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Brown marmorated stink bug (Hemiptera: Pentatomidae) attraction to various light stimuli

John E. Cambridge^{1,*}, Laurie Francoeur², and George C. Hamilton³

Abstract

Light trapping is a common method for monitoring and capturing insects such as the invasive agricultural pest, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae). Efforts to develop more effective trapping methods for *H. halys* have led to research investigating the response of this insect to potentially exploitable stimuli. A behavioral study was conducted to examine the response of *H. halys* to various light stimuli. Seven intensities (0 [control: dark], 0.1, 10, 50, 75, 100, and 155 lx) of white light were tested. The most attractive intensity for *H. halys* was 75 lx for adult males and females. Nymphal instars 2 to 5, adult males, and adult females were also exposed to 75 lx white light. Adult males were significantly more attracted to the light than any other life stage. Adults were also exposed to green, orange, red, white, and yellow light. All colors tested were attractive to *H. halys*. White light was significantly more attractive than the other tested colors. The findings of this study suggest that the incorporation of a white light into *H. halys* traps may increase the number captured.

Key Words: *Halyomorpha halys*; light trap; intensity; wavelength; life stage

Resumen

La trampa de luz es un método común para monitorear y capturar insectos como la plaga agrícola invasora, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae). Los esfuerzos para desarrollar métodos de captura más eficaces para *H. halys* han llevado a investigar la respuesta de este insecto a estímulos potencialmente explotables. Se realizó un estudio de conducta para examinar la respuesta de *H. halys* a diversos estímulos de luz. Se ensayaron siete intensidades (0 [oscuro], 0,1, 10, 50, 75, 100 y 155 lx) de luz blanca. La intensidad más atractiva fue de 75 lx para machos y hembras adultas. Los estadios de ninfas de 2 a 5 instares, los machos adultos y las hembras adultas también fueron expuestos a 75 lx de luz blanca. Los machos adultos estaban significativamente más atraídos por la luz que cualquier otro estadio de vida. Los adultos también fueron expuestos a luz verde, anaranjada, roja, blanca y amarilla. Todos los colores probados fueron atractivos para *H. halys*. La luz blanca fue significativamente más atractiva que los otros colores probados. Los hallazgos de este estudio sugieren que la incorporación de una luz blanca en las trampas de *H. halys* puede aumentar las capturas.

Palabras Clave: *Halyomorpha halys*; trampa de luz; intensidad; longitud de onda; estadio de vida

For over one hundred years, light has been used to influence insect behavior in a variety of ways (Roth 1891; Harding et al. 1966). Insects may exhibit positive or negative phototaxis. These behaviors can be used to either attract or repel individuals (Jander 1963; Kim et al. 2013). Traps that employ light to catch insects are most effective at capturing individuals during the night time as sunlight can negate or mask their attractive influence (Shimoda & Honda 2013). Many insects have regular circadian rhythms or other behavioral patterns which are governed by the presence or absence of light. These can be exploited to disrupt undesirable pest activities (Walcott 1969; Shimoda & Kiguchi 1995). For example, some insects use light cues to orient themselves during flight or to identify suitable habitats. An understanding of these triggers has allowed growers to effectivity cloak green houses and other structures from certain nearby pests (Goodman 1965; Legarra et al. 2010). Moreover, researchers have evaluated the consistency of these types of behavioral responses across the visual spectrum and found that different insects express peak reactions at different wavelengths (von Helversen 1972; Coombe 1981; Hardie 1989; Kinoshita & Arikawa 2000).

Investigation into the underlying biology that is responsible for these behaviors has provided insight on why variation among species

occurs. While most of the insects which have been studied can be generally described as having an ultraviolet (UV)-blue-green trichromacy, there are several different pigments and configurations which insects may have within their compound eye (Briscoe & Chittka 2001; Koshitaka et al. 2008). Even within a species, males and females have been shown to have different wavelength sensitivities (Bernard & Remington 1991). Developers of light traps can use this type of information to tailor new devices to the optical peaks of the desired insect (Duehl et al. 2011).

Trapping for mosquitoes typically uses white, UV, or yellow lights (Li et al. 2015). In agricultural settings, UV light traps are commonly used to monitor population levels of pest species (Nielsen et al. 2013). Even when pheromones or other species-specific methods for trapping an insect have been developed, it is helpful to identify the effect of light as an additional incorporation into trapping devices or protocols (Duehl et al. 2011; Leskey et al. 2015b). *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), the brown marmorated stink bug, is a prime candidate for this type of investigation. Several semiochemicals, mainly pheromones and kairomones, have been identified and shown to be attractive to *H. halys* adults when used in baited traps at certain times. How-

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ever, this species is such a serious pest that the need still exists for trap refining and identification of non-pheromonal synergists to attract *H. halys* and increase catches (Leskey et al. 2015a).

H. halys is a highly polyphagous, invasive agricultural pest native to Asia whose introduction into North America has been traced back to eastern Pennsylvania in or before 1996 (Hoebeke & Carter 2003). Over the past 18 years, it has spread across the continent and established populations in 42 states, the District of Columbia, and portions of Canada (Leskey 2015). This insect is responsible for damage to numerous crops including soybeans, tomatoes, peppers, apples, peaches, corn, and cane berries (Rice et al. 2014). Population monitoring and both preemptive and responsive pesticide application are currently the primary strategies employed for control of this pest (Leskey et al. 2012a, 2012b). Furthermore, UV light traps have been successfully used to monitor for *H. halys* on both local and regional scales (Nielsen et al. 2011; Nielsen et al. 2013; Wallner et al. 2014).

Recent studies have called for more comprehensive research into how different wavelengths of light affect the behavior of stink bug pest species (Shimoda & Honda 2013). Leskey et al. (2015b) investigated the potential trapping uses of different colors of light and various intensities under field conditions and in individual laboratory-based choice trails, but called for future studies to look into the dynamics of these types of responses and specifically the area of arrestment around the light stimulus source. This current study further explores the potential use of light as an attractant for this insect by investigating how movement of *H. halys* differs between life stages and sexes in response to different intensities of white light and different colors of light across the visual spectrum in a laboratory setting.

Materials and Methods

Halymorpha halys adults and nymphs used in this study were taken from a lab colony maintained by the Rutgers Department of Entomology since 2004. Genetic diversity is maintained in this lab colony by continuously introducing wild stink bugs throughout multiple generations. Colony individuals were sustained on green beans (*Phaseolus vulgaris* L.; Fabaceae), organic sunflower seeds (*Helianthus annuus* L.; Asteraceae), carrots (*Daucus carota* L.; Apiaceae), and water at approximately 25 °C on a 16:8 h L:D photoperiod as described by Nielson et al. (2008). Standard maintenance protocols were used in accordance with Niva and Takeda (2003) in BugDorm2 cages (BioQuip™, Rancho Dominguez, California).

The study arena consisted of a 2.3 × 1.3 × 3.0 m room that was gridded into 10 cm squares over all walls, the ceiling, and floor using black paint (Valspar™, Minneapolis, Minnesota). A light socket (3M Company™, Flemington, New Jersey) was affixed to the center of four grid cells on one of the 2.3 m walls with a 1 cm disk of hot glue (3M Company™, Flemington, New Jersey). The distance from the light source was measured for each cell from the center of the square. The room was held at 27 °C and 30% relative humidity during all tests. No light was visible within the arena other than the light stimulus. All trials were conducted between Jun and Nov between 9 AM and 6 PM.

GENERAL PROTOCOL

For each trial, 10 *H. halys* individuals of a predetermined life stage were placed into an empty 1 L polyethylene cubical holding container for 10 min to allow them to acclimate to the environment and limit their alarm responses. Individuals which exhibited deformities, such as missing legs at any stage of development or wing malformations at the adult stage, were excluded. All individuals used in the experiments were released at a standardized point on the floor of the arena which was consistent between all trials. This standardized location was 155 cm away from the light

source. Following release, individuals were allowed to move freely within the room for 30 min. At the end of each 30 min trial, the distance from the light source of each stink bug was recorded according to the grid cell which it was found in. After each trial was completed, the room was aired out for 10 min in preparation for the following trial. Individual stink bugs were not used in a trial more than once per day and were placed back into the colony at the end of each testing day. Each trial was replicated 4 times. Individuals who died during the trial were excluded from the data set.

LIGHT BULBS

Color trials were conducted using compact fluorescent light bulbs (Brightech International™, Somerset, New Jersey) of a predetermined peak wavelength (560 nm: green, 590 nm: yellow, 750 nm: red, 460 nm: blue, and 640 nm: orange). The white light bulb used in the color trial was an incandescent light bulb. During dark trials, the light bulb was turned off. The tests investigating light intensity, life stage, and sex used an incandescent light bulb.

COLOR

One hundred and fifty eight mixed sex adult *H. halys* with an approximately 1:1 male to female ratio were tested for each color. Light bulbs in these trials had a luminosity of 30 lx. A digital lx meter (DrMeter™, Union City, California) was placed 0.50 m away from the light source to verify the intensity for each trial.

LIFE STAGE

The 2nd, 3rd, 4th, and 5th instars, as well as adult *H. halys*, were tested using a white light set to 75 lx. Trials were conducted in a randomized order. First instars were excluded from consideration as they do not move away from the egg mass under natural conditions.

INTENSITY

As described above, a digital lux meter (DrMeter™, Union City, California) was placed 0.50 m away from the white light source to determine the intensity for each trial. Trials were conducted in a randomized order. Lux readings of 0.1, 75, and 155 were used for the low, medium, and high intensity trials, respectively. An average of 40 males and 40 females were tested at each intensity using the general protocol. Males and females were tested separately to allow for comparison of the sexes.

DATA ANALYSES

The effect of the various light conditions, life stages, and sex was assessed by comparing the average distance of the individuals from the source being tested for each trial type in SAS® (SAS Institute Inc. 2014). Data were analyzed using a generalized linear model which assessed the effect of life stage, light color, adult sex, or light intensity, and their interactions on the average distance of *H. halys* from the light source. The generalized linear model was used to calculate means for each trial type. An ANOVA was used to determine any differences within the trials, and then a Tukey honest significant difference (HSD) test was used to separate these means and determine statistical groups (SAS Institute Inc. 2014). The relative attractiveness of each light type was evaluated using the results from the Tukey HSD, and statistically distinct groups ($P \leq 0.05$) with a closer average distance to the light stimulus were determined to be more attractive.

Results

COLOR

Of all the colors tested, white light was the most attractive stimulus tested as determined by it having the smallest ending average distance

at 83 cm from the light source. The dark trials had an average ending distance of 156.3 cm. All colored lights had a small ending distance and had an attractive effect on *H. halys* ($P < 0.0001$, $df = 5$, $F = 23.96$). Green, orange, red, yellow, and blue all had closer ending average distances than the initial release distance, which were 104.3 cm, 112.8 cm, 112.1 cm, 107.8 cm, and 94.4 cm respectively. All measured travel ending distances for individuals used in the colored light trials were significantly different than ending travel distances in the dark trials. The ending distances of the white light trials were significantly different than the ending distances recorded from all other color light trials ($P < 0.05$) (Fig. 1).

LIFE STAGE AND SEX

The attractive influence of 75 lx white light was significantly different between life stages ($P < 0.0001$, $df = 5$, $F = 15.95$). Nymphs were not attracted to the light and the responses of 2nd, 3rd, 4th, and 5th instar *H. halys* were not significantly different from one another ($P > 0.05$). Nymphs averaged 113.6 cm, 136.1 cm, 136.6 cm, and 140.1 cm away from the light source at the end of the trial for 2nd, 3rd, 4th, and 5th instars, respectively. Adult males averaged 59.2 cm and adult females averaged 103.5 cm away from the light source. Males were significantly more attracted to the light source than all other life stages ($P < 0.005$; ANOVA). (Fig. 2).

INTENSITY AND SEX

The interaction of sex and light source intensity had significant influence on the response of *H. halys* ($P < 0.05$ $df = 4$, $F = 3.47$). Intensity alone showed a significant difference between the tested lux levels ($P < 0.0001$, $df = 4$, $F = 9.88$); however, sex did not ($P = 0.06$, $df = 1$, $F = 3.53$). Males had a significantly different response to the changes in light intensity than did females ($P < 0.05$). When white light was dimmed to 20 or 0.1 lx, it did not induce a significant response from either sex of adult *H. halys* ($P > 0.05$). Both males and females had the shortest average distance away from the light source at 75 lx (Fig. 3).

Discussion

The results of the laboratory studies conducted here are consistent with the previous findings that *H. halys* individuals exhibit a positive

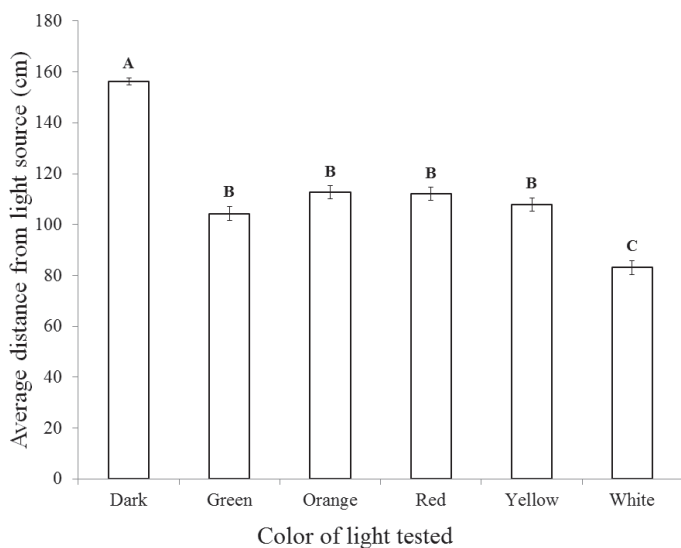


Fig. 1. Average distance *Halyomorpha halys* adults were found away from the light source for each color tested at 30 lx using mixed sex adult *H. halys* populations. Dark (0 lx) trials were used as the no-attractive-stimulus control. Error bars represent the standard error. Bars with the same letter are not significantly different ($P < 0.05$; ANOVA and Tukey HDS).

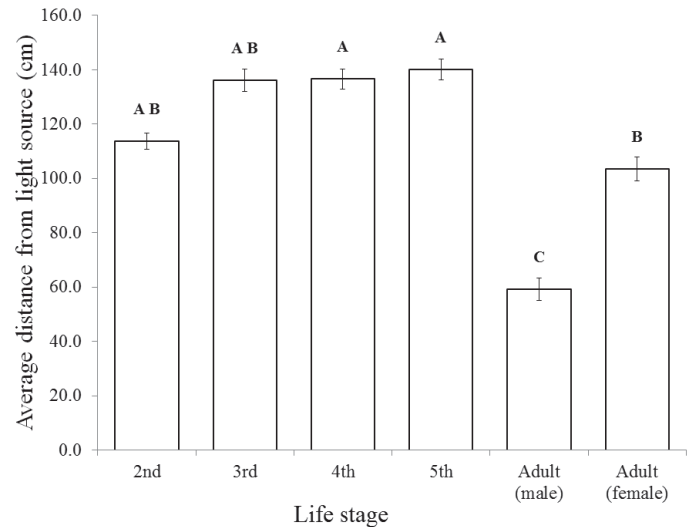


Fig. 2. Average distance *Halyomorpha halys* individuals were found away from the light source for each life stage tested at 75 lx using white light. Error bars represent the standard error. Bars with the same letter are not significantly different ($P < 0.05$; ANOVA and Tukey HDS).

phototactic response, orient themselves towards visual light sources, and show a distinct preference for white light over other colors in a laboratory setting (Leskey et al. 2015b). This investigation further explored several of the behaviors described in Leskey et al. (2015b) and identified that the average distance of *H. halys* around a light source depended on the light type and intensity. The area of arrestment around a light source for *H. halys* is of particular interest, because it can provide valuable insight into how new traps should be designed to effectively monitor this pest. The data from this study suggests that when *H. halys* are attracted to a light source, some of the individuals will cluster directly around it while others will stay a short distance away (Fig. 4). More research should be done to investigate the individuals that do not cluster directly around the light and see if the average proportion exhibiting this behavior is consistent. If traps use light sources as an attractant, the data they produce must be interpreted

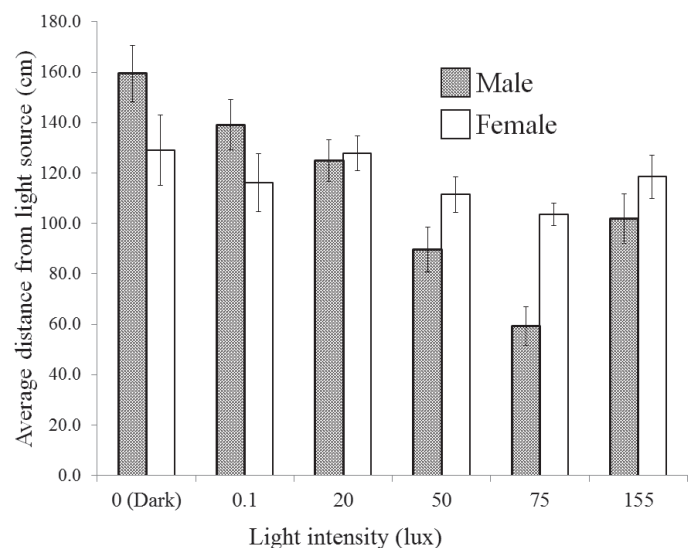


Fig. 3. Average distance *Halyomorpha halys* adults were found away from the light source for each combination of sex and intensity. Error bars represent the standard error.

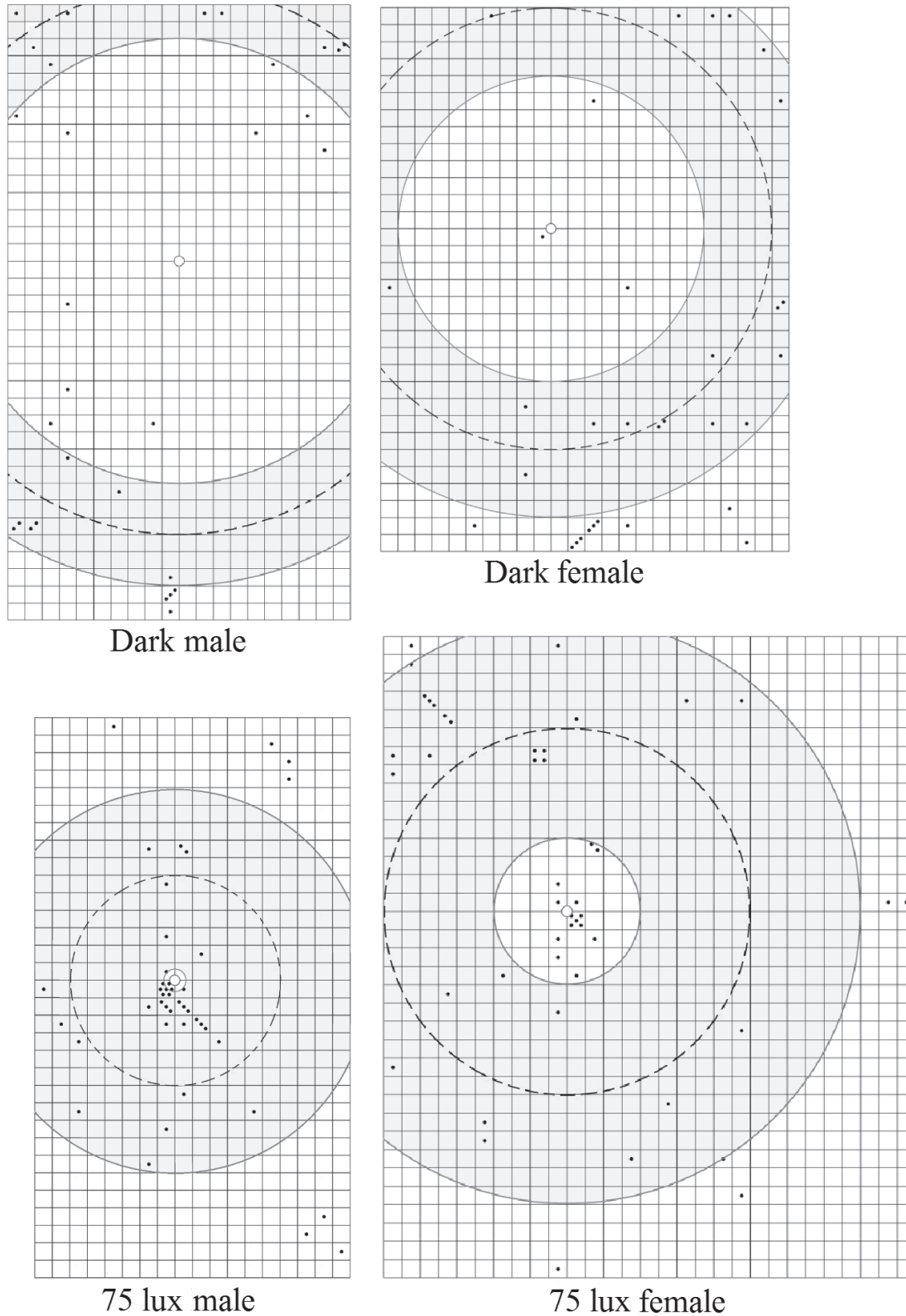


Fig. 4. The distribution of *Halyomorpha halys* around the light source for the adult male and female trials for both the 0 (dark) and 75 lx trials. The grid shown is a two dimensional representation of the distances for each stink bug as the actual data was not gathered on a flat plane. The light source is demarcated by the small circle in the center. The average distance for the group is shown with the black dashed circle. Grey filled-in circles show 1 SD from the mean. Means and SD values were rounded to the nearest 10 cm, and the angle of *H. halys* that were not found on the light wall were estimated to show general direction in this figure. The ending position of each *H. halys* adult is represented by a black dot.

in a manner that accounts for the individuals that were within the attractive range, but not induced to enter the trap. If the percentage of *H. halys* that are sufficiently attracted to a light source such that they enter a trap is not consistent, it may indicate that light is not a suitable lure for monitoring this species. The data collected in the study suggests that more research is needed to assess whether or not light is a reliable attractant for *H. halys*.

Light does not appear to be an effective way to attract nymphal *H. halys*. As light trapping for this species has traditionally failed to capture nymphs (Nielsen et al. 2011; Nielsen et al. 2013; Wallner et al. 2014; Leskey et al. 2015b), there is limited data on the behavior of nymphs to light stimulus. This is the first study to investigate the response of *H. halys* nymphs to white light. The 2nd, 3rd, 4th, and 5th instar nymphs did not respond differently to the light source and appeared not to show any positive phototaxis throughout the trials (Fig. 2). Adult *H. halys* males were significantly more attracted to the light source than any of the nymphal life stages, and adult females were significantly more attracted to the light source than 4th and 5th instars. An explanation for these findings is that *H. halys* adults may use astronavigation for orientation during nighttime flight as has been documented in various other insects (Sotthibandhu & Baker 1979; Wehner 1984; Dacke et al. 2003). If this is the case, nymphal instars would have no use for such a behavior as they are incapable of flight. Further research is needed in this area in order to further understand the response of *H. halys* nymphs to light.

Adults *H. halys* showed variable responses to white light at different intensities. As the light was dimmed to 20 lx and below, it appeared that the stimulus was too faint to elicit a response as average distance from the light source was not significantly different from the dark control. Females did not show as strong an attraction to the light as did males. As the intensity increased from 20 lx to 75 lx, the average distance around the light decreased by 5.6% in females and 40.2% in males. As the intensity was increased further to 155 lx, the trend changed and the average distance increased, although there remained a significant overall attractive response of males to the light source as compared with males released in the lower light intensity trials. These findings support the idea that there is a minimum detection threshold of light intensity at which *H. halys* does not exhibit a positive phototaxis.

The large variability of stopping distances at all intensities presents an issue for assigning an optimal intensity to attract these insects. Many of the individuals tend to disregard the stimulus, while others cling to the blub. Further investigation is needed to clarify these issues. The authors also note that temperature played a role in the behavior of the insects in these conditions. All trials reported in this study were held at 27 °C. However, several of the initial trials had to be excluded from data analysis, because the temperature controls failed and the room either increased to temperatures over 30 °C or decreased to temperatures below 23 °C. During the trials that experienced these temperature extremes, the insects did not appear to move at all. However, other research into the dispersal behaviors of this insect show that flight is indeed possible at lower temperatures (Lee & Leskey 2014). One interpretation of this data is that *H. halys* is less likely to respond to light stimulus during periods of temperature fluctuation, regardless of the suitability of the temperatures themselves.

This study showed that *H. halys* response to light is different between life stages, light colors, and light intensities. These findings can be used to develop better sampling methods for this insect, but much more research is needed to fully understand all the intricacies of phototactic behaviors of this insect. In order to explore some of these issues, it may be useful to conduct a contained field study to acquire a more applied understanding of the response of *H. halys* to light stimuli.

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