

## BEHAVIORAL RESPONSES OF LARVAE AND ADULTS OF *ESTIGMENE ACREA* (LEPIDOPTERA: ARCTIIDAE) TO LIGHT OF DIFFERENT WAVELENGTHS

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### ABSTRACT

We investigated the behavioral responses of neonate and mature (6th instar) larvae, and mated females of *Estigmene acrea* (Drury) (Lepidoptera: Arctiidae) to a range of wavelengths under laboratory conditions. The behavioral responses of *E. acrea* were determined by means of choice tests, exposing the insects to 2 different wavelengths, ranging from 340-670 nm (ultraviolet to red colors), of the same intensity in selection chambers. Both neonate and mature larvae were significantly more attracted to 380, 400, and 520 nm than to the control wavelength of 570 nm. Because *E. acrea* is a generalist species that moves between plants to feed, it may be important for a larva to detect and move towards green foliage in preference to the ground or other objects. Mated females were significantly more attracted to the wavelengths of 340, 350, 370, 380, 420 and 460 nm than to the control wavelength. Females may use ultraviolet and blue lights as orientation cues during the searching behavior for oviposition sites.

Key Words: Lepidoptera, Arctiidae, larvae, generalist, phototaxis

### RESUMEN

En este estudio, investigamos la respuesta comportamental de las larvas neonatas y maduras (6to instar), y hembras apareadas de *Estigmene acrea* (Drury) (Lepidoptera: Arctiidae) a una gama de longitudes de onda en condiciones de laboratorio. Las respuestas comportamentales de *E. acrea* se determinaron por medio de pruebas de doble elección, exponiendo los insectos a dos diferentes longitudes de onda, que van desde 340-670 nm (de ultravioleta a rojo), de la misma intensidad, en cámaras de elección. Tanto las larvas neonatas como las maduras fueron significativamente más atraídas a las longitudes de onda de 380, 400, y 520 nm que la longitud de onda control de 570 nm. Las hembras fueron significativamente más atraídas a las longitudes de onda de 340, 350, 370, 380, 420, y 460 nm que la longitud de onda control.

Translation provided by the authors.

The selection of a food source or a site for oviposition is a crucial decision in the life of herbivorous insects. This selection is based on the perception of visual, olfactory, mechano-sensory, and gustatory cues (Schoonhoven et al. 2005). Generally, these types of cues are often used in an integrated way. In addition, internal and external biotic and abiotic factors may affect the host plant selection of herbivorous insects (Bernays & Chapman 1994).

The saltmarsh caterpillar moth, *Estigmene acrea* (Drury), is a generalist species feeding on more than 60 plant species. Previous studies have shown that mated females are attracted to host plant volatiles (Castrejón 2006) and neonate larvae use chemical cues during host plant searching behavior (Castrejón et al. 2006). *Estigmene acrea* larvae use chemical cues for the specific rec-

ognition of plants that contain pyrrolizidine alkaloids (Hartmann et al. 2005). In contrast, nothing is known about the role of visual cues during host plant selection behavior displayed by *E. acrea*, although it is accepted that visual cues are important during host searching behavior by herbivorous insects (Prokopy & Owens 1983). Insects may use shape, dimension, color, and the pattern or architecture of the host plants during its host searching behavior. Because of the rather poor resolution of insect eyes, color is considered the most important long-range visual cue (Brown et al. 1998).

In humans, color can be characterized from a psychophysical perspective by its hue, saturation, and intensity (Long et al. 2006). We do not have an equivalent psychophysical knowledge of invertebrate sensory perception, but we do know that

the insect eye is sensitive to the wavelength and intensity of light and that insects are differentially attracted to light of different wavelengths (Briscoe & Chittka 2001).

We have investigated the behavioral responses of neonate and mature (6th instar) larvae, and mated females of *E. acrea* to a range of ecologically relevant wavelengths of constant intensity under laboratory conditions.

