Journal of Insect Physiology 95 (2016) 133–139

Contents lists available at ScienceDirect

Journal of Insect Physiology

journal homepage: www.elsevier.com/locate/jinsphys

Photoreceptor spectral sensitivity of the compound eyes of black soldier fly (*Hermetia illucens*) informing the design of LED-based illumination to enhance indoor reproduction

D.G.A.B. Oonincx [a,*], N. Volk [b], J.J.E. Diehl [a], J.J.A. van Loon [a], G. Belušič [b]

[a] *Laboratory of Entomology, Plant Sciences Group, Wageningen University, PO Box 16, 6700 AA Wageningen, The Netherlands*
[b] *Department of Biology, Biotechnical Faculty, University of Ljubljana, Večna pot 111, SI-1000 Ljubljana, Slovenia*

A R T I C L E I N F O

Article history:
Received 2 June 2016
Received in revised form 22 September 2016
Accepted 13 October 2016
Available online 14 October 2016

Keywords:
Black soldier fly
Spectral sensitivity
UV light
Light Emitting Diode
Indoor breeding

A B S T R A C T

Mating in the black soldier fly (BSF) is a visually mediated behaviour that under natural conditions occurs in full sunlight. Artificial light conditions promoting mating by BSF were designed based on the spectral characteristics of the compound eye retina. Electrophysiological measurements revealed that BSF ommatidia contained UV-, blue- and green-sensitive photoreceptor cells, allowing trichromatic vision. An illumination system for indoor breeding based on UV, blue and green LEDs was designed and its efficiency was compared with illumination by fluorescent tubes which have been successfully used to sustain a BSF colony for five years. Illumination by LEDs and the fluorescent tubes yielded equal numbers of egg clutches, however, the LED illumination resulted in significantly more larvae. The possibilities to optimize the current LED illumination system to better approximate the skylight illuminant and potentially optimize the larval yield are discussed.

1. Introduction

The black soldier fly (*Hermetia illucens* (L.); Diptera: Stratiomyidae; BSF) is a tropical fly species with great potential for the processing of several types of organic waste and byproducts (Larde, 1990; Oonincx et al., 2015a,b; St-Hilaire et al., 2007). Their larvae and prepupae are rich in protein and fat, and can serve as feed for a wide range of animals, such as fish, pigs, poultry, as well as dogs and cats (Bosch et al., 2014; Makkar et al., 2014). In order to successfully breed the BSF, mass rearing protocols need to be developed (Pastor et al., 2015). Several studies report on temperature and humidity requirements of larvae and adults, as well as on conditions for pupation (Holmes et al., 2012, 2013; Sheppard et al., 2002; Tomberlin et al., 2009; Tomberlin and Sheppard, 2002). Providing these circumstances in production facilities is feasible, even in temperate regions. However, one bottleneck for the mass production of BSF is the efficient production of eggs (Pastor et al., 2015). The BSF depends on direct sunlight for mating and therefore adult colonies are normally kept in greenhouses with considerable costs of climate control (Sheppard et al., 2002; Tomberlin and Sheppard, 2002; Zhang et al., 2010).

Some attempts have been made to induce mating under artificial conditions. Zhang et al. (2010) tested a so-called rare earth-lamp (500 W, emitted wavelengths between 350 and 450 nm) which failed to stimulate mating. However, a quartz-iodine lamp (450 W, emitted wavelengths between 350 and 2500 nm), did induce mating (Zhang et al., 2010). The spectrum of the latter lamp supposedly was similar to sunlight. Because the rare earth lamp was ineffective, the researchers concluded that wavelengths between 450 and 700 nm influence mating behaviour. Similarly, Tomberlin and Sheppard (2002) tried two artificial light sources (a 40-w Sylvania Gro Lux® and a 430-w ProUltralight Light System®), but did not observe mating, and obtained only infertile eggs. When their colony was provided with direct sunlight through a window, mating was observed and fertilized eggs were obtained. As 75% of all matings took place when the light intensity was above 200 μmol $m^{-2}s^{-1}$ and adult BSFs are active on sunny days only, Tomberlin and Sheppard (2002) concluded that intense sunlight was a major determining factor for mating, and they suggested that to induce mating male BSF need specific wavelengths which were absent in the artificial light sources.

In diurnal insects, the visually mediated behaviour is supported by the detection of shapes, colours, patterns or movement by the visual system (Engelmann and Kerkut, 1970). The visual system of adult BSF consists of three simple eyes (ocelli) and a pair of large compound eyes. In many insect species mating involves the

* Corresponding author at: Laboratory of Entomology, Plant Sciences Group, Wageningen University, PO Box 8031, 6700 EH Wageningen, The Netherlands.
E-mail address: dennisooonincx@gmail.com (D.G.A.B. Oonincx).

http://dx.doi.org/10.1016/j.jinsphys.2016.10.006
0022-1910/

airborne interception of females (Horridge and Mclean, 1978; Ruttner and Ruttner, 1965; Vogel, 1957). Similarly, the BSF males exhibit "lekking behaviour", i.e. they sit on leaves and intercept flying conspecifics. If a male is intercepted, a fight ensues; if a female is intercepted, copulation is initiated in flight (Tomberlin and Sheppard, 2001). From below, the bodies of conspecifics are perceived as dark spots against the sky. The contrast between the sky and the insect silhouette is strongest in the ultraviolet part of the light spectrum (Belušič et al., 2013). The dorsal retina of many insects predominantly contains blue- or ultraviolet-sensitive photoreceptors (Stavenga, 1992; Stavenga et al., 2001). Insect behaviour, including mating, can be influenced by UV light (Hunt et al., 2001; Kemp, 2008; Obara et al., 2008; Shimoda and Honda, 2013).

