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ScienceDirect
Journal of Insect Physiology 53 (2007) 509–516
Journal of Insect Physiology
www.elsevier.com/locate/jinsphys

Chromatic cues to trap the oriental fruit fly, *Bactrocera dorsalis*

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Received 27 December 2005; received in revised form 5 February 2007; accepted 5 February 2007

Abstract

Various colors have been used as visual cues to trap insect pests. For example, yellow traps for monitoring and control of the oriental fruit fly (*Bactrocera dorsalis*) have been in use for a very long time. However, the chromatic cue of using color traps has never been meticulously investigated. In this study, the spectral sensitivities of the photoreceptors in the compound eyes of *B. dorsalis* were measured intracellularly, and the theory of receptor quantum catch was applied to study the chromatic cue of fly attracting. Responses to five wavelength categories with peak wavelengths of 370, 380, 490, and 510 nm, and one with dual peaks at 350 and 490 nm were recorded. Based on spectral sensitivities, six colored papers were chosen to test the color preference of the fly, and an additional UV preference test was done to confirm the effect of the UV stimuli. It was concluded that UV and green stimuli (spectra: 300–380 nm and 500–570 nm) would enhance the attractiveness of a colored paper to the oriental fruit fly, and blue stimuli (380–500 nm) would diminish the attractiveness.

Keywords: Spectral sensitivity; Color preference; Oriental fruit fly; *Bactrocera dorsalis*

1. Introduction

Several species of fruit flies (Tephritidae) are major and widespread destructive pests in agriculture around the world (Christenson and Foote, 1960). Their oviposition causes serious damage to fruit crops, costing millions of dollars every year. To control and monitor these pests, many studies have been devoted to trap-designs (Katsoyannos, 1989; Roessler, 1989; Heath et al., 1993). Since host-finding is the indispensable stage for discovering a suitable place for oviposition, and since it is strongly influenced by both visual and olfactory cues (Prokopy and Owens, 1983; Prokey et al., 1990), the best strategy to attract these flies is by using these cues to develop effective traps to lure them.

The oriental fruit fly, *Bactrocera dorsalis*, is the main fruit fly pest in the Pacific Rim (Haramoto and Bess, 1970; Liu, 1981), and numerous lure-and-kill traps with yellow colored surfaces have been developed and applied in the field, such as yellow sticky papers and the famous male-specific methyl eugenol-baited traps (Cornelius et al., 1999; Alyokhin et al., 2000; Chen and Dong, 2001). Both Vargas et al. (1991) and Cornelius et al. (1999) demonstrated that yellow fruit-mimicking spheres were an excellent device to lure the oriental fruit fly, and Alyokhin et al. (2000) demonstrated that the visual cues of a yellow fruit-mimicking sphere trap could increase the attractiveness of hydrolyzed liquid protein odor. It has been shown that visual and olfactory cues can function separately and have a complementary effect to each other for attracting flies (Jang and Light, 1991; Vargas et al., 1991; Cornelius et al., 1999; Alyokhin et al., 2000; Corneluis et al., 2000a, b). Thus, to optimize lure-and-kill traps, these cues must be investigated carefully using experiments based on neuroethological aspects. However, most of these studies were done only on olfactory cues for tephritids (Light et al., 1992; Raptopoulos et al., 1995). Also, it should be noted that using a color name as a reference to describe a fly's preference is not correct, since our color vision is quite

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doi:10.1016/j.jinsphys.2007.02.003

different from that of an insect, and it is better to define the fly's chromatic preference using the insect visible spectrum. In this paper, we report the wavelength preference of the oriental fruit fly based on the evidence from electrophysiological recordings complete with behavioral demonstration.

Since the host-finding behavior is closely related to mate-finding and oviposition behaviors in fruit flies (Prokopy and Owens, 1983), it can be assumed that the inherent wavelength preference is also closely related to host-finding behavior. From a physiological perspective, the chromatic cues of host-finding behavior can be taken as a different proportion of stimulation for each type of photoreceptor. Thus, selecting the chromatic stimuli according to the spectral sensitivity of the photoreceptors for testing the fly's wavelength preference will yield more specific results than studies referring to the host fruit of the fly only (Vargas et al., 1991; Cornelius et al., 1999). We tested the fly's preference for colored papers that were selected based on the spectral sensitivities of a fly's photoreceptors, and analyzed the results to reveal possible chromatic cues.