## MATERIALS AND METHODS

### Insects

The insects were reared on an artificial diet based on maize flour, wheatgerm, and Brewer's yeast as described by Castrejón et al. (2006). The insects were reared under controlled conditions at 24-26.5°C, 60-90% relative humidity, and a photoperiod of 12: 12 (L:D) h. Recently-emerged larvae were reared on the diet in groups of about 200 individuals in plastic containers (30 × 20 × 7 cm) until the fourth instar, after which they were individually transferred to plastic cups (45 × 30 mm) with lids, containing about 2 mL of diet until pupation. Pupae were carefully extracted from the silken cocoons, sorted by sex, and held separately in Petri dishes. Before eclosion, 40 pupae (20 per sex), were placed in separate screen cages (30 × 30 × 30 cm) for adult emergence. The newly emerged moths were allowed to mate and lay eggs until the insects died. The moths were fed with a 10% sugar solution dispensed on cotton wool on the floor of the cage. The neonate larvae used for experiments were directly removed from the mating cages with a paintbrush. Mature larvae were taken from the plastic cups with lids. Mated females were obtained by pairing virgin females and males in a cage, and 2- to 4-day-old females were used in the bioassays. All experiments were performed between 0800 and 1900 h at 25 ± 1°C, and 55 ± 5% r.h.

### Larval Response

The behavioral response of larvae of *E. acrea* was determined by means of choice tests, exposing the insects to 2 different wavelengths of the same intensity in a linear selection chamber. The selection chamber used was adapted from that described by Brown et al. (1998). The selection chamber consisted of a T-shaped glass tube (20 mm internal diameter). The side arm was 50 mm long and was at the center of the main arm (300 mm long), the main arm was marked in three 100-mm sections. The selection chamber was placed on a wood base. A 20-nm bandpass filter (Andover Corp., NH) was placed at each end of the main arm of the selection chamber. The light was provided by 2 fiber optic illuminators (Fiber-Lite PL-750, Dolan-Jenner Industries, MA), one at

each end of the main arm. In order to assure that light intensity at each end of the main arm was the same, independent of wavelength, light intensity was measured with a solar cell and was adjusted with the adjustment button incorporated in each illuminator so that the cell generated a direct current of 0.1 µA and 2.3 mV, measured with a digital microamperimeter (Master® Mod. MAS830L). This system of measurement was used because a conventional photometer offers reliable readings only within the visible spectrum, however, the solar cells produce direct electrical current (measured in µA in this system) almost proportionally in relation to the intensity of the incident light, even in wavelengths outside the visible spectrum (whereas the voltage, measured in mV, remains almost constant) (Duffie & Beckman 1991). During the bioassays, the selection chamber was covered with a black cardboard box, so that the only light in the chamber originated from the fiber optic illuminators.

One end of the main arm was illuminated with a control wavelength of 570 nm, a yellow color for which most insects have photoreceptors (Briscoe & Chittka 2001). The opposite end was illuminated by one of 14 test wavelengths: 340, 350, 370, 380, 400, 420, 460, 490, 520, 540, 590, 640, 650, and 670 nm. Either a group of 10 neonate larvae or 1 sixth instar, depending on which were the most active, was introduced into the selection chamber through the side arm. The lights were turned off and the selection chamber was covered with the cardboard box. Larvae were allowed to move freely inside the selection chamber during 5 min (3 min in the case of mature larvae as they moved faster than neonate larvae). The number of individuals in each section of the tube was recorded. This was considered a replication; the position of the filters was reversed every 5 replications. The expected theoretical attraction to a test wavelength of 570 nm is 50%.

Periodically, the selection chamber was washed with acetone to eliminate some possible remains left by the larvae. For each wavelength tested, 10 replications were made for the neonate larvae, and 50 replications for the sixth instars. In both cases the insects were used only once. The wavelengths were tested randomly.

### Female Response

The behavioral response of mated females was evaluated in a selection chamber that consisted of a cardboard box (30 cm long, 11 cm wide and 6 cm high) painted with black acrylic paint (Createx Colors, CT). The filters were placed in wood supports in windows in the center of the narrow sides of the box. The fiber optic illuminators were placed outside the chamber. One end of the chamber was illuminated with a control wavelength of 570 nm, and the other by 1 of 14 test wavelengths

ranging from 340-670 nm. Females were allowed to move freely inside of the chamber for 5 min, and then the number of individuals lying on the wall where the filter was placed was recorded. Females were tested individually and used only once. For each wavelength 25 replications were used. All the observations were performed between 0.5 and 3 h after commencing scotophase, the peak oviposition period displayed by *E. acrea* females (Castrejón 2006).

#### Statistical Analysis

The *G* test with William's correction (Sokal & Rohlf 1998) was used to compare the responses of neonate and mature larvae, and females to the test wavelengths versus the control wavelength.

### RESULTS

#### Larval Response

Neonate larvae were more attracted to wavelengths between 370 and 540 nm than to the control wavelength (570 nm), with the exception of 490 nm. In contrast, neonate larvae were more attracted to the control wavelength than to the wavelengths of 340, 350, 590, 640, 650, and 670 nm (Table 1).

