Light attraction of the Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), and regional spectral sensitivity of its compound eye

by

Thomas Cowan
BSc Trent University 1998

THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF PEST MANAGEMENT

In the
Department of Biological Sciences

SIMON FRASER UNIVERSITY

Summer 2009

Approval

Name: **Thomas Cowan**

Degree: **Master of Pest Management**

Title of Thesis: **Light attraction of the Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), and regional spectral sensitivity of its compound eye**

Examining Committee:

Chair: Dr. Rolf. Mathewes
Professor, Department of Biological Sciences,
S.F.U.

_____

Dr. G. Gries, Professor, Senior Supervisor
Department of Biological Sciences, S.F.U.

_____

Dr. I. Novales Flamarique, Associate Professor,
Department of Biological Sciences, S.F.U.

_____

Dr. G. J. R. Judd, Research Scientist,
Pacific Agri-Food Research Centre,
Agriculture and Agri-food Canada
Public Examiner

Date Defended/Approved: July_30_2009

Declaration of
Partial Copyright Licence

Abstract

I tested the hypothesis that the Indian meal moth, *Plodia interpunctella*(Hübner), uses wavelengths of visible blue/violet light as orientation cues. In four-choice laboratory experiments, blue light (400–475 nm) was significantly more effective than green (475–600 nm), orange (575–700 nm) or red (590–800 nm) light in attracting males and mated females. The 405-nm “violet” light emitting diode (LED) was significantly more effective than the 435-, 450- or 470-nm “blue” LED in attracting males as well as virgin and mated females. A 405-nm wavelength also significantly enhanced the known attractiveness of UV light. In electroretinograms, standardized responses of dorsal, equatorial and ventral eye regions to UV, violet and green light were similar, but the equatorial region was most sensitive. Occluding regions of the eye did not affect the moths’ behavioural orientation to violet light, indicating that phototactic responses are not dependent on a single eye region.

Keywords: regional specialization, orientation, violet light, foraging, Indian meal moth, *Plodia interpunctella*, Lepidoptera, Pyralidae

Acknowledgements

I would like to thank Dr. Gerhard Gries and Regine Gries for their support, patience, editing and helpful insights in this project; Dr. Inigo Novales Flamarique for valuable advice; Pilar Cepida and Bryan Jackson for technical assistance in bioassays and electrophysiology; Bob Birtch for graphical illustrations and Cory Campbell for advice and insight into bioassay design. I would also like to thank my family and friends for their encouragement and support. This research was made possible through financial support in form of a Simon Fraser University Graduate Fellowship, the Professor Thelma Finlayson Graduate Entrance Scholarship, the Professor Thelma Finlayson Fellowship, and the Dr. John Yorston Memorial Graduate Scholarship in Pest Management. Additional funding has been provided by the Natural Sciences and Engineering Research Council of Canada (NSERC) – Industrial Research Chair to Gerhard Gries with Pherotech International Inc., SC Johnson Canada, and Global Forest Science as industrial sponsors.

Table of Contents

List of figures

1: Indian meal moth pest status and control

1.1.1 Pest status

The Indian meal moth (IMM) *Plodia interpunctella* (Hübner) is one of the most important pests of stored food products worldwide. The larvae are able to infest a wide variety of stored food products including numerous grains and grain-based products, as well as dried fruits and nuts (Williams, 1964; Doud and Phillips, 2000) and have even been reported to infest bee hives, feeding on the bee pollen (Yong Jung et al., 2003). Infestations can cause substantial economic loss in production and storage facilities by product spoilage, contamination and pest control costs (Mohandass et al., 2007). The wide variety of potential sources that are infested and the damage associated with infestations present a great challenge for pest managers to minimize the incidence and extent of infestations.

1.1.2 Biology

In a controlled environment with no predation and little disease, populations of IMM can proliferate. Briggs et al. (2000) provided a summary of the basic life cycle of IMM. Mated females produce up to 200 eggs, which hatch in 3–4 days. The larvae develop through 5 instars in 23 days before they reach the pupal stage which lasts up to 7 days, after which adults eclose. Adult moths survive

approximately 5–6 days. The developmental time can be highly variable, depending on temperature and rearing conditions. In this study, generation times of 28–35 days were common and adult moths survived for 3–12 days depending on temperature and size of the containment area.

1.1.3 Control strategies

Protecting food in storage sites and processing plants against IMM infestations can be problematic because the use of insecticides and fumigants are restricted. Control tactics for IMM consist include sanitation and exclusion methods and the use of pheromone traps for monitoring and mass trapping (Bennett et al., 1997). Insecticide application is less common due to potential contamination of food products but fumigants such as phosphine and methyl bromide are commonly applied in food processing plants (Mohandass et al., 2007). UV light traps are widely used in structural pest management for control of flies (Bennett et al., 1997) and research into the response of IMMs to light traps has demonstrated that the moths prefer to orientate and land on traps emitting UV and green light (Stremer, 1959; Soderstrom, 1970; Kirkpatrick, 1970; Sambaraju and Philips, 2008). Unlike pheromone baited traps, light traps attract both male and female moths, and thus, might be more effective in controlling populations of IMM. Light has not been used extensively as an operational control in managing IMM but has significant potential as part of an integrated management program by reducing populations of both male and female moths. To use light trapping

technology for IMM most effectively, the attractive wavelength(s) and optimal intensity of light should be identified and the visual system of IMM investigated.

2: Insect visual systems

2.1 Introduction to insect vision

The ability to detect objects, colours, movement, prey or conspecifics using the properties of light provides an incredible spatial advantage as critical information is collected and responded to from an extended scene. Visual systems of insects have adapted to operate in a wide range of intensities and spectrally diverse visual conditions, such as diurnal and nocturnal periods, dense forests or aquatic environments (Briscoe and Chittka, 2001). Many of these adaptations have likely evolved from the basic compound eye and photoreceptor design to take advantage of specific environmental information contained in the available light sources. Here I will (*i*) give an overview of the basic structure of the compound eye and the molecular basis for visual reception, (*ii*) discuss the information contained in light sources and (*iii*) discuss some eye designs that take advantage of visual cues.

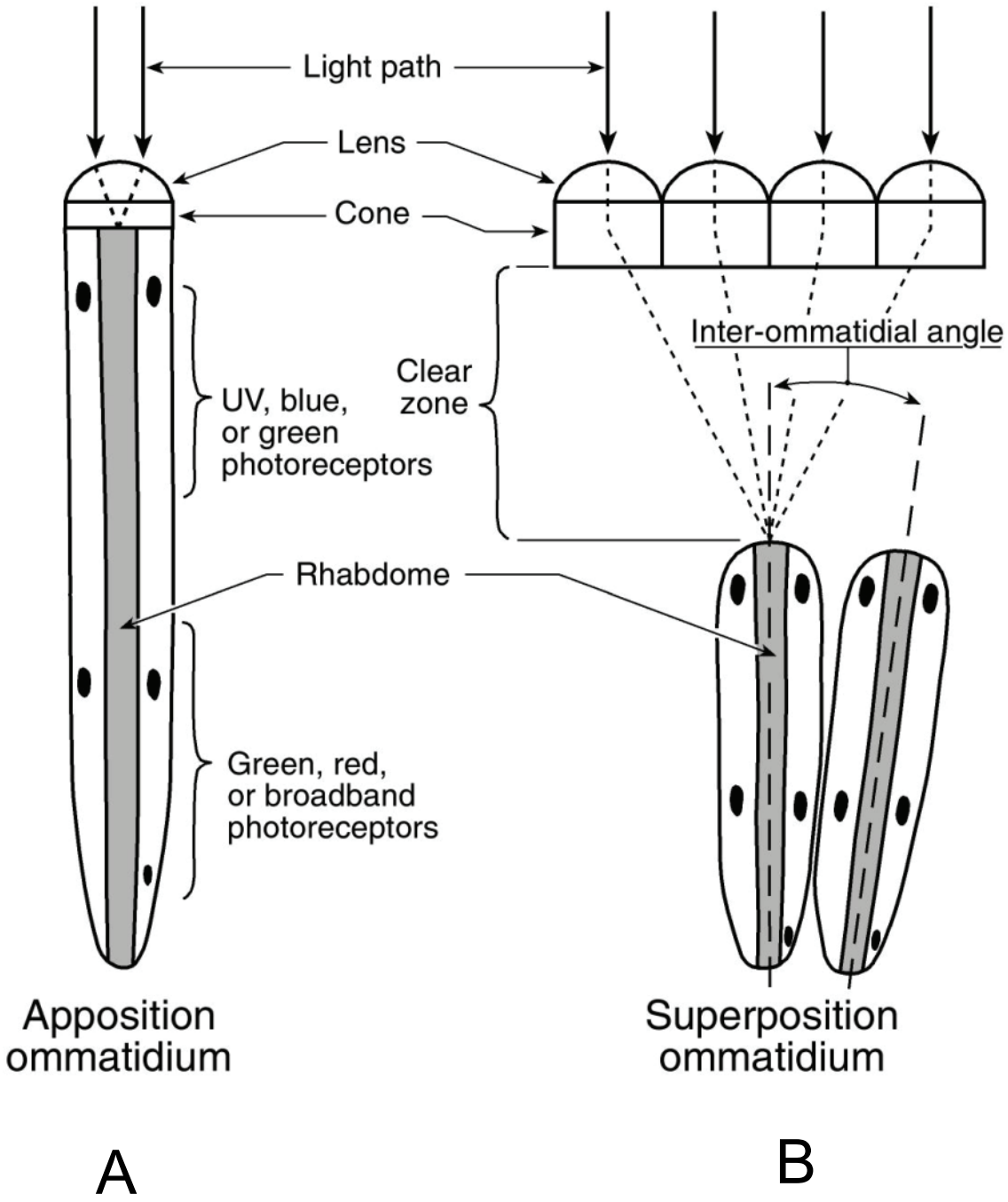
2.1.1 Compound eye and ultrastructure

On the surface, the compound eye is a somewhat spherical structure containing a large number of facet lenses. Beneath the surface, it is a much more complex structure. Lenses gather light and focus it through a crystalline cone

(Lepidoptera) or directly onto a light guide (other insects) where photoreceptors capture the photons. The entire structure of lens, crystalline cone, light guide and photoreceptors is referred to as an ommatidium. The phototransductive light guide that runs through the centre of the ommatidium is constructed of tightly packed microvilli which direct light to retinula cells containing the photoreceptors. This structure is termed rhabdom. In a typical non-specialized lepidopteran ommatidium, the rhabdom can be divided into nine photoreceptor regions. Four are located in the proximal one third of the rhabdom and usually contain UV, blue and green receptors. The last two thirds of the rhabdom contain the five distal photorectoptor sections that contain green, red or broad band receptors (Arikawa, 2003).

