blue (SW6806, Rhythmic Blue), dark blue (SW6967, Frank Blue), and red (9963828, Ruby Red; Ace Hardware, Oak Brook, IL). Purple corrugated plastic (Coroplast Inc., Dallas TX), found to be attractive to *A. planipennis* in previous studies by Francese et al. (2005, 2008) and Crook et al. (2008a) were used as a control treatment in both assays.

Three-panel "prism" traps (35.0 by 58.75 cm each) of 3-mm-thick corrugated plastic (described by Crook et al. 2008a, Francese et al. 2008) were used for field assays. Before painting, translucent prism traps were coated with a plastic-bonding primer (Preprite B51W50; Sherwin Williams Co.) that was gray-colored to a P5 Sherwin Williams scale. Three to five coats of a single paint were then applied to a primed trap until the primer was no longer visible. Once dry, subsamples of painted, primed traps were checked with the spectrophotometer to ensure the primer did not affect the required wavelength or reflectance of the paint colors to be tested. After placement in the field, Tanglefoot insect trapping glue (The Tanglefoot Co., Grand Rapids, MI) was applied to the outer surface of all traps.

Color Comparison Study. Twelve randomized trap lines were established along the edges of infested ash stands. Traps were hung at a height of 1.5 m from L-shaped pieces of rebar. Each line (treated as a replicate) consisted of a purple control and seven painted prism trap treatments: 1) dark purple, 2) light purple, 3) dark green, 4) light green, 5) dark blue, 6) light blue, and 7) red. Traps in each line were spaced 15 m apart. Trap lines were spaced at least 30 m apart.

Comparison of Color Traps at Two Heights. Nine lines of traps were hung near the ground at 1.5 m and in the mid-canopy (≈13 m high) of ash trees along the edge of infested stands using methods similar to those described by Francese et al. (2008) and Crook et al. (2008a). Traps in each line were placed on adjacent trees. Each line consisted of a purple control, and four painted prism trap treatments: 1) dark purple, 2) light purple, 3) dark green, and 4) light green. Each trap set at 1.5 m was the same color as the trap placed in the mid-canopy above it. Traps in each line were spaced 15 m apart. Trap lines were spaced at least 30 m apart.

Statistical Analyses. All statistical analyses were performed using JMP version 5.1 (SAS Institute 2003). Trap catch was recorded weekly from 29 May 2007 to 18 July 2007. Beetles were sexed and catch was totaled by trap over the entire field season. Summed catch on each trap in both studies was transformed as log (n + 1) and analyzed by analysis of variance (ANOVA) (GLM). For the color comparison study, the analysis included main effects for color and block, whereas in the study comparing color at two heights, the analysis included main effects for color, block and height, and an interaction term for height x color. Tukey's honestly significant difference (HSD) ($\alpha = 0.05$) test was used to compare differences in catch between treatments.

For each study, the median date of capture was calculated for all colors combined (but separately by height in the second study). The number of *A. pla-*