Mature larvae were more attracted to wavelengths of 380, 400, and 520 nm than to the control wavelength (570 nm). In contrast, mature larvae were more attracted to the control wavelength than to the 340, 350, 370, 590, 640, 650, and 670 nm wavelengths. The responses of mature larvae to wavelengths of 420, 460, 490 and 540 nm showed no differences when compared to the control wavelength (Table 1).

#### Female Response

Females were significantly more attracted to wavelengths of 340, 350, 370, 380, 420 and 460 nm than to the control wavelength (570 nm). In contrast, females were less attracted to wavelengths of 640, 650 and 670 nm than to the control wavelength. Female responses to wavelengths of 400, 490, 520, 540 and 590 nm were not different to those observed with the control wavelength (Table 1).

### DISCUSSION

We have investigated the behavioral responses of immature stages and adult females of *E. acrea* to light of different wavelengths. Although neonate and mature larvae, and adult females showed similar responses to the wavelengths tested, there are some differences in responses of the different biological stages. Within the UV region, neonate larvae were more attracted to the wavelengths of 370 and 380 nm, mature larvae were more attracted to the 380 nm wavelength, and mated females were more attracted to 340, 350, 370, and 380 nm when compared to the control wavelength of 570 nm. Within the violet and blue regions, neonate larvae were more attracted to wavelengths of 400, 420, and 490 nm, mature larvae were more attracted to 400 nm, and females were more attracted to the 420 and 460 nm wavelengths in comparison to the 570 nm, the wavelength control. Within the green region, neonate larvae were more attracted to the wavelengths of 520 and 540 nm, mature larvae were more attracted to 520 nm in comparison to the wavelength control, while females did not show any preferences for any of the wavelengths tested.

TABLE 1. BEHAVIORAL RESPONSES OF NEONATE, SIXTH INSTARS, AND FEMALES OF *ESTIGMENE ACREA* TO LIGHT OF DIFFERENT WAVELENGTHS COMPARED WITH A WAVELENGTH CONTROL OF 570 NM.

Wavelength (nm)	Neonate	G	P	Sixth instars	G	P	Females	G	P
340 (ultraviolet)	0	—	—	0	—	—	83.33	11.41	<0.001
350	1.61	75.11	<0.001	23.40	13.86	<0.001	77.78	8.66	<0.01
370	75.61	11.15	<0.001	30.43	7.16	<0.01	84.62	13.46	<0.001
380	94.52	69.71	<0.001	67.39	5.62	<0.05	70.97	5.53	<0.05
400 (violet)	83.02	24.95	<0.001	65.12	3.95	<0.05	61.54	1.37	NS
420	70.21	7.82	<0.01	61.11	1.77	NS	86.21	16.64	<0.001
460 (blue)	82.81	29.76	<0.001	46.94	0.18	NS	72	4.91	<0.05
490	59.42	2.45	NS	64.44	3.77	NS	52	0.04	NS
520 (green)	93.51	69.28	<0.001	67.39	5.62	<0.05	47	0.12	NS
540	75	18.71	<0.001	56.53	0.78	NS	52	0.04	NS
590 (orange)	18.97	23.86	<0.001	29.79	7.82	<0.01	40	0.99	NS
640	0	—	—	0	—	—	20	9.44	<0.01
650 (red)	0	—	—	0	—	—	30.77	3.87	<0.05
670	0	—	—	0	—	—	12	15.99	<0.001

Finally, the immature stages were more attracted to the wavelength control than to wavelength 590 nm (orange), whereas females did not show any preference.

The observed peaks of attraction of the larval stages of *E. acrea* to the wavelengths tested agree with the results obtained with other Lepidoptera. The larvae of Lepidoptera so far investigated showed responses in 3 regions of the spectrum: 340-370 nm, 440-450 nm, and 520-540 nm, although these values have been obtained by means of electrophysiological intracellular recordings (Gilbert 1994; Lin et al. 2002, and references therein). Electrophysiological recordings can indicate that the photoreceptors are present in the visual system of insects, but they will not necessarily indicate if wavelengths are attractive to the insects (Cowan & Gries 2009). For example, *B. mori* larvae showed electrophysiological responses to 350, 450 and 530 nm, but they were only attracted to wavelengths of < 364 and to 557 nm (Kitabatake et al. 1983; Shimizu 1981).