There are two main types of ommatidia (Figure 1). In the apposition type, a single lens focuses light onto a single rhabdom. In the superposition type a clear zone is present between the lens and the light guide, allowing multiple lenses to focus light onto a single rhabdom, vastly increasing the light gathering ability of the receptors (Warrant, 2006). The apposition type is most often found in diurnal insects where light-saturated environments provide ample photons for each section of the visual field viewed by an ommatidium. The superposition type is most often found in crepuscular and nocturnal insects that require improved sensitivity to available light (Land, 2003). An additional adaptation to increase light sensitivity in some species of nocturnal Lepidoptera includes a reflective tapetum at the base of the ommatidium that allows light not absorbed by the photoreceptors in the first pass down the rhabdom to be reflected and

Figure 1 Simplified ommatidial structure of superposition **(A)** and apposition **(B)** eye designs, modified from Arikawa (2003) and Land (2003).


Light path
Lens
Cone
Inter-ommatidial angle
Clear zone
UV, blue, or green photoreceptors
Rhabdome
Green, red, or broadband photoreceptors
Apposition ommatidium
Superposition ommatidium
A
B

absorbed on the way back out (Land, 2003). The light reflected from the tapetum is responsible for the eye shine phenomenon noticed in dark-adapted eyes of nocturnal moths.

The resulting image produced by the compound eye, regardless of ommatidum type, is a whole erect image comprised of the various sections of the visual field knitted together to form the complete image (Land 2003). A somewhat crude but effective analogy would be a large scoreboard comprised of hundreds of television screens each of which producing only a small part of the overall image. The image is not continuous but is in fact comprised of many smaller sections of the visual field.

Each lens of the compound eye collects photons from a certain portion of the visual field and focuses them onto a series of photoreceptors. It is the response of receptors to various wavelengths of light that allows insects to perceive their surroundings. The basis for light reception in insects are photopigments, also referred to as rhodopsins. Rhopsins consist of an opsin protein and a light-sensitive chromophore, usually retinal or 3-hydroxyretinal (Stavenga, 2006). Chromophores by themselves absorb maximally in the UV range but the combination of chromophore and opsin amino acid sequence can alter the peak sensitivity of a pigment to give various maximal absorbancies, ranging from UV to red (Briscoe and Chittka, 2001). The presence of the UV-sensitive chromophore gives the visual pigment two absorption peaks, the alpha band with strong sensitivity in the visible spectrum and the beta band with reduced sensitivity in the UV range (Stavenga, 2006).

The modification of a rhodopsin's peak wavelength absorbance allows insects to identify and discriminate between different colours. When light strikes the visual pigment, a series of rapid reactions takes place to convert the absorption of a photon into an electrical nerve impulse. This process is known as the phototransduction cascade. When the visual pigment absorbs a photon, the chromophore photoisomerizes from its rhodopsin (R) state to the metarhodopsin (M) all-trans state (Hardie, 2006). There is an interesting relationship between the maximal absorbance by the R and corresponding M state. Rhodopsins with maximal absorbencies in the UV and blue spectrum upon photoconversion from R to M, are bathochromatic, meaning that the M state will now absorb light at longer wavelengths than does the original R state. Rhodopsins with maximal absorbencies in the green and orange spectrum have M that are hypsochromatic, absorbing at shorter wavelengths than the original R (Stevenga 2006). After photoisomerization, M is phosphorylated and binds to the G-proteins resulting in stimulation of the nerve. Arrestin, a soluble protein, is then bound to M facilitating its removal from the G-protein binding site and halting the nerve action. Thereafter, M is reconverted to R by absorbing a second photon, dephosphorylated and ready for absorption of another photon to begin the process again (Hardie, 2006).

2.2 Information contained in light

The visual field of diurnal and nocturnal insects is extended, meaning that light reaches the eye from various directions at the same time (Warrant, 2004). From this extended scene of sky and ground, the light reaching the eye contains a variety of information important to insects such as wavelength, intensity, direction of emitted light, and even polarization pattern.

2.2.1 Colour discrimination

Despite the diversity of environments insects inhabit, electrophysiological studies have shown that most insects are trichromatic possessing three different classes of photoreceptors that absorb light maximally in three different regions (UV, blue and green) of the electromagnetic spectrum (Briscoe and Chittka, 2001). Receptors in these three regions allow insects to identify colours based on the ratio of responses between different receptors. For example, a butterfly with these three receptor types can determine the difference between green and blue light but would have more difficulty determining the difference between similar hues in the blue spectrum where the dominant wavelength of one colour is close the other. The difficulty of distinguishing between these wavelengths is due to the similar ratio of responses given by the blue and green photoreceptors. Some butterflies such as *Papilio xuthus* (Linnaeus) have photoreceptors that absorb violet light (Arikawa et al.,1987), and with a violet light receptor can more accurately identify hues in the violet range of the blue spectrum. Discrimination in the violet range may help these butterflies differentiate between flower colours

and identify certain violet flowers of higher nectar content, thus increasing foraging efficiency.

The importance of colour discrimination might be explained, in part, by the limited resolution of the compound eye. The main limitation is diffraction, which is due to the small aperture of the lens (Larsson and Svensson, 2005). Smaller lenses cannot focus light to a very fine point and project a circle with poor focus. This circle is referred to as an Airy disk which is the smallest point to which light can be focused for the given lens. The size of the Airy disk projected by the small lenses in the compound eye give rise to large “pixels” (Land, 2003). Due to this limitation in optics, visual systems with compound eyes generate a very grainy representation of the visual field compared to those with a single lens, as mammals use (Larsson and Svensson, 2005). Due to the lower quality of the image that the optics of the compound eye produce, insects are likely limited to identifying basic shapes of objects. If this lower quality image were coupled with monochromatic reception, identification of food, mates or oviposition sites would be problematic due to the lack of contrast between the target and the background. With the ability of colour identification, coarse objects can stand out against a background allowing insects to identify targets even with the low resolution of compound eyes (Larsson and Svensson, 2005).

2.2.2 Polarized light

The polarization pattern of the sky provides important information for insect navigation. Light can be conceived as an electric and magnetic wave oscillating

in phase, perpendicular to each other. Because light travels in straight lines, the angle at which the wave travels does not change from the source to the receptor. The orientation of the electric wave relative to the direction of travel (from vertical through horizontal) is referred to as the electric vector or e-vector. If only a certain orientation is reflected or passes through the atmosphere, most of the electric waves are aligned in the same direction (perpendicular to the direction of travel). Such light is said to be polarized. Several species including desert ants, bees and butterflies use the polarized light pattern of the sun produced by the scattering effect of air molecules for directional orientation (Homberg, 2004). These insects detect polarized light with specialized ommatidia found in a specific region of the eye known as the dorsal rim area (DRA). Microvilli in these ommatidia are highly aligned at a distinct angle, allowing photoreceptors to absorb light maximally at specific e-vectors (Wehner and Labhart, 2006). Insects that detect polarization patterns are capable of using this information as a reliable visual compass reference or as a method of course control even under difficult conditions, such as haze or patchy clouds (Henze and Labhart, 2007).

Water produces a largely horizontal polarization pattern of reflected sunlight, which is attractive to certain water dwelling insects such as mayflies and may explain why they swarm and lay eggs on substances that reflect a similar polarization pattern, such as cars and asphalt (Kriska et al., 1998). *Notonecta* bugs have a similar attraction to polarized light reflected from water surfaces and initiate a “plunge response” when polarizing photoreceptors experience maximum absorbance regardless of intensity (Wehner and Labhart, 2006). The

ability to detect the polarization pattern of water from a distance would be advantageous to flying insects that require water for reproduction, especially if water bodies are patchy. It has also been suggested that migrating locusts are capable of detecting the polarization pattern of water in order to avoid flying out over the sea (Shashar et al., 2005).

2.2.3 Directional light

Directional light from a celestial or artificial point source is used by some nocturnal insects as a directional guidance cue. Foraging along odour trails, black carpenter ants, *Camponotus pennsylvanicus* (DeGeer), are capable of using directional moon light to take short-cuts off the trail to return to their nests (Klotz and Reid, 1993). The subsocial shield bug, *Parastrachia japonensis* (Scott), exhibits a similar behaviour when it returns to its burrow from foraging (Heronaka et al., 2007). By using light sources as a method of positional orientation, these insects improve their ability to locate their nests or burrows and become less dependent on semiochemical cues.