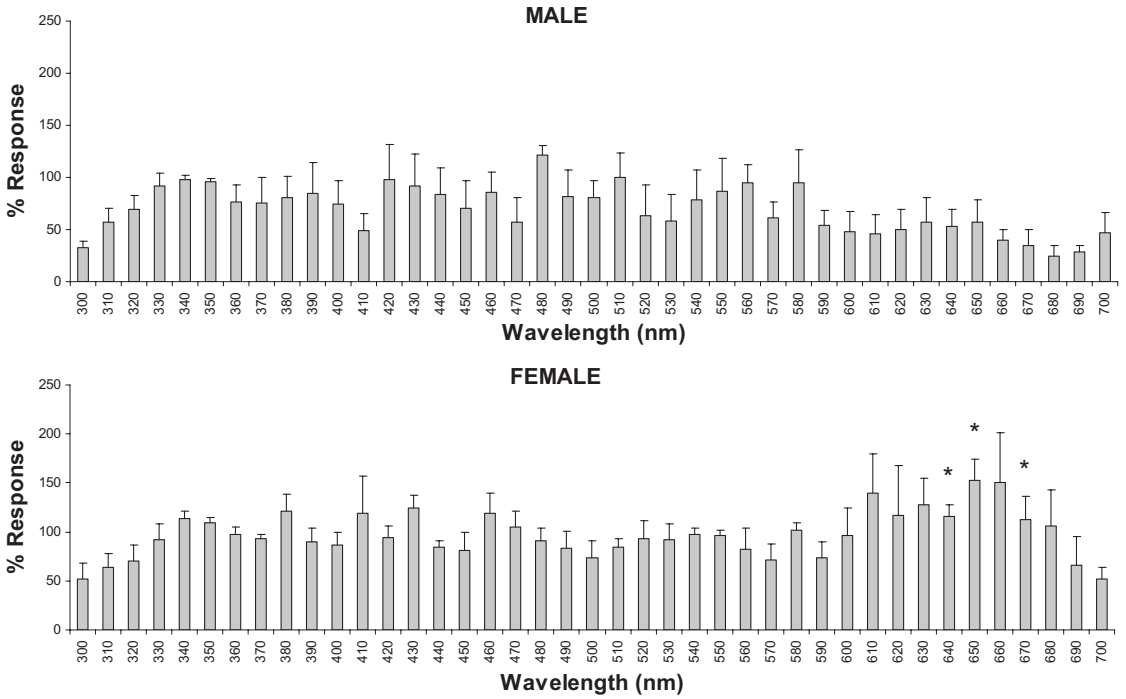

Fig. 1. ERG responses from dark adapted compound eyes of male and female ($n = 4$) *A. planipennis* to a range of different wavelengths (300–700 nm). Stimulus duration, 1 s. Response data were normalized against control recordings at 350 nm (100%). Asterisk (*) indicates females were significantly more sensitive to these wavelengths (640, 650, and 670 nm) than males ($P < 0.05$; *t*-test).

nipennis caught before and after the median date on traps of each color was then calculated. Traps were checked at intervals of several days, not daily. Beetles captured in the interval including the median date were assigned to two groups, before and after (according to a uniform distribution). We performed χ^2 analyses to determine whether there was a difference in the overall timing of catch among colors ($\alpha = 0.05$). For the comparison study at two heights, pair-wise comparisons were made between colors, separately at the two heights, with an adjusted α-value (0.005) by means of Bonferroni's correction.

The χ^2 analyses also were performed to determine whether there were differences in the overall sex ratio of males to females caught on traps in both studies ($\alpha = 0.05$). Pairwise comparisons were made using χ^2 analysis of sex ratios by color in the color comparison study, with a Bonferonni-corrected α-value of 0.0018. For the comparison of sex ratio among color traps at two different heights, the adjusted α-value was 0.005.

Results

Electroretinogram Recordings. Electrophysiological retinal recordings from the eyes of adult male and female *A. planipennis* showed some similarities as well as differences in sensitivity between the sexes (Fig. 1). Both sexes showed peaks in sensitivity at 340 nm (UV range), 420–430 nm (violet), and 460 nm (blue). Within the green range of the visible spectrum, males seemed to be most sensitive between 540 and 560 nm, whereas females showed a peak in sensitivity at 540 nm. The most obvious differences in retinal sensitivity between the sexes were observed in the red visible range. Females were significantly more sensitive to 640, 650, and 670 nm than males ($P < 0.05$; *t*-test).

Wavelength and Reflectance Measurements. *Emerald Ash Borer Body Surface.* Spectrophotometer readings showed both male and female *A. planipennis* elytron surfaces had a wavelength peak of between 530 and 540 nm, with a reflectance of 5% (Fig. 2). Spectrophotometer readings of dorsal abdomen sections also showed little difference between males and females, peaking between 650 and 660 nm. Female abdomens had a reflectance of 7.4%, whereas males had a reflectance of 6.5%.

Ash Foliage. Wavelength scans of green ash foliage taken at different times of the *A. planipennis* flight season showed a consistent peak between 545 and 555 nm (Fig. 3). Reflectance recorded from green ash leaf samples in May 2006 showed a peak reflectance of 14.6% at 550 nm. Recordings taken the previous year from the same trees showed there was an almost 1% decrease in reflectance levels per month through June, July, and August. Peak reflectance at 550 nm averaged 9.7% for June, 9.0% for July, and 8.1% for August.