Although the results obtained cannot be extrapolated to specific behavior in natural conditions, it can be argued that they may be related to important aspects of insect behavior. For example, it is important for neonate *E. acrea* larvae to orient themselves and to locate a suitable food source. Some Lepidoptera neonate larvae show a phase of dispersion before they feed for the first time, and this dispersion can occur in the same plant where they hatched or towards other plants located at greater distances (Berger 1992; Saxena 1990). *Estigmene acrea* is a generalist species that moves between plants to feed (Bernays et al. 2004) and therefore it would be very important for the larvae to detect green foliage and to differentiate it from the surrounding plants, the sky, ground, and other objects. Mature larvae showed positive phototaxis and spectral preference, probably because of their foraging habits. They tend to disperse presumably from the original sites of feeding in search for plants with pyrrolizidine alkaloids; initially they disperse at random until finding a potential host, which is tasted before being accepted as food (Bernays et al. 2004). In these circumstances, it must be important for the larvae to orient themselves and to visually differentiate the possible hosts from other non-relevant objects. In other Lepidoptera species, mature larvae move away from the light in search of refuge (Ruiter & van der Horn 1957; Omino et al. 1973).

Unlike larvae, phototaxis in *E. acrea* females was observed virtually for all tested wavelengths, although the highest responses occurred in the UV and blue regions. The attraction of females to UV and blue may indicate that *E. acrea* use these lights as orientation cues as reported for other moths. Catch of mated females of *Lambina fiscelaria* in UV traps was attributed to searching for oviposition sites (Delisle et al. 1998). *Plodia inter-*

*punctella* adults take long-distance foraging flights in the twilight hours of scotophase when blue light dominates the irradiance spectrum of the sky (Cowan & Gries 2009). We cannot rule out that the attraction towards UV may simply be a response to a possible escape route when the insects are in confined places (Mikkola 1972; Kevan et al. 2001).

The fact that *E. acrea* adults showed a greater sensitivity in all the tested wavelengths may be due to greater complexity of the compound eye compared with the ocelli of the larvae, and possibly to a greater amount of photoreceptor pigments. Adult Lepidoptera possess 6 different photoreceptor pigments (Arikawa et al. 1999; Briscoe & Chittka 2001). Electrophysiological recordings in 35 species of Lepidoptera showed that they responded to wavelengths of 360-400, 420-460, and 520-560 nm (Eguchi et al. 1982). Field experiments showed that *Coloradia pandora* adults were attracted to but did not discriminate between colors within the visible spectrum (Gerson & Kelsey 1997).

In conclusion, this study showed that neonate and mature larvae, and adult females of *E. acrea* were able to discriminate between luminous stimuli of the same intensity but different wavelengths. From a practical point of view the information obtained in this study may eventually be used to improve the efficiency of traps used for monitoring lepidopteran insect pests.

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#### REFERENCES CITED

- ARIKAWA, K. S., MIZUNO, D., SCHOLTEN, M., KINOSHITA, T., SEKI, J., KITAMOTO, J., AND STAVENGA, D. 1999. An ultraviolet absorbing pigment causes a narrow-band violet receptor and a single-peaked green receptor in the eye of the butterfly *Papilio*. *Vision Res.* 39: 1-8.
- BERGER, A. 1992. Larval movements of *Chilo partellus* (Lepidoptera: Pyralidae) within and between plants: timing, density responses and survival. *Bull. Entomol. Res.* 82: 441-448.
- BERNAYS, E. A., AND CHAPMAN, R. F. 1994. Host-plant Selection by Phytophagous Insects. Chapman & Hall, NY. 312 pp.
- BERNAYS, E. A., SINGER, M. S., AND RODRIGUES, D. 2004. Foraging in nature: foraging efficiency and attentiveness in caterpillars with different diet breadths. *Ecol. Entomol.* 29: 389-397.
- BRISCOE, A. D., AND CHITTKA, L. 2001. The evolution of color vision in insects. *Annu. Rev. Entomol.* 46: 471-510.
- BROWN, P. E., FRANK, C. P., GROVES, H. L., AND ANDERSON, M. 1998. Spectral sensitivity and visual conditioning in the parasitoid wasp *Trybliographa rapae* (Hymenoptera: Cynipidae). *Bull. Entomol. Res.* 88: 239-245.