2.2.4 Sensitive regions in the compound eye

Although compound eyes are limited in resolution, their visual acuity can behigh in certain regions. When ommatidia as the basic sampling unit arearranged close together, a greater resolution of the image is achieved (Land, 1997). The angle at which ommatidia are positioned relative to each other (Figure 1B) can either increase or decrease the number of sampling points. Therefore, smaller

interommatidial angles give higher resolution and larger interommatidial angles give lower resolution (Land, 1997). When in flight, objects projected onto the retina from farther away move more slowly across the visual field than objects that are closer. Closer objects appear to move at higher speed and have a blurring effect across the retina, a phenomenon encountered by ommatidia in the dorsal and ventral regions that are not positioned to detect objects in the flight path (Land, 2003). The blurring effect makes higher resolution unnecessary in the dorsal and ventral regions and, in general, interommatidial angles increase as the distance from the equatorial region increases (Land, 1997).

Most general-purpose insect eyes have some division of labour, which is usually restricted to specific regions (Land and Nilsson, 2006). The distribution of photoreceptor types in some insects can be homogeneous throughout the eye (Arikawa, 2003) but the resolution in different regions can vary depending on the ecology of the insect (Land, 2003). Acute zones for forward-flight exist at the frontal equatorial region in many flies and wasps, providing higher resolution during forward flight navigation, tracking, and chasing of prey and mates. Some insects such as dragonflies have two acute zones, one in the forward equatorial region for forward flight and one in the dorsal area for pursuit of prey (Land, 2003).

Although the total amount of light available to crepuscular and nocturnal insects is low, such insects are still able to function with considerable reliability (Warrant, 2004). The main problem in obtaining a visual image in dim light is the lack of available photons. Fewer photons available for absorption by the

receptors affect the signal-to-noise ratio of the detector. The more photons that are available, the lower the incidence of false signals (Warrant, 2006). With a few exceptions (Greiner, 2006), most nocturnal insects have superposition eyes. As previously noted, the superposition eye focuses light from many lenses onto a single photoreceptor. The convergence of many lenses to gather and focus light on a single point greatly increases the number of photons available to photoreceptors, thereby boosting the light signal and reducing noise. However, when the eye increases its light-sensitivity, resolution decreases (Warrant, 2006). The decrease in resolution is due to the number of available detectors. As seen in the formation of acute zones, increasing the number of sampling points increases the amount of information that is taken from the extended scene, producing a finer image. In low light, with fewer photons available for each detector, each sampling point does not obtain sufficient photons to form an image thus reducing the sensitivity of the eye. By focusing available photons from several sampling points onto fewer receptors, a coarser image is produced but the ability to detect light from the extended scene is increased (Warrant, 2004).

Despite the reduction in resolution, the eyes of nocturnal insects can be highly sensitive to various environmental cues. A remarkable example is nocturnal colour vision in the hawk moths *Deilephila elpenor* (L.), *Hyles lineata* (Fabricius) and *Hyles gallii* (Rott). These insects are capable of using colour vision when foraging at starlight intensities that would render most diurnal insects and mammals colour blind (Kelber et al., 2003). An example of nocturnal

navigation using superposition eyes is the African dung beetle *Scarabaeus zambesianus* (Péringuey). This beetle is capable of using polarization patterns of moonlight, which are up to ten million times dimmer than that of the sun to maintain a straight course from the dung patty to its burrow (Warrant, 2004). Clearly, the advantages gained by increased light sensitivity far outweigh the loss of resolution in nocturnal habitats.

3: Ultraviolet and violet light: attractive orientation cues for the Indian meal moth *Plodia interpunctella*[1]

3.1 Introduction

The use of light as a navigational or directional orientation cue has been well studied in diurnal insects (Wehner, 1984; Wehner and Muller, 2006;Hironaka et al., 2007; Pfeiffer and Homberg, 2007), but has been investigated for only a few insects active in crepuscular or nocturnal light with inherently diverse irradiance spectra (Warrant et al., 2004; Theobald et al., 2007). Blue wavelengths become dominant ("blue-shifted") as the solar elevation angle decreases and the sun disappears below the horizon. Under starlight, irradiance spectra are "red-shifted" and strongly influenced by the presence or absence of the moon (Johnson et al., 2006). For one to two hours between sunset and astronomical twilight, blue-shifted twilight offers a constant polarization pattern in non-cloudy skies that provides insects with orientation cues (Cronin et al., 2006).

The specialized dorsal rim area of the eye of the desert locust, *Schistocerca gregaria* (Forskal), with peak sensitivity for polarized blue light, is likely an adaptation for nocturnal flight (Homberg, 2004). Moonlight and artificial light are also known to serve as directional cues. Black carpenter ants,

[1] A modified version of this chapter has been published in Entomologia Experimentalis et Applicata 138, 148-158.

Camponotus pennsylvanicus (DeGeer), can use moon light or other artificial light to orient themselves along trails (Klotz and Reid, 1993). Similarly, polarized moon light as well as non-polarized natural and artificial light sources serve as orientation cues for foraging dung beetles, *Scarabaeus zambesianus* Péringuey, as orientation cues when they return to their harborage (Dacke et al., 2004). Attraction of nocturnal moths to light may be due, in part, to a shift in orientation response from moonlight to artificial light (Baker and Sadovy, 1978).

The Indian meal moth (IMM), *Plodia interpunctella*, is one of the most serious and widespread pests of stored products (Zhu et al., 1999; Nansen and Phillips, 2004). It is most active in the first two hours of the scotophase (twilight conditions). As an enduring flyer, it can travel over a large spatial scale (Campbell and Abogast, 2004). I argue that during long-distance flights, the IMM is dependent upon visual cues that are received by photoreceptors adapted to function under blue-shifted twilight.

Previous studies have investigated the response of IMMs to ultraviolet light (UVa; 345–400 nm) and green light (480–580 nm). In electoretinogram (ERG) studies, Marzke et al. (1973) demonstrated that IMM eyes respond to wavelengths ranging between 350 nm to 650 nm, with the strongest responses to green light at 550 nm. In behavioural studies, Stremer (1959) demonstrated that IMMs are most strongly attracted to UV (365 nm) and green (580 nm) lights, suggesting that the eyes are potentially dichromatic with UV and green receptors. He further showed that high-intensity lights are more effective than low-intensity lights in attracting moths from the same distance. Kirkpatrick et al. (1970)

confirmed that IMMs are attracted to UV light alone and in combination with green light, with no significant preference for either stimulus. Using non-standardized stimuli with respect to light energy, Soderstrom (1970) showed that traps fitted with eight green lights captured significantly more IMMs than traps fitted with one UV light.

In this chapter I show that (*i*) blue light (400–475 nm) is more attractive to IMMs than green (475–600 nm), orange (575–700 nm) or red (590–800 nm) light; (*ii*) a 405-nm "violet" light emitting diode (LED) is more attractive than the 435-, 450- or 470-nm "blue" LEDs; (*iii*) the 405-nm LED elicits stronger receptor potentials from female and male eyes than the 350-nm UV LED; and (iv) that at maximum light intensities a 405-nm LED is significantly more attractive than a 350-nm LED.

3.2 General materials and methods

3.2.1 Origin and maintenance of IMM colony

IMM larvae were obtained from infested cereal bars from a processing plant. Larvae were reared at 25-27ºC at a photoperiod of 17(L):7(D). The rearing diet was modified from LeCato (1976), and consisted of whole-wheat flour (27.5% by volume), yellow cornmeal (27.5%), Purina One dog food (13.5%), brewers yeast (6.9%), honey (6.9%), glycerine (6.9%; 96% pure), Quaker rolled oats (6.8%), and wheat germ (3.4%). Fifth-instar larvae were separated by sex and placed in groups of 12–15 specimens in Petri dishes (10 cm diam) containing corrugated

cardboard as pupation sites. Male larvae were identified by the presence of gonads visible through the dorsum. Eclosed adults were kept at both reversed and staggered photoperiods to allow experimentation throughout the entire day. To obtain gravid females, 2–3 virgin females and 3–4 virgin males were confined in small cages (10 × 1 × 10 cm) during the scotophase. The next day, females were assumed mated and used for colony rearing or laboratory experiments. All adult moths used in experiments were 2–5 days old.

3.2.2 General experimental design

Two- or four-choice laboratory experiments (Figure 2, A-B) were conducted in a modified wind tunnel (1.10 × 1.10 × 3.30 m long), with air entry and exit sections covered by mesh screens and by black paper to minimize light reflections. For each replicate, two Petri dishes with five insects each were placed on a 50 cm tall, black felt-covered platform (23 × 30 cm) in the centre of the tunnel. Light sources as test stimuli were randomly assigned to, and mounted within, adhesive-lined green paper Delta traps (Contech International Inc., Delta, BC, Canada), assuming that a strong orientation cue would be attractive to flying moths and that trap catches could serve as a measure of the strength of the orientation response. In four-choice experiments, traps were randomly placed in each corner of the tunnel ~80 cm apart from each other and 1.5 m from the release platform (Figure 2, B). They were positioned at a 15º angle directed towards the release stand (Figure 2, A). Each stimulus was rotated clockwise

after every replicate to determine whether IMM orient to a particular light source regardless of its position inside the wind tunnel.