2. Materials and methods

2.1. Animals

Experiments were carried out on both male and female adult oriental fruit flies, *B. dorsalis*, derived from a laboratory stock maintained at $28 \pm 1\,^{\circ}C$ in a rearing room with 12:12 (L:D) photoperiod and fed with the peptone–sugar mixture.

2.2. Spectral sensitivity measurement

Before electrophysiological manipulation, the experimental fly was immobilized by placing it in a freezer with chipped ice at 4 °C for 15–30 min. Then the head of the fly was mounted on a brass pedestal, with a beeswax/rosin (3:1) mixture, so that the head and thorax were rigidly secured, and the abdomen was free to perform ventilatory movements. A small window was cut in the dorsal–lateral part of the left compound eye to expose the retinula for inserting the recording microelectrode, and a silver wire was inserted into the thorax as the indifferent electrode. The damaged surfaces were covered immediately with vaseline to prevent drying. The brass pedestal with the mounted animal was placed on a metal plate with the animal's head at the center of a Cardan arm perimeter, which was mounted with an optical fiber-guided stimulating light source.

The microelectrode was made of microfilament aluminosilicate capillary glass (O.D. = 1.0 mm, I.D. = 0.68 mm, AF100-68-10, Sutter Instrument Co.) pulled on a Flaming-Brown microelectrode puller (P-97 Flaming/Brown Micropipette Puller, Sutter Instrument Co.), and had a resistance of 140–160 MΩ when filled with 1 M lithium chloride solution. Using a micromanipulator (MWS-32, Narishige Scientific Instrument Lab.), the microelectrode was adjusted and lowered vertically to insert into the retinula through the small window in the compound eye. After inserting the microelectrode, the fly was dark-adapted for at least 10 min. The following manipulations were performed in the dark room to keep the fly dark-adapted. The microelectrode was advanced meticulously, and a gentle tapping was performed when the tip of microelectrode was going to penetrating through the cell membrane. Each time the baseline potential shifted, a series of flash stimuli were used to check if the tip of microelectrode was impaling the photoreceptor. If a depolarization response was detected, the Cardan arm perimeter would be adjusted to align with the visual axis of the recorded cell by obtaining the maximum response. The recorded cell would be further verified as a photoreceptor that the cell had a depolarized graded response waveform, which was composed of an initial on-transient depolarizing peak and a sustained plateau, to an on-axis saturated "white light" stimulus of 200 ms duration. The signals measured by the microelectrode were preamplified $10\times$ by an amplifier (Neuropobe, Model 1600, A-M Systems Inc.) and then sent via a 12-bit multifunction data acquisition system (PCI-6024E, National Instruments) to an IBM compatible PC.

A Xenon-short arc lamp (XBO 1000W/HS/OFR, OSRAM) was used as the stimulating light source. The polychromatic "white light" was guided to pass a quartz circular variable neutral-density wedge filter (Acton Research Co.), which was capable of varying the intensity of the light over a range of approximately 3 log units, and then sent into the monochromator (SP-150-M with 150-030-300 grating, Acton Research Co.). The separated monochromatic light with half-band widths below 10 nm was controlled by a magnetic shutter (SH-150, Acton Research Co.) to form the flash stimulation with 20 ms duration and was then guided by a UV-VIS fiber optic bundle (LG-455-020-3, Acton Research Co.) to project on the recorded eye. Since the terminal of the fiber optic bundle with a diameter of approximately 1.5 mm was 230 mm away from the recorded eye, the terminal of the fiber optic bundle therefore subtended an angle less than 0.37° at the eye.

The optic instruments and the multifunction data acquisition system were controlled by a program developed by LabVIEW software (ver. 6i, National Instruments) and executed on the PC. Thus, it could produce equal quanta of flux stimuli at 41 wavelengths from 300 to 700 nm, with 10 nm steps, and record the respective electrophysiological responses from the photoreceptor. The amplitude of the recorded responses were measured on-line and calculated as the spectral sensitivity by the same program. All the measured spectral sensitivity curves were calculated as the mean of squared errors with the theoretical visual pigment absorption curves, which were derived from the nomogram by Ebrey and Honig (1977), at 41 peak wavelengths from 300 to 700 nm. The λ_{max} was determined at the wavelength with the minimum mean square error.