Painted Traps. Wavelength and reflectance measurements for control and painted traps are shown in Figs. 4–6. Painted light and dark green traps

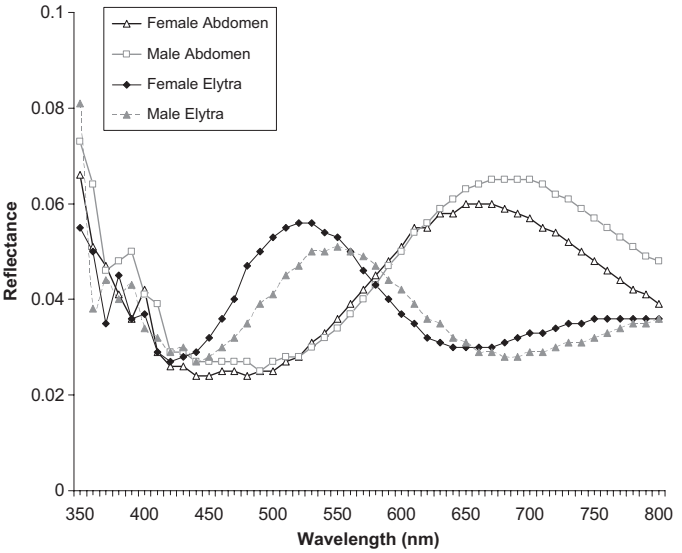

Fig. 2. Reflectance spectra of *A. planipennis* abdomen and elytra. (0.1 = 10% reflectance).

(540–550 nm) showed peak reflectance values of 64 and 24%, respectively (Fig. 4). The peak reflectance values for light and dark blue traps (450–460 nm) were 80 and 25%, respectively (Fig. 5). Light and dark purple traps exhibited peak reflectance values in the 430–440 nm range of 75% and 23%, respectively, with a second peak at 670 nm with readings of 78% and 12%, respectively (Fig. 6). The purple control trap had a similar profile to the dark purple paint (Fig. 6). Red painted traps showed a peak reflectance of 50% at 650 nm and 58% at 750 nm (Fig. 6). The addition of glue to the trapping surface increased reflectance of all traps by ≈2.5%.

Color Comparison Study. Trap color significantly affected trap catch in the color comparison trapping study ($F = 4.67$; df = 7, 77; $P = 0.0002$). Although light green traps caught more *A. planipennis* than light or dark blue traps, there was no significant difference between light green traps and traps of the remaining colors (Table 1). Dark blue traps caught significantly less than control, dark purple, light green and red traps, but there were no other significant differences among colors.

The median catch occurred 28.8 d after the start of the study. Overall differences in timing of catch were not significant ($\chi^2 = 12.6$, df = 7, $P = 0.08$), so additional pairwise χ^2 analyses were not performed (Table 1). The overall ratio of males to females (448:617 = 0.73:1) among traps of all colors was significantly female-biased ($\chi^2 = 1{,}466.8$; df = 7; $P < 0.0001$). Light blue, dark green, light green, and control traps caught a significantly higher ratio of males to fe-

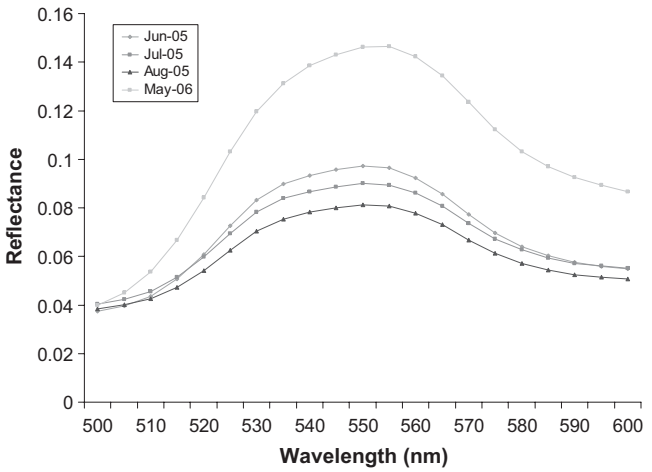

Fig. 3. Average reflectance spectra of green ash leaf foliage sampled and measured on four different dates from June 2005 to May 2006. Mean is based on 10 leaves sampled from four trees ($n = 40$).