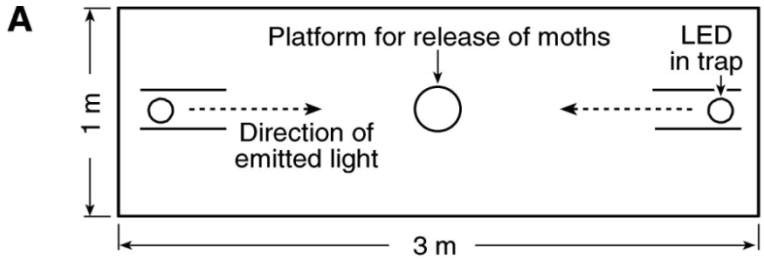
For two-choice experiments, light sources were placed inside adhesive-lined green Delta traps, and positioned at opposite ends of the wind tunnel (Figure 2, B). Lights were randomly assigned to each side and then alternated for each replicate. All experiments were conducted in the first 2 h of the 7-h dark phase, when IMMs are searching for mates (males) or suitable oviposition sites (females).

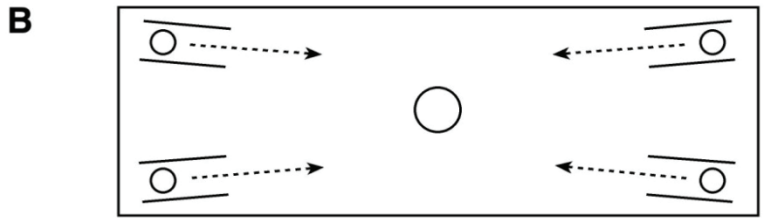
An experimental replicate was initiated by lifting the lid of each Petri dish on the release platform, and was terminated by scoring the number of moths captured in each trap 2 h later. All moths not responding were removed from the wind tunnel prior to initiating a new experimental replicate. After each set of four replicates, the wind tunnel was cleaned with 70% ethanol and left to dry over night.

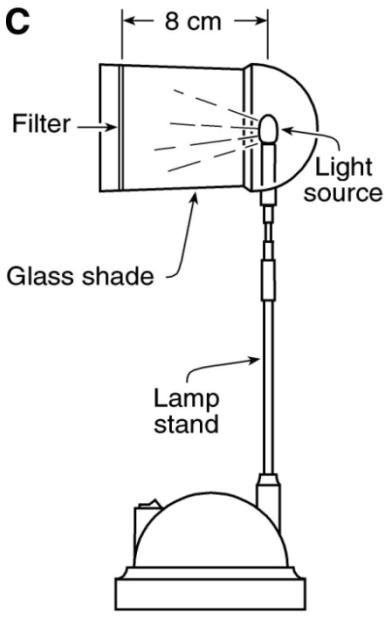
3.2.3 Electroretinogram recordings

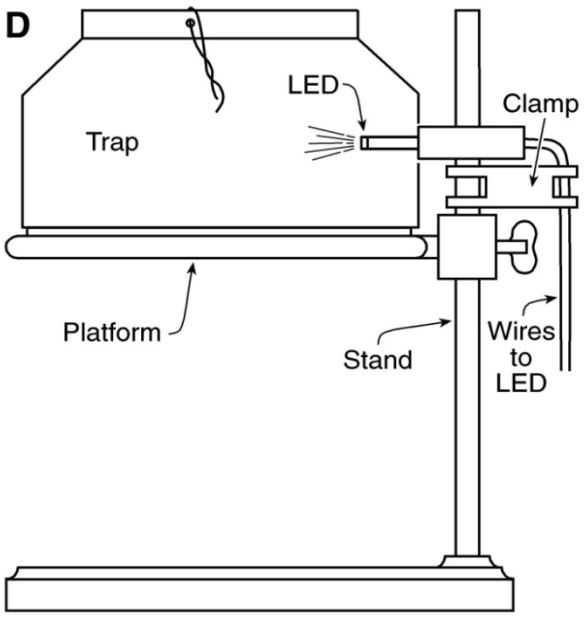
Insects were immobilized laterally with modelling clay (Sargent Art Inc., Hazleton, PA, USA) on an adhesive-coated glass slide. Both antennae and the left wing were removed to ensure access of tungsten electrodes that had been electrically sharpened (Cool et al., 1970). The indifferent electrode was micro-manipulated (Leitz micromanipulator M, Vienna, Austria) into the thorax and the recording

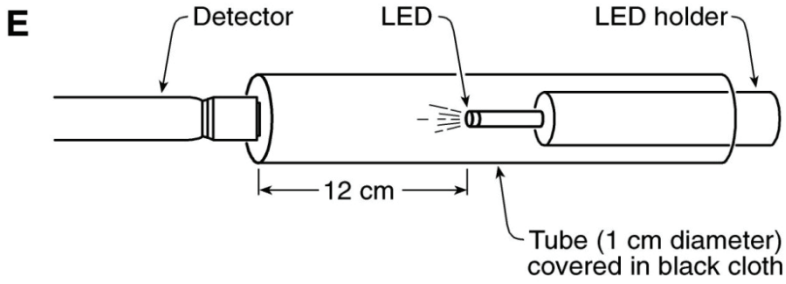
Figure 2 (**A, B)** Experimental design to test behavioural responses of *Plodia interpunctella* in two- and four-choice experiments; **(C)** light source for testing portions (blue, green, orange, red) of visible light; **(D)** mounting of a light emitting diode (LED) within a green Delta trap; **(E)** set-up for measurement of spectral composition and light intensity of LEDs. All drawings are not to scale.


A
Platform for release of moths
LED in trap
1 m
Direction of emitted light
3 m


B


C
8 cm
Filter
Light source
Glass shade
Lamp stand


D
LED
Clamp
Trap
Platform
Stand
Wires to LED


E
Detector
LED
LED holder
12 cm
Tube (1 cm diameter) covered in black cloth

electrode into the equatorial region of the eye near the left edge, thus preventing light shadows. Electrical potentials from the eye in response to test stimuli were preamplified (Syntech Auto Spike, Syntech, Hilversum, The Netherlands) and recorded with an EAD oscilloscope program (Syntech). Based on the signal-to-noise ratio, potentials of > 5 mV were considered responses. Experiments were conducted inside a light-proofed Faraday cage mounted on a steel table to limit vibration.

3.2.4 Statistical analyses

Ten replicates were conducted for each behavioural experiment, but replicates without any responding insects were excluded from statistical analysis. Percent trap captures in all replicates of four-choice experiments with responding insects were arcsine-transformed and subjected to the Kruskal-Wallis test for non-parametric data followed by the Student-Newman-Keuls'' analog for multi-comparison analysis (Zar, 1999). Data obtained in binary-choice experiments and in ERG recordings were analyzed by the Wilcoxin rank sum test. All data analyses employed JMP software (SAS®, Cary, NC, USA).

3.3 Specific methods and results

Specific methods and results are reported in the same section because results of preceding experiments affected the design of subsequent experiments.

Experiments 1–3: Attractiveness of blue, green, orange and red light

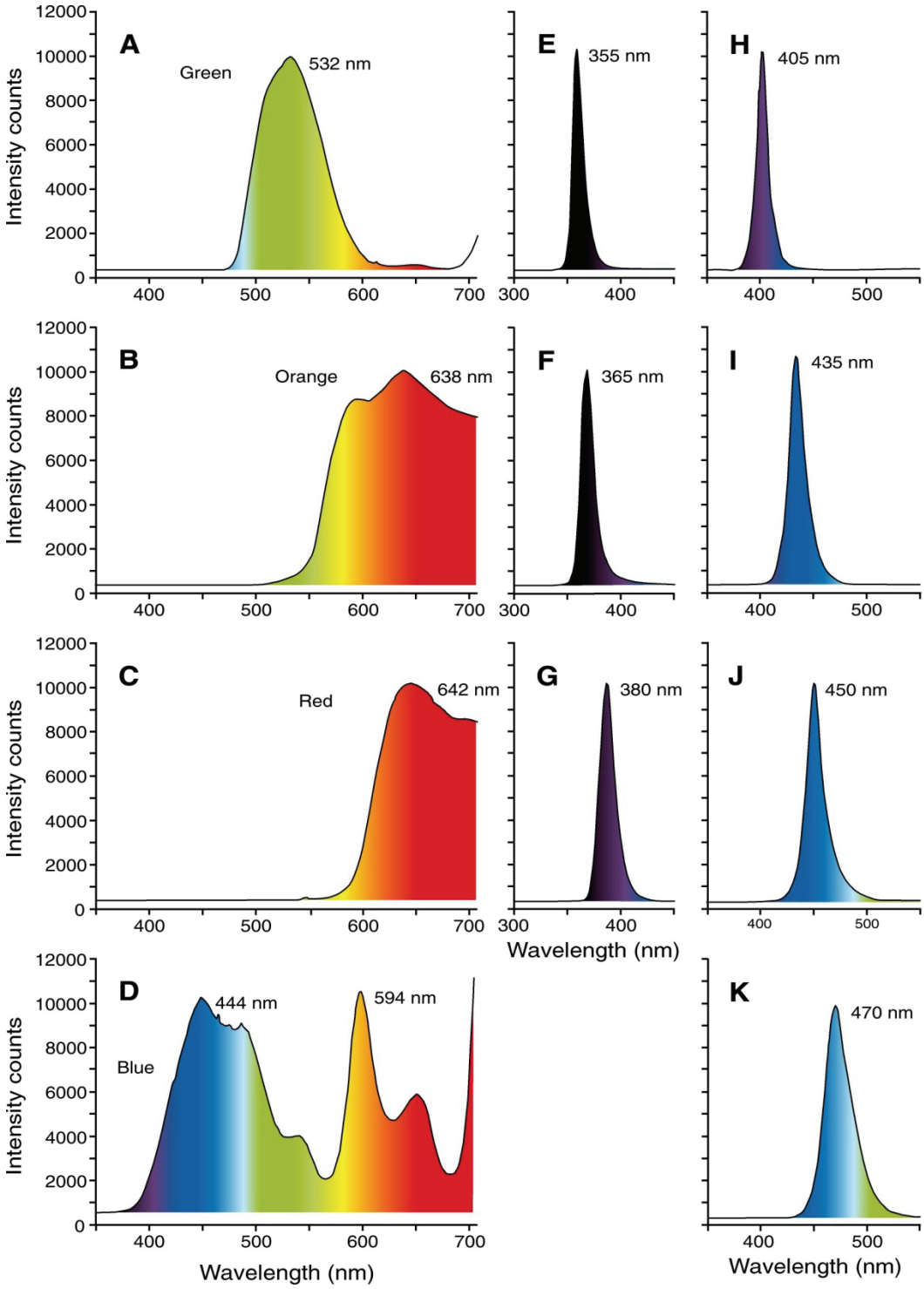
To determine the portion of visible light that constitutes the strongest visual orientation cue for IMMs, a modified desk lamp (Espressivo, Ikea) with a 20-watt halogen bulb was used as a light source (Figure 2, C). Males (Exp. 1, n = 10), virgin females (Exp. 2, n = 10) and mated females (Exp. 3, n = 10) were used as bioassay insects. The desk lamp was connected to a rheostat to adjust light intensities, and the halogen bulb was fitted with a black cardboard cylinder (8 × 12 cm wide), with the light filters mounted at the front, 8 cm apart from the bulb (Figure 2, C). The cylinder projected the light in one direction.