Efforts to breed BSF indoors have to be based on an understanding of the physiological properties of the BSF visual system. The BSF belongs to a suborder of Diptera, the Brachycera. All studied species belonging to this suborder share a common design of the retina (Boschek, 1971; Hardie et al., 1989; Kuiper, 1962; Trujillo and Melamed, 1966). Each ommatidium of their compound eye contains six large cells (R1-6) of which the photosensitive parts, the rhabdomeres, are positioned at the periphery. The rhabdomeres of two smaller cells (R7, R8) reside in the centre of the ommatidium, R7 situated distally and R8 proximally. The cells R1-6 represent a spectrally homogeneous class of photoreceptors with a broad spectral sensitivity. Their fast temporal responses presumably are used for motion detection, while their broad spectral sensitivity is less suitable for colour discrimination (Gao et al., 2008; Heisenberg and Buchner, 1977; Yamaguchi et al., 2010), but at least in *Drosophila*, R1-6 do contribute to colour vision (Schnaitmann et al., 2013). The central photoreceptors R7 and R8 have narrow spectral sensitivities and their temporal responses are slower (Autrum et al., 1985). Photoreceptors R7 (subclasses R7y and R7p) have peak sensitivity in the UV, while the two subclasses of R8 are most sensitive to blue (R8p) or green (R8y) light. Thus, they enable trichromatic vision, based on the three channels, UV – blue – green (Gao et al., 2008; Heisenberg and Buchner, 1977; Yamaguchi et al., 2010). A similar division might be expected in the retina of the BSF, with motion and wavelength detection photoreceptor classes. In some Diptera, particularly in the true flies, the spectral sensitivity of the photoreceptors is modified by additional chromophores bound to the outer side of visual pigment molecules, the opsins. These act as sensitizing pigments, increase the UV-sensitivity and contribute a detailed spectral signature to the spectral sensitivity, the 'fine vibronic structure' with three sharp peaks of sensitivity between 330 and 370 nm (Stavenga et al., 1989). The sensitizing pigments have not been reported so far in the family Stratiomyidae.

Small animals such as insects cannot afford neural redundancy and their senses and brains are narrowly tuned to their natural environment. A low mating rate of BSF indoors is likely due to the indoor illumination failing to produce the necessary natural visual cues. It is crucial to understand the physiological properties of the BSF visual system in order to create the correct light circumstances that induce indoor mating in order to produce fertilized eggs. Therefore, the aims of this study were to determine the spectral sensitivity of BSF compound eyes and subsequently, design an illumination system with the appropriate wavelengths using LED technology. Illumination by LEDs and the conventional fluorescent artificial light sources were compared to the natural illuminant (skylight) in terms of the relative photoreceptor excitation. Lastly, two artificial illumination systems were provided to BSF adults to compare their effectiveness in obtaining fertilized BSF eggs.

2. Materials and methods

Black soldier flies were obtained from the breeding colony at the Laboratory of Entomology (Wageningen University, Wageningen, The Netherlands). This colony was maintained for over five years in a climate chamber at a temperature of 26 °C and a relative humidity of 70% illuminated by an array of fluorescent tubes (TLD18W840NG, Philips, Eindhoven, The Netherlands) resulting in a light intensity of approximately 23 W m^{-2}. Containers with pupae from this colony were kept in the dark and were checked daily for newly eclosed adults. Gender was visually determined based on genitalia (Fig. 1).

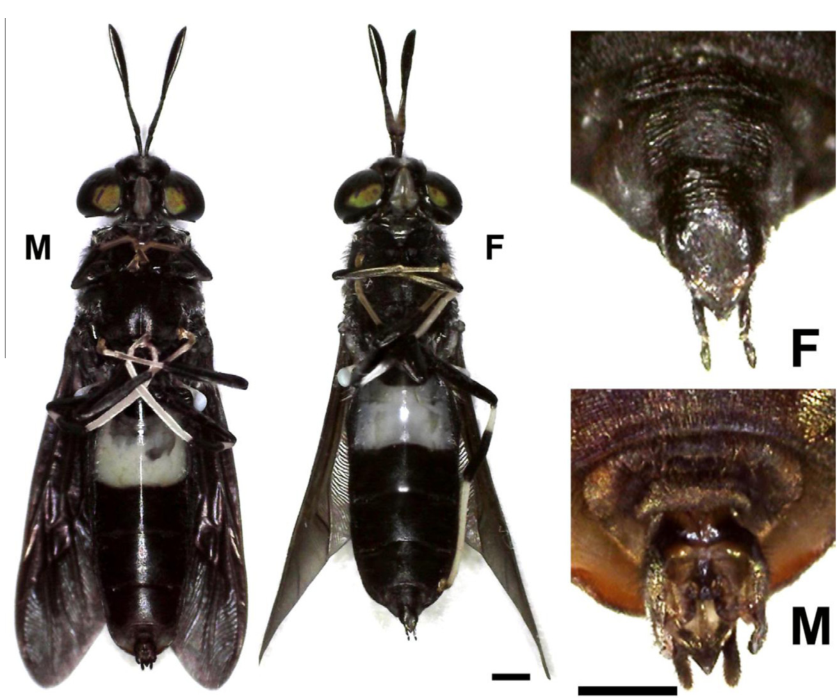

Fig. 1. Left, adult female (F) and male (M) black soldier flies (*Hermetia illucens*); right, magnified genitals. Scale bars, 1 mm.