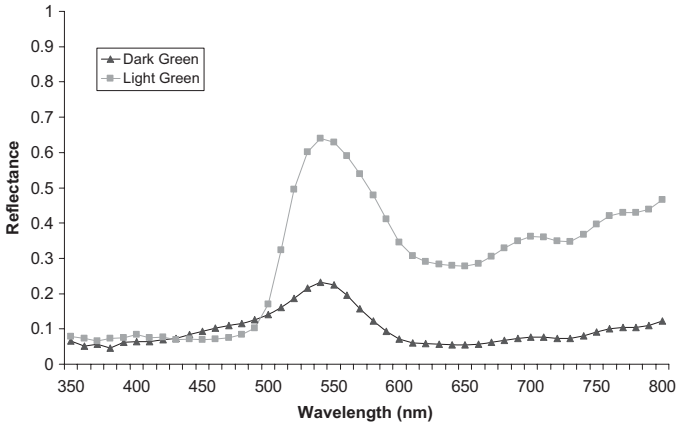

Fig. 4. Reflectance spectra of dark green and light green painted traps.

males than red traps, but there were no other differences in the male to female ratio between any other traps (Table 1).

Comparison of Color Traps at Two Heights. Trap color ($F = 6.53$; df = 4, 72; $P = 0.0002$), trap height ($F = 39.95$; df = 1, 72; $P < 0.0001$), and the interaction between the two factors ($F = 5.02$; df = 4, 72; $P = 0.0013$) played a significant role in trap catch (Table 2). Because the interaction effect was significant, separate analyses of variance were performed to compare catch at each height.

Among traps placed in the mid canopy, dark green traps caught significantly more beetles than control, dark purple and light purple traps, and light green traps caught more than the light purple traps (Table 2) ($F = 7.54$; df = 4, 32; $P = 0.0002$). There were, however, no significant differences in beetle catch among any of the other colors in the mid-canopy. Among traps placed at 1.5 m, control and dark purple traps caught significantly more beetles than light purple traps, but there were no significant differences among traps of other colors ($F = 5.06$; df = 4, 32; $P = 0.0028$).

The median catch occurred 23.9 and 26.4 d after the start of the study for high (mid-canopy) and low (1.5 m) traps, respectively. Differences in timing of catch among colors were significant for both the high ($\chi^2 = 90.9$, df = 4, $P < 0.0001$) and low traps ($\chi^2 = 66.5$, df = 4, $P < 0.0001$). The percentage of beetles caught before the median date was highest on light green traps at both trap heights (Table 2). Among the other colors, there were no differences in percentage of beetles caught before the median date at 1.5 m, but in the mid-canopy the percentage of beetles caught before the median date was significantly lower on dark green traps when compared to light green and dark purple traps (Table 2).

Sex ratio (M:F) depended significantly on color, both in the mid-canopy ($\chi^2 = 751.0$, df = 4, $P < 0.0001$) where it was male-biased overall (4,282: 3,314 = 1.29:1) and near the ground ($\chi^2 = 83.9$, df = 4, $P < 0.0001$) where it was female-biased overall (611:785 = 0.78:1) (Table 2). At both heights, dark green traps had the highest male to female ratio and dark purple traps had the lowest (Table 2). Sex ratio did not differ between light green and control traps at either height. Sex ratio on light purple traps relative to other colors varied with trap height; in the mid-canopy it was lower than light green and dark green but higher than dark purple whereas near the ground it was similar to all colors except dark purple.

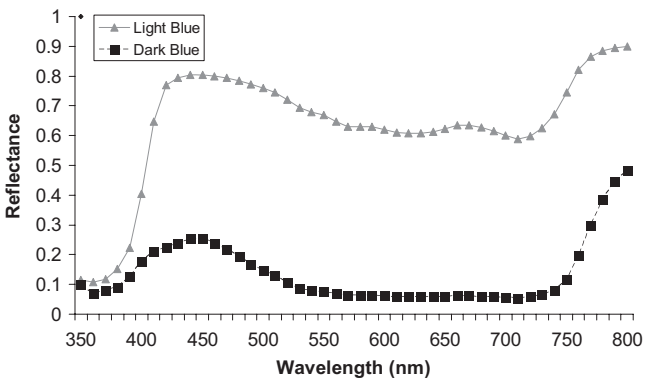

Fig. 5. Reflectance spectra of dark blue- and light blue-painted traps.