Flexible filters (Lee Filters, Hamshire, England) transmitted light spectra in the range of blue (400–475 nm, peaks at 444 nm and 594 nm; "Rose Purple 7"), green (475–600 nm, peak at 532 nm; "lime 8"), orange (525–750 nm; "orange 9") and red (590–750 nm; “light Red”) (Figure 3). The orange filter, permitting passage of 560- to 600-nm yellow wavelengths, substituted for a narrow-band yellow filter which was not obtainable. Any attraction to the orange filter was assumed to be due to yellow wavelengths because IMMs have a low relative response to red wavelengths (Stremer, 1959).

Light intensities[2] were measured with an HR4000 high-resolution spectrometer (Ocean Optics, Dunedin, Florida) fitted with a cosine corrector at the detector. All light sources were tested at an intensity of 15 μW/cm^2 (the

[2] Using photon counts (photons/cm^2/s) instead of light energy (μW/cm$^{2)}$ to calibrate light sources would not have significantly altered the response of insects and the interpretation of results. For example, a 405-nm LED at 200 μW/cm^2 produces 4.14 x 10^{14} photons/cm^2/s, and a 470-nm LED at 200 μW/cm^2 produces 4.87 x 10^{14} photons/cm^2/s.

Figure 3 Spectral composition of light sources bioassayed in experiments 1–16. Intensity counts on y-axes are relative and provide a standardized reference for all light sources. A–D are transmission spectra from green, orange, red and blue filters, respectively. E–K are spectra from Light Emitting Diodes emitting UV and violet light.


A
Green
532 nm
E
355 nm
H
405 nm
B
Orange
638 nm
F
365 nm
I
435 nm
C
Red
642 nm
G
380 nm
J
450 nm
D
Blue
444 nm
594 nm
K
470 nm
Intensity counts
Wavelength (nm)

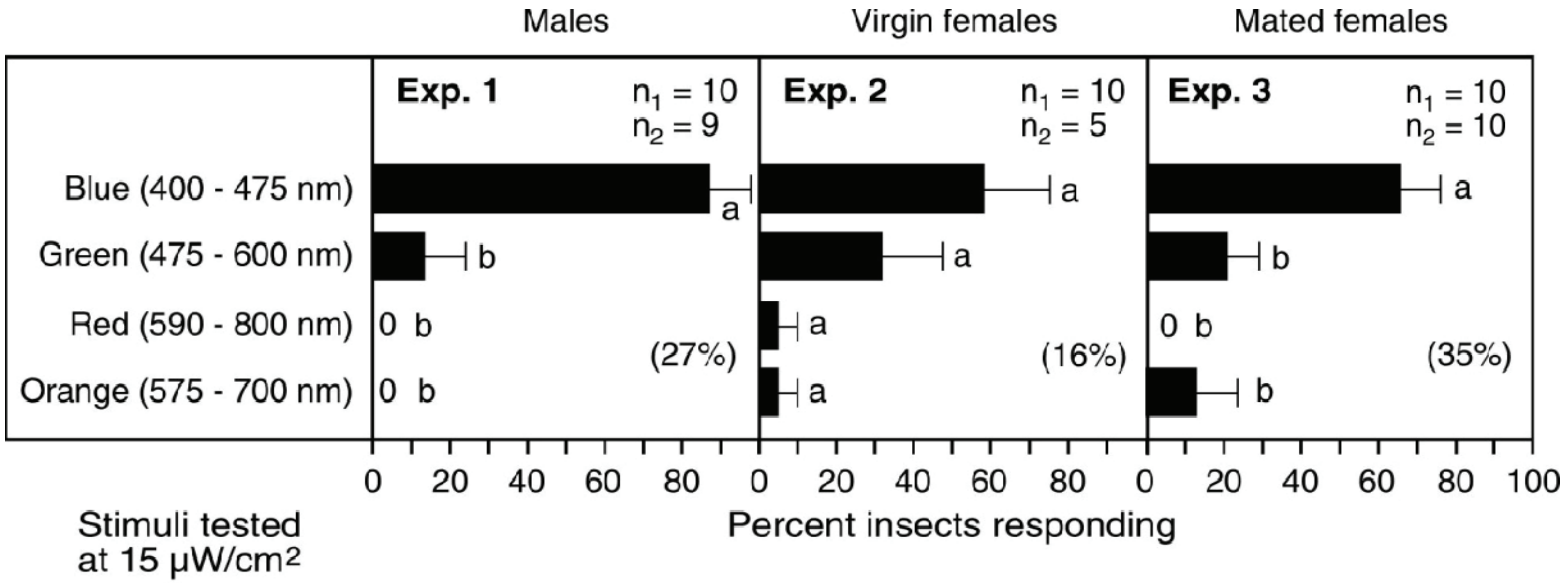
maximum intensity of light obtainable from the blue filter), integrated from 350–700 nm, and measured at the filter held 8 cm from the halogen bulb.

In experiments 1–3, significantly more males (Exp. 1) and gravid females (Exp. 3) were captured in traps emitting blue light than in traps emitting green, red or orange light. Virgin females (Exp. 2) were not very responsive and exhibited no preference for either spectrum of light (Figure 4).

Experiments 4–6: Attractiveness of wavelengths in the blue range (400–475 nm)

Experiments 4–6 were designed to determine the wavelengths in the blue range most effective in attracting males (Exp. 4, n = 10), virgin females (Exp. 5, n = 10) and mated females (Exp. 6, n = 10). LEDs (Roithner Lasertechnik, Vienna, Austria) with peak wavelengths of 405 nm (range 400–410 nm), 435 nm (range 410–470 nm), 450 nm (range 440–460 nm) and 470 nm (range 465–475 nm) (Figure 3) were tested in four-choice experiments. For each replicate, one of the four LEDs was randomly assigned to, and mounted within, green Delta traps (Figure 2, D; see general experimental design), using an LED controller to adjust the intensity of each LED to 200 $\mu W/cm^2$ integrated from 350 nm to 550 nm. Each LED was calibrated by inserting it into a ridged black cloth-covered plastic tube (28 × 1 cm) 12 cm apart from the light detector positioned at the opposite end of the tube (Figure 2, E). Compared to experiments 1–3, light intensities were increased to help maintain or even increase the high percentage of responding insects.

Figure 4 Mean (+ SE) percent of male, virgin female and mated female *Plodia interpunctella* responding to spectra of visible light in four-choice experiments 1–3. In each experiment n_1 is the number of replicates that were tested; n_2 is the number of replicates that did yield responding insects; the number in parenthesis is the overall percentage of responding insects; bars with the same letter are not statistically different (Kruskal-Wallis test followed by the Student-Newman-Keuls' analog: $P < 0.05$).


Males
Virgin females
Mated females
Exp. 1
n1 = 10
n2 = 9
Exp. 2
n1 = 10
n2 = 5
Exp. 3
n1 = 10
n2 = 10
Blue (400 - 475 nm)
Green (475 - 600 nm)
Red (590 - 800 nm)
Orange (575 - 700 nm)
a
b
0 b
a
(27%)
(16%)
(35%)
0 20 40 60 80 0 20 40 60 80 0 20 40 60 80 100
Stimuli tested
at 15 µW/cm2
Percent insects responding

In experiments 4–6, significantly more males (Exp. 4), virgin females (Exp. 5) and mated females (Exp. 6) were captured in traps fitted with the 405-nm LED than in traps fitted with the 435-nm, 450-nm or 470-nm LED (Figure 5).

Experiments 7–9: Attractiveness of wavelengths in the UV- and violet-light range (350–405 nm)

To determine the wavelength(s) in the UV- and violet-light range that are the most attractive, LEDs with peak wavelengths of 350 nm, 365 nm, 380 nm and 405 nm (integrated from 300 nm to 450 nm) were tested with males (Exp. 7, n = 10), virgin females (Exp. 8, n = 10) and mated females (Exp. 9, n = 10). Experimental design and calibration method were identical to those described for experiments 4–6 (Figure 6).