- CASTREJON, A. F. 2006. Comportamiento de Oviposición y de Búsqueda de Hospedero de *Estigmene acrea* (Drury) (Lepidoptera: Arctiidae). Doctoral Thesis. El Colegio de la Frontera Sur. México. 122 p.
- CASTREJON, A. F., VIRGEN, A., AND ROJAS, J. C. 2006. Influence of chemical cues from host plants on the behavior of neonate *Estigmene acrea* larvae (Lepidoptera: Arctiidae). *Environ. Entomol.* 35: 700-707.
- COWAN, T., AND GRIES, G. 2009. Ultraviolet and violet light: attractive orientation cues for the Indian meal moth, *Plodia interpunctella*. *Entomol. Exp. Appl.* 131: 148-158.
- DELISLE, J., WEST, R. J., AND BROWERS, W. W. 1998. The relative performance of pheromone and light traps in monitoring the seasonal activity of both sexes of the eastern hemlock looper, *Lambdina fiscellaria fiscellaria*. *Entomol. Exp. Appl.* 89: 87-98.
- DUFFIE, J. A., AND BECKMAN, W. A. 1991. *Solar Engineering of Thermal Processes*. 2nd Ed. John Wiley & Sons, Inc. 944 pp.
- EGUCHI, E., WATANABE, K., HARIYAMA, T., AND YAMAMOTO, K. 1982. Comparison of electrophysiologically determined spectral responses in 35 species of Lepidoptera. *J. Insect Physiol.* 28: 675-682.
- GERSON, E. A., AND KELSEY, R. G. 1997. Attraction and direct mortality of pandora moths, *Coloradia pandora* (Lepidoptera: Saturniidae), by nocturnal fire. *Forest Ecol. Manag.* 98: 71-75.
- GILBERT, C. 1994. Form and function of stemmata in larvae of holometabolous insects. *Annu. Rev. Entomol.* 39: 323-49.
- HARTMANN, T., THEURING, C., BEUERLE, T., KLEWER, N., SCHULZ, S., SINGER, M. S., AND BERNAYS, E. A. 2005. Specific recognition, detoxification and metabolism of pyrrolizidine alkaloids by the polyphagous arctiid *Estigmene acrea*. *Insect Biochem. Mol. Biol.* 35: 391-411.
- KEVAN, P. G., CHITTKA, L., AND DYER, A. G. 2001. Limits to the salience of ultraviolet: lessons from colour vision in bees and birds. *J. Exp. Biol.* 204: 2571-2580.
- KITABATAKE, S., SHIMIZU, I., AND KATO, M. 1983. Wavelength-dependent properties of phototaxis in larvae of *Bombyx mori*. *Photochem. Photobiol.* 37: 321-327.
- LIN, J. T., HWANG, P. C., AND TUNG, L. C. 2002. Visual organization and spectral sensitivity of larval eyes in the moth *Trabala vishnou*, Lefebur (Lepidoptera: Lasiocampidae). *Zool. Stud.* 41: 366-375.
- LONG, F., YANG, Z., AND PURVES, D. 2006. Spectral statistics in natural scenes predict hue, saturation, and brightness. *Proc. Natl. Acad. Sci. USA* 103: 6013-6018.
- MIKKOLA, K. 1972. Behavioural and electrophysiological responses of night-flying insects, especially Lepidoptera, to near-ultraviolet and visible light. *Ann. Zool. Fenn.* 9: 225-254.
- OMINO, T., YOKOI, S., AND TSUJI, H. 1973. Experimental studies on the daytime behaviour of noctuid larvae, the cabbage armyworm, *Mamestra brassicae*, the tobacco cutworm, *Spodoptera litura*, and the black cutworm, *Agrotis ipsilon*. *Japanese J. Appl. Entomol. Zool.* 17: 215-220.
- PROKOPY, R. J., AND OWENS, E. D. 1983. Visual detection of plants by herbivorous insects. *Annu. Rev. Entomol.* 28: 337-364.
- RUITER, L. DE, AND HORN VAN DER, I. J. 1957. Changes in phototaxis during the larva life of the eyed hawk moth. *Nature* 179: 1027.
- SAXENA, K. N. 1990. Mechanisms of resistance/susceptibility of certain sorghum cultivars to the stem borer *Chilo partellus*: role of behaviour and development. *Entomol. Exp. Appl.* 55: 91-99.
- SCHOONHOVEN, L. M., VAN LOON, J. J. A., AND DICKE, M. 2005. *Insect-plant Biology*. Oxford University Press, Oxford. 421 pp.
- SHIMIZU, I. 1981. Phototactic behavior in the silkworm, *Bombyx mori*. I. Adaptive significance of phototaxis in newly hatched larvae. *Environ. Ctrl. Biol.* 19: 41-49.
- SOKAL, R. R., AND ROHLF, F. J. 1998. *Biometry*. Third edition. W. H. Freeman & Company, NY. 880 pp.