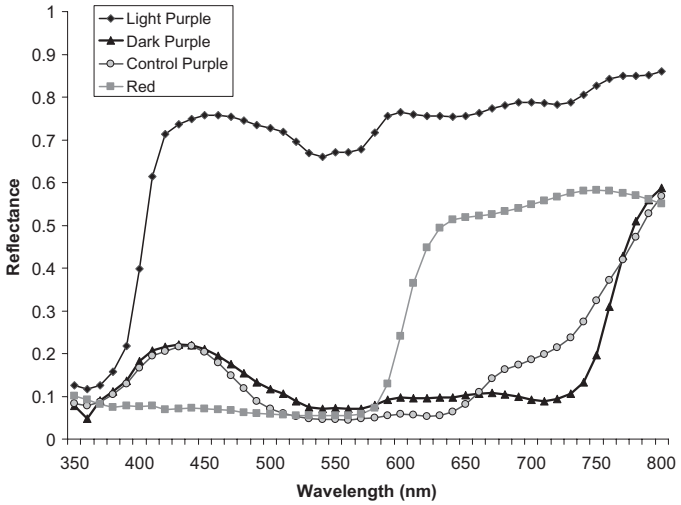

Fig. 6. Reflectance spectra of dark purple-, light purple-, and red-painted traps and an unpainted purple plastic trap (control).

Discussion

Trichromatic color vision provides the ability to see different colors. It is mediated by interactions among at least three types of color-sensing receptors. Trichromacy that uses roughly equidistant receptor peaks is a useful multipurpose vision system that has been adopted by many different insects (Barlow 1982, Vorobyev 1997). The most common type of trichromacy uses receptors with maximal sensitivity of ≈350 nm (UV), 450–480 nm (blue), and 500–550 nm (green) (Kelber 2006), as shown in the moth *Manduca sexta* (L.) (White et al. 2003) and butterfly *Vanessa cardui* (Briscoe et al. 2003). Spectral receptors in the Apidae (λ_{max} = 340, 430, and 540 nm) have been shown to be close to optimal for the discrimination of several sets of sympatric and simultaneously blooming flower colors, as well as the discrimination of green foliage (Chittka 1996). Our electrophysiological studies indicate that *A. planipennis* has these three spectral receptors along with a potential fourth receptor (particularly in females) that is sensitive to 640–650 nm. However, to determine the precise number of color receptors present (and their spectral properties) in *A. planipennis* will require more precise intracellular recording methods on single retinular cells (Meinertzhagen et al. 1983).

The retinal sensitivity of *A. planipennis* to the 430-nm region of the spectrum may explain why purple (dark) control traps (430-nm main peak) are attractive to both sexes of *A. planipennis* (Francese et al. 2005, 2008; Crook et al. 2008a). However, it is difficult to explain the biological significance of the attraction of *A. planipennis* to purple. Varying the reflectance in purple paint did seem to be an important factor in trap catches between light and dark purple traps. The dark purple traps, with fairly low reflectance of around 23%, did seem to catch more insects than the lighter colored 75%-reflectance purple (only at traps placed near the ground). Purple traps generally captured more females than males, especially in the canopy. This contrasts with the reported sex ratio of male to female beetles collected from trees, which have been biased

Table 1. Number of *A. planipennis* caught on prism traps in the color comparison study

Trap color	Mean trap catch[a] (SE)	No. males[b]	No. females[b]	Ratio M:F[c]	No. after the median date[d]	No. before the median date[d]	% before the median date
Dark Blue	4.2 (1.2)c	14	34	0.41ab	25	23	52.1
Light Blue	7.7 (1.8)bc	41	39	1.05a	47	33	58.8
Dark Green	7.5 (1.5)abc	50	39	1.28a	38	51	42.7
Light Green	23.9 (6.8)a	134	147	0.91a	154	128	54.6
Dark Purple	12.0 (2.7)ab	56	85	0.66ab	65	73	47.1
Light Purple	8.3 (2.5)abc	34	62	0.55ab	38	59	39.2
Red	16.3 (4.3)ab	56	140	0.40b	99	97	50.5
Control	11.2 (2.5)ab	63	71	0.89a	71	63	53.0

[a] Mean trap catch (±SE) of *A. planipennis* on 1.5-m-high colored traps. Means with different letters indicate significance (α = 0.05; Tukey's HSD test); analyses performed on data transformed by ln (n + 1).

[b] Does not include *A. planipennis* of unidentifiable sex.

[c] Pairwise χ^2 analyses performed using a Bonferroni-adjusted α value (0.0018). Letters represent significant differences between color treatments.

[d] Includes all *A. planipennis* adults removed from traps.

Table 2. Number of *A. planipennis* caught on prism traps at two heights ($n = 9$)

Trap ht and color	Mean trap catch[a] (SE)	No. males[b]	No. females[b]	Ratio M:F[c]	No. before the median date[d]	No. after the median date[d]	% before the median date[c]
Mid-canopy							
Dark Green	306.7 (76.7)a	2030	730	2.78:1a	1216	1,558	43.8c
Light Green	297.9 (98.1)ab	1474	1206	1.22:1b	1521	1,166	56.6a
Dark Purple	131.9 (83.7)bc	342	845	0.41:1d	603	589	50.6b
Light Purple	29.9 (11.1)c	118	151	0.78:1c	128	148	46.4bc
Control	77.8 (26.5)bc	318	382	0.83:1bc	345	356	49.2bc
Ground (1.5 m)							
Dark Green	12.9 (3.9)ab	81	36	2.25:1a	39	77	33.6b
Light Green	30.1 (11.0)ab	119	153	0.78:1b	188	83	69.4a
Dark Purple	56.9 (31.1)a	157	358	0.44:1c	217	296	42.3b
Light Purple	7.3 (2.3)b	42	25	1.68:1ab	33	33	50.0b
Control	47.2 (17.2)a	212	213	1.00:1b	197	229	46.1b

[a] Mean trap catch (±SE) of *A. planipennis* on colored traps placed in the mid canopy and at 1.5 m. Means with different letters indicate significance ($\alpha = 0.05$; Tukey's HSD test); analyses performed on data transformed by ln (n + 1). Control = purple plastic trap.

[b] Does not include *A. planipennis* of unidentifiable sex.

[c] Pairwise χ^2 analyses were performed using a Bonferroni-adjusted α value (0.005). Letters represent significant within-ht differences between color treatments.

[d] Includes all *A. planipennis* adults removed from traps.

toward males (2.8:1; Rodriguez-Saona et al. 2006). Females may be more highly attracted to the secondary red peak that is reflected from purple plastic traps as they do seem to have an increased sensitivity to the 640–650 nm region of the visible spectrum compared with males. Red receptors ($\lambda_{max} > 565$ nm) seem to be relatively rare in insect vision systems but have been reported in the Odonata, Hymenoptera, Lepidoptera, and Coleoptera (Briscoe and Chittka 2001). The solitary bee *Callonychium petuniae* Cure & Wittmann, which is sensitive to 600 nm, is interesting because it visits purple *Petunia* flowers (Peitsch et al. 1992, Briscoe and Chittka 2001). The largest recorded λmax value recorded, 630 nm, was from a glaphyrid beetle, *Amphicoma* sp. (Briscoe and Chittka 2001). This beetle obtains its pollen diet from red, UV-light–absorbing flowers (Dafni et al. 1990). Based on our findings, female *A. planipennis* seem to have the highest λ_{max} value measured from an insect eye to date.

The reason for female *A. planipennis* to be more sensitive to red wavelengths (640–650 nm) is unclear. This region of the red spectrum does match the wavelength recorded from the characteristic red surface of both male and female abdomens. Wing fanning and basking behavior by both sexes (fully open wings, displaying the abdomen) have been reported to occur under laboratory and field conditions, but to date no obvious link has been made to suggest it is associated with precopulatory behavior (Lelito et al. 2007). The abdomen of *A. planipennis* is highly iridescent, varying from red to purple when lit from above, to bright gold when lit from the side (D.J.C., unpublished observation). Further studies need to be done to elucidate whether *A. planipennis* uses its abdomen coloration to attract and aggregate other adults to the tree canopy on bright sunny days. The fact that female *A. planipennis* are more sensitive to 640–650 nm than males suggests they may use this red region of the spectrum when seeking oviposition sites on the bark of ash trees. Apple (*Malus* spp.) tree bark, for example, reflects uniformly little energy at all wavelengths except red, where it rises slightly (Prokopy and Owens 1983). A similar trend has been seen for measurements taken from several types of pine (Strom et al. 1999, Campbell and Borden 2005) as well as varieties of black walnut, elm, hickory, and ash (D.W.B., unpublished results). Ongoing studies will examine the spectral properties of bark tissue from several ash species.

When placed in the mid-canopy, green traps may constitute a foliage-type stimulus that elicits food-seeking and/or host seeking behavior by *A. planipennis.* The light green paint we used for this experiment has very similar coloration and reflectance to freshly emerged ash leaves. We initially predicted that the light green trap would be more effective as the flight season progressed. We speculated that, as surrounding tree foliage matured and darkened, the light green traps would stand out as a contrasting source of younger leaf material that would potentially be a "supernormal" type stimulus (Prokopy and Owens 1983) that would be attractive to flying adult *A. planipennis.* Our results show that, for traps placed in the mid-canopy, most beetle catches on light green traps occurred early in the field season, whereas the majority of catch on dark green traps occurred later in the season. This may suggest that *A. planipennis* prefer darker, mature leaves for feeding and/or aggregation. Leaves of different plants differ in their levels of reflectance (or tint—amount of white) and intensity (amount of black). They do not, however, differ much in their wavelength (or hue), which is always at a yellow-green of ≈550 nm (Moericke 1969). This consistency is due to the absorption properties of chlorophyll, which is responsible for the dominant reflectance-transmittance hue of 500–580 nm (peak at 550 nm, with ≈20% of peak at 350 nm and 60% at 650 nm) (Prokopy and Owens 1983). Based on the ranges of spectral sensitivity reported here, it seems that *A. planipennis* is visually adapted to respond to the reflectance-transmittance of foliage. It is most likely that as a phytophagous and host-specific insect, *A. planipennis* is capable of visually locating host ash based

on a very specific combination of hue, luminance and saturation (Moericke 1969).

The spectral composition of ash leaves also coincides with the spectral wavelength range of *A. planipennis* elytra, although foliage does have a marginally higher reflectance. In the ash canopy, initial short-range attraction of flying males to sedentary females is visual (Lelito et al. 2007). Males have been shown to rapidly descend from a height of 30–100 cm directly onto a female. Male *A. planipennis* are therefore capable of visually distinguishing the subtle differences in reflectance between sedentary female adults and the lighter colored background ash foliage.

Olfactometer studies have shown that there is currently no strong behavioral evidence to suggest that there is a sex or aggregation pheromone for *A. planipennis* (Rodriguez-Saona et al. 2006). It should be noted, however, that dark green traps caught more than twice as many males than females both in the mid-canopy (2.78:1) and near the ground (2.25:1). This ratio was nearer 1:1 for light green traps, suggesting that males are more attracted to a darker green, possibly one that matches the wavelength and reflectance of adult elytra.

Traps in our height study caught more insects when placed in the mid canopy than at 1.5 m, supporting previous findings that showed that traps are more effective when placed higher up in the ash canopy (Crook et al. 2008a, Francese et al. 2008). Based on data from these studies, the most effective trap for detecting *A. planipennis* (throughout an entire field season) would be a green trap (540–550 nm) placed in the mid canopy. If detection is required early on in the flight season our results suggest it is more effective to use a lighter green trap (540–550 nm, reflectance of 64%) placed in the mid canopy. Ongoing work will aim to produce and test a green plastic trap based on the wavelengths and reflectance described here, and field test it with the latest lure attractants for *A. planipennis* (Crook et al. 2008a). It is hoped that this will result in a more sensitive and efficient trapping system that can be used in state-wide monitoring programs to detect new infestations of *A. planipennis.*

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