In experiment 7, the 350-nm and 380-nm LEDs were equally effective in attracting males, but the latter was not more effective than the 365- and 405-nm LED (Figure 6). In experiment 8, the 350-, 365-, 380- and 405-nm LEDs all were equally ineffective in attracting virgin females (Figure 6). In experiment 9, the 350-nm LED attracted significantly more mated females than did the 365-, 380- and 405-nm LEDs (Figure 6).

Experiments 10–13: Attractiveness of UV and violet light singly or in combination

Given the strong attraction of IMMs to the 405-nm (violet) and 350-nm (UV) LEDs in experiments 4–6 and 7–9, respectively, experiments 10–13 explored whether

Figure 5 Mean (+ SE) percent of male, virgin female and mated female *Plodia interpunctella* responding to light emitting diodes (LEDs) with peak wavelengths of 405 nm, 435 nm, 450 nm or 470 nm in four-choice experiments 4–6. Additional information is provided in the caption of figure 3. In each experiment, bars with the same letter are not statistically different (Kruskal-Wallis test followed by Student-Newman-Keuls' analog: $P < 0.05$).

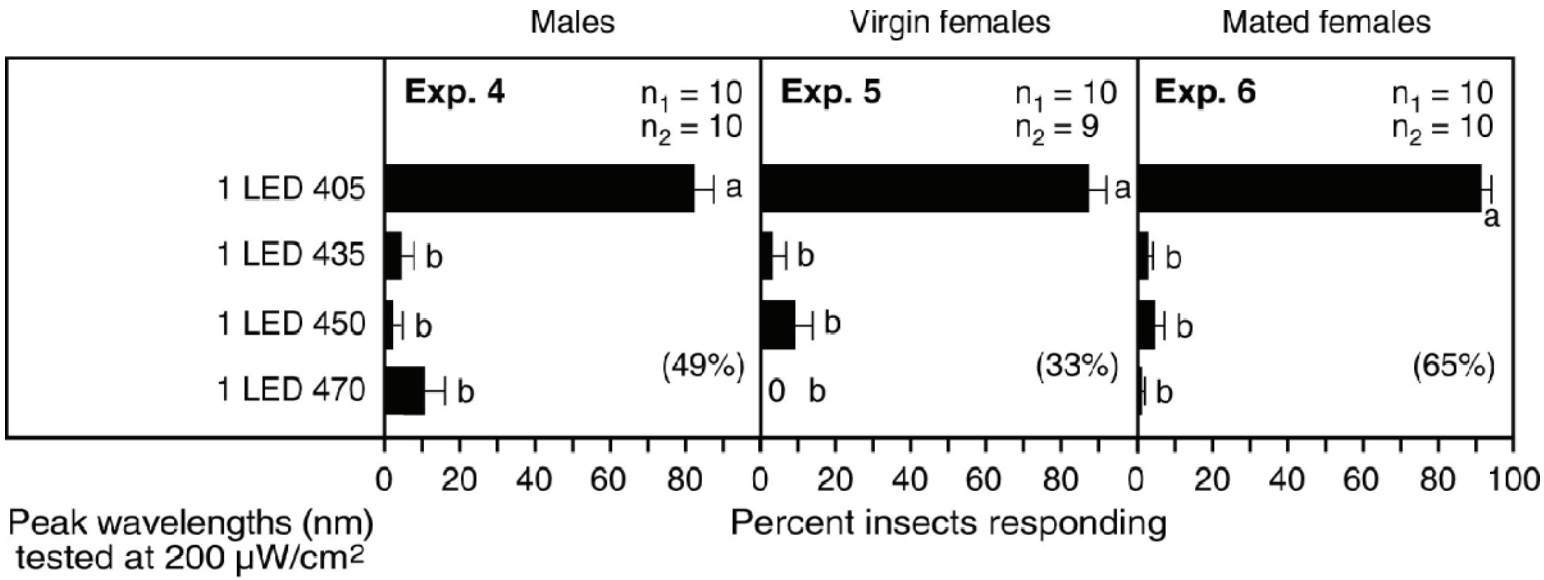
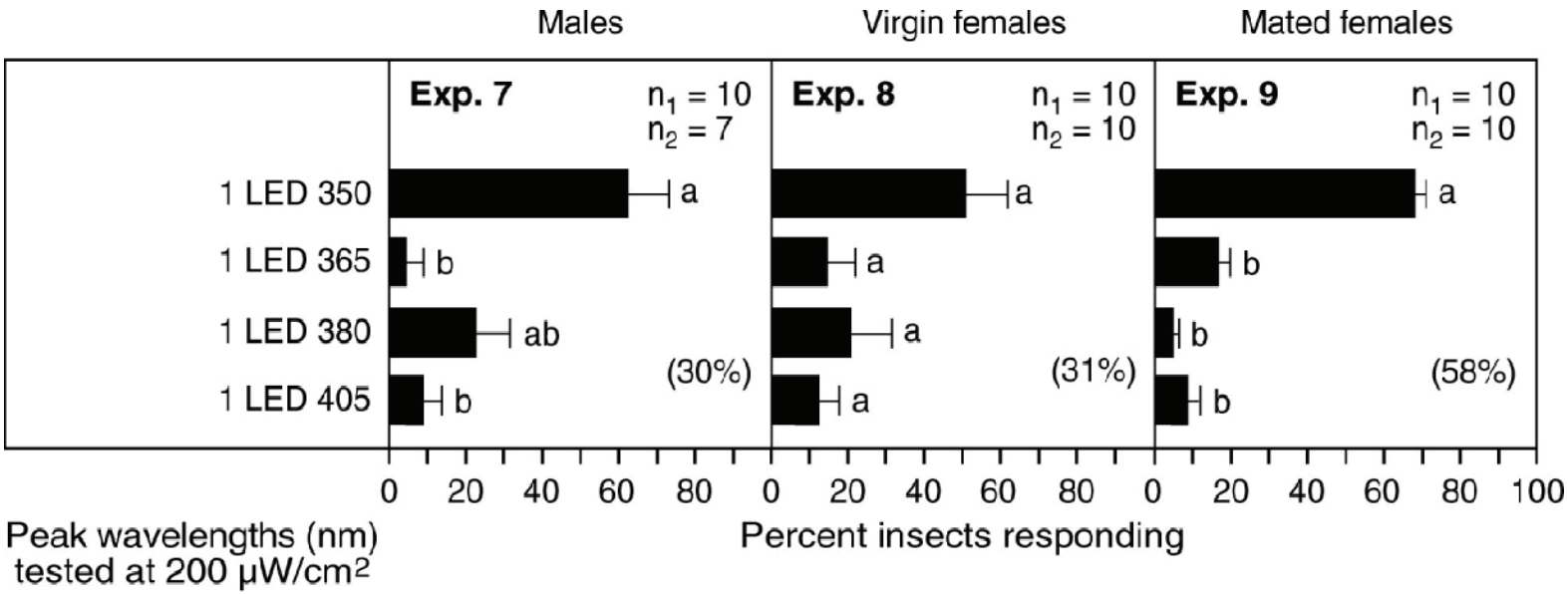

Males
Virgin females
Mated females
Exp. 4
n1 = 10
n2 = 10
Exp. 5
n1 = 10
n2 = 9
Exp. 6
n1 = 10
n2 = 10
1 LED 405
1 LED 435
1 LED 450
1 LED 470
a
b
b
b
a
b
b
0 b
a
b
b
b
(49%)
(33%)
(65%)
0 20 40 60 80 0 20 40 60 80 0 20 40 60 80 100
Peak wavelengths (nm)
tested at 200 µW/cm2
Percent insects responding

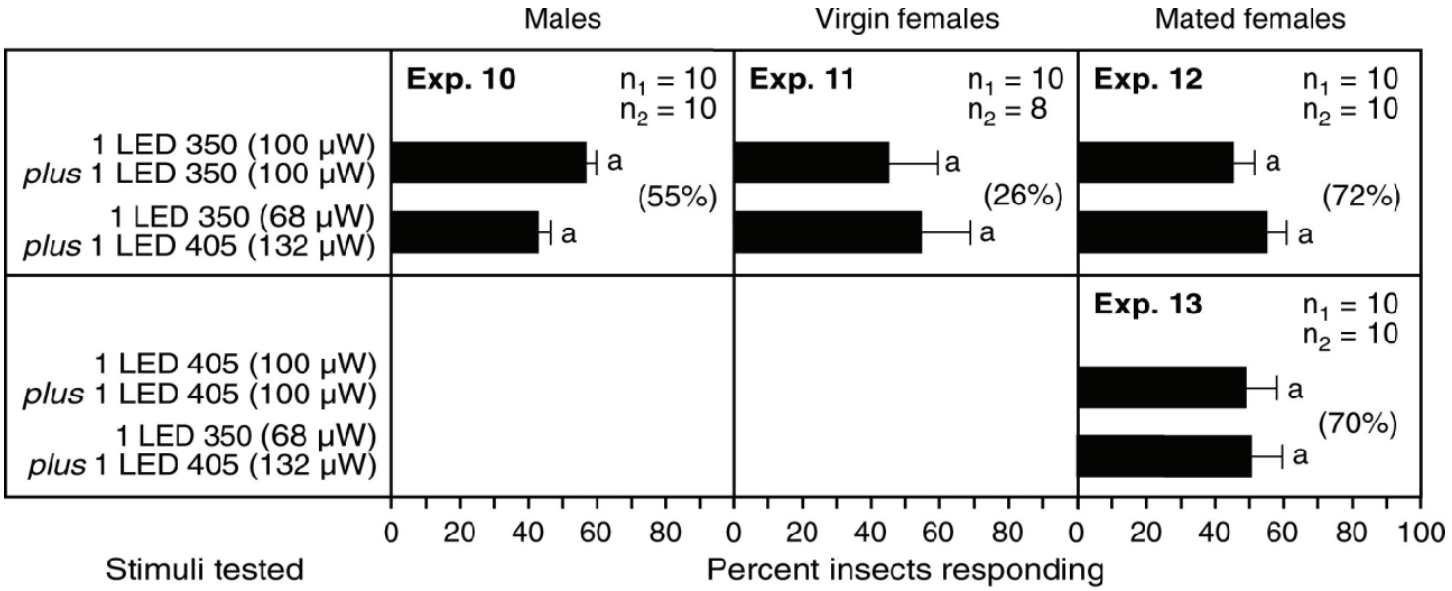
Figure 6 Mean (+ SE) percent of male, virgin female and mated female *Plodia interpunctella* responding in four-choice experiments 7–9 to light emitting diodes (LEDs), emitting ultraviolet and violet light at peak wavelengths of 350 nm, 365 nm, 380 nm or 405 nm. Additional information is provided in the caption of figure 3. In each experiment, bars with the same letter are not statistically different (Kruskal-Wallis test followed by Student-Newman-Keuls' analog: $P < 0.05$).


Males
Virgin females
Mated females
Exp. 7
n1 = 10
n2 = 7
Exp. 8
n1 = 10
n2 = 10
Exp. 9
n1 = 10
n2 = 10
1 LED 350
1 LED 365
1 LED 380
1 LED 405
a
b
ab
b
a
a
a
a
a
b
b
b
(30%)
(31%)
(58%)
0 20 40 60 80
0 20 40 60 80
0 20 40 60 80 100
Peak wavelengths (nm)
tested at 200 µW/cm2
Percent insects responding

violet and UV wavelengths are more attractive in combination than on their own. Two different experimental designs were deployed. The first design tested one 405-nm LED (132 μW/cm^2) and one 350-nm LED (68 μW/cm^2) with a combined light intensity of 200 μW/cm^2/trap as the treatment stimulus versus a control stimulus that consisted of two 350-nm LEDs with a combined light intensity of 200 μW/cm^2/trap (integrated from 300–450 nm) for attraction of males (Exp. 10, n = 10), virgin females (Exp. 11, n = 10), and mated females (Exp. 12, n = 10). The second design tested the same treatment stimulus versus a control stimulus that consisted of two 405-nm LEDs with a combined light intensity of 200 μW/cm^2/trap (integrated from 300–450 nm) for attraction of mated females (Exp. 14, n = 10). Only mated females were tested for the second design because mated females appeared to respond best in preceding experiments. Emission of the 405-nm wavelength at intensities twice as high as the UV wavelength in combined light sources was based on proportionally higher levels of violet light at sunset and twilight (Robertson, 1966; Johnsen et al., 2006).

In experiments 10–12, the two 350-nm LEDs attracted as many males, virgin females and mated females as did the 350- and 405-nm LEDs in combination (Figure 7). In experiment 13, the two 405-nm LEDs attracted as many gravid females as the combined 350- and 405-nm LEDs (Figure 7).

Figure 7 Mean (+ SE) percent of male, virgin female and mated female *Plodia interpunctella* responding in two-choice experiments 10–13 to combinations of light emitting diodes (LEDs), emitting ultraviolet and violet light at peak wavelengths of 350 nm and 405 nm. Additional information is provided in the caption of figure 4. In each experiment, bars with the same letter are not statistically different (Wilcoxin rank sum test: $P < 0.05$).


Males
Virgin females
Mated females
Exp. 10
n1 = 10
n2 = 10
Exp. 11
n1 = 10
n2 = 8
Exp. 12
n1 = 10
n2 = 10
1 LED 350 (100 µW)
plus 1 LED 350 (100 µW)
a
(55%)
a
(26%)
a
(72%)
1 LED 350 (68 µW)
plus 1 LED 405 (132 µW)
a
a
a
Exp. 13
n1 = 10
n2 = 10
1 LED 405 (100 µW)
plus 1 LED 405 (100 µW)
a
(70%)
1 LED 350 (68 µW)
plus 1 LED 405 (132 µW)
a
0 20 40 60 80
0 20 40 60 80
0 20 40 60 80 100
Stimuli tested
Percent insects responding

Experiment 14: Attractiveness of single and dual light sources of identical intensities

In preparation for experiment 15, where the maximum output from one LED was provided by two LEDs, experiment 14 (n = 10) determined whether stimuli of identical light intensity emitted from one or two light sources are equally effective in attracting IMMs. Thus, one 405-nm LED at 200 μW/cm^2 (integrated from 350 nm to 450 nm) was tested *versus* two 405-nm LEDs each at 100 μW/cm^2 (integrated from 350-450-nm). Only males were tested because in experiments 4–6 the insects' response to the 405-nm wavelength appeared not affected by their gender or mating status. In experiment 14, one 405-nm LED at 200 μW/cm^2 and two 405-nm LEDs at 100 μW/cm^2 each were equally effective in attracting males (Figure 8).

Experiments 15–17: Effects of light intensity and wavelength combination

Considering *(i)* that high-intensity lights are more effective than low-intensity lights in attracting IMMs (Stremer, 1959), and *(ii)* that a 350-nm LED emits on average not more than 100 μW/cm^2, experiment 15 investigated whether attractiveness of a 350-nm LED (100 μW/cm^2) could be enhanced by combining it with a 405-nm LED that emitted a light intensity of 600 μW/cm^2 (integrated from 300 nm to 450 nm) instead of merely 132 μW/cm^2, as in experiments 10–13. The control stimulus consisted of two 350-nm LEDs with a combined light intensity of 100 μW/cm^2 (integrated from 300 nm to 450 nm).

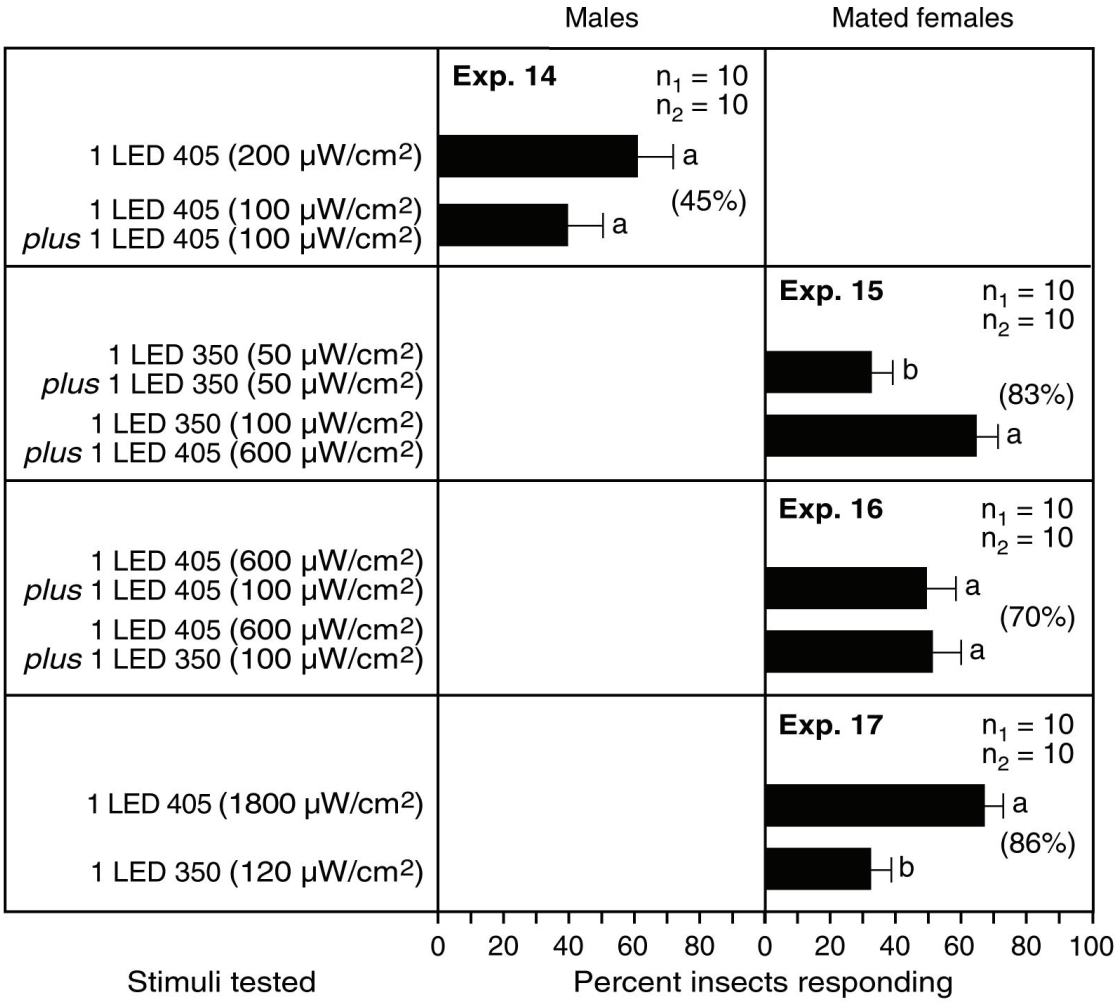
The significantly stronger response of mated females in experiment 15 to the 350- and 405-nm LED combination could have been due to its higher light

intensity or particular wavelength combination. Thus, experiment 16 (n = 10) tested both stimuli at identical light intensity (700 μW/cm^2) but contrasting light composition. Stimulus 1 consisted of two 405-nm LEDs at 600 and 100 W/cm^2, respectively (integrated from 300 nm to 450 nm), whereas stimulus 2 consisted of one 405-nm LED at 600 μW/cm^2 and one 350-nm LED at μ100 W/cm^2 (integrated from 300 nm to 450 nm).

With light intensity affecting the attractiveness of multiple LED light sources in experiments 15 and 16, experiment 17 (n = 10) tested one 405-nm LED *versus* one 350-nm LED at maximum intensities of respectively ~100 μW/cm^2 and ~1800 μW/cm^2.

In experiment 15, the combination of one 350-nm and one 405-nm LED at a combined light intensity of 700 μW/cm^2 attracted significantly more mated females than did two 350-nm LEDs at a combined light intensity of 100 μW/cm^2. In experiment 16, with each stimulus at the same combined light intensity of 700 μW/cm^2, the two 405-nm LEDs attracted as many mated females as did one 350-nm and one 405-nm LED (Figure 8). In experiment 17, one 405-nm LED at ~1800 μW/cm^2 attracted significantly more mated females than did one 350-nm LED at ~100 μW/cm^2 (Figure 8).

Figure 8 Mean (+ SE) percent of male or mated female *Plodia interpunctella* responding in two-choice experiments 14–17 to light emitting diodes (LEDs), emitting violet light and ultraviolet light at peak wavelengths of 405 nm and 350 nm. Additional information is provided in the caption of figure 3. In each experiment, bars with the same letter are not statistically different (Wilcoxin rank sum test: $P < 0.05$).


Males
Mated females
Exp. 14
n1 = 10
n2 = 10
1 LED 405 (200 µW/cm2)
a
1 LED 405 (100 µW/cm2)
plus 1 LED 405 (100 µW/cm2)
a
(45%)
Exp. 15
n1 = 10
n2 = 10
1 LED 350 (50 µW/cm2)
plus 1 LED 350 (50 µW/cm2)
b
(83%)
1 LED 350 (100 µW/cm2)
plus 1 LED 405 (600 µW/cm2)
a
Exp. 16
n1 = 10
n2 = 10
1 LED 405 (600 µW/cm2)
plus 1 LED 405 (100 µW/cm2)
a
(70%)
1 LED 405 (600 µW/cm2)
plus 1 LED 350 (100 µW/cm2)
a
Exp. 17
n1 = 10
n2 = 10
1 LED 405 (1800 µW/cm2)
a
(86%)
1 LED 350 (120 µW/cm2)
b
0 20 40 60 80 0 20 40 60 80 100
Stimuli tested
Percent insects responding

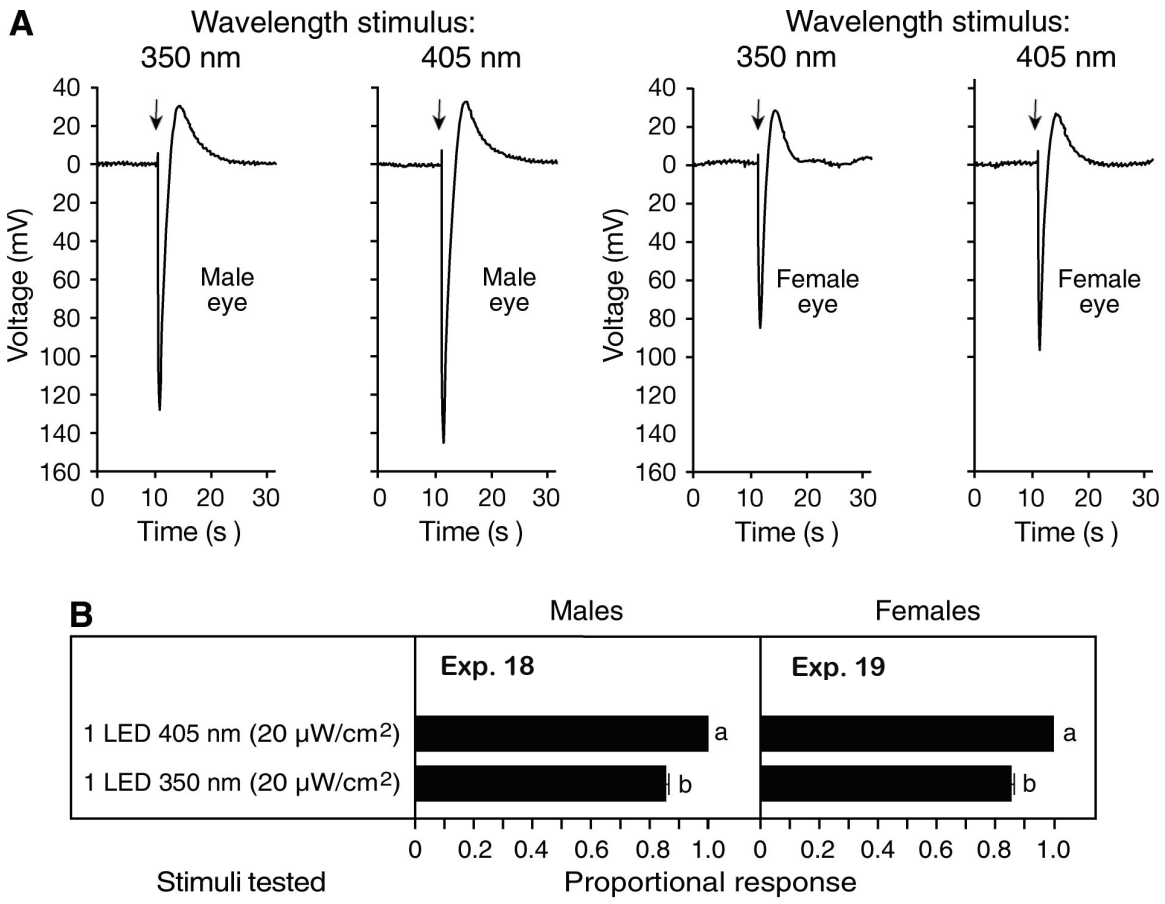
Experiments 18 and 19: Electroretinogram recordings

Eyes of males (Exp. 17, n = 6) and females (Exp. 18, n = 6) were exposed in alternating sequence to 350-nm and 405-nm wavelengths at a light intensity of 20 μW/cm^2 emitted from LEDs. Each LED was inserted into a reflective foil-covered 2-ml pipette tip (T-100-C; Axygen Scientific, Union City, CA, USA), with 2.3-cm clearance between the LED and the tip. The LED was calibrated to 20 μW/cm^2 using an HR4000 high-resolution spectrometer (Ocean Optics, Dunedin, Florida) fitted with a cosine corrector at the detector. For calibration, the pipette tip was positioned 1 mm apart from the detector, with the light spot focused at the middle of the detector surface, and intensities were measured using an integration of 300 nm to 450 nm. For ERG recordings, the pipette tip was positioned 1 mm above the eye, delivering a 0.5-s flash of light controlled by a programmable timer. Between exposures, insects were dark-adapted for 20 min. Electrical potentials in response to light stimuli were normalized to the greatest response amplitude. Eyes of males (Exp. 18) and females (Exp. 19) responded significantly more strongly to the 405-nm LED than to the 350-nm LED (Figure 9).

3.4 Discussion

My data support the conclusion that the IMM uses violet light in addition to UV light as an orientation cue. Gender and mating status of IMMs appear to affect their responsiveness to light. My observations that males and mated but not

Figure 9 **(A)** Representative electroretinograms of eyes of male (left) and female (right) *Plodia interpunctella* responding to 405-nm and 350-nm wavelengths at 20 μW/cm^2 each; arrows indicate the onset of the 0.5-s light stimulus; **(B)** mean (+ SE) proportion of electrical potentials elicited by eyes of male and female *Plodia interpunctella* in response to 405-nm and 350-nm wavelengths. Bars with a different letter are statistically different (Wilcoxin rank sum test: $P < 0.05$).


A
Wavelength stimulus:
350 nm
405 nm
Wavelength stimulus:
350 nm
405 nm
Voltage (mV)
Male eye
Female eye
Time (s)
B
Males
Females
Exp. 18
Exp. 19
1 LED 405 nm (20 µW/cm2)
1 LED 350 nm (20 µW/cm2)
a
b
Stimuli tested
Proportional response

virgin females engage in prolonged flights are well reflected in the percentage of IMMs that are captured in light-emitting traps (Figures 4–7). Similarly, pheromone-emitting virgin females of the almond moth, *Ephestia cautella* (Walker) hardly take flight, whereas conspecific males engage in prolonged flights (Hagstrum and Davis, 1980).

Captures of males and gravid females of the hemlock looper, *Lambdina fiscellaria* (Guenée), in UV light traps (Delisle et al., 1998) were attributed to foraging behaviour for mates and oviposition sites, respectively. In all my experiments (Figures 4–7), the percentage of gravid females responding to attractive light exceeded that of males or virgin females. These results contrast with those reported by Soderstrom (1970) that 2–3 times more males than females responded to traps emitting green or UV light. Based on my results and those cited above, it appears that mating induces a behavioural reversal from sedentary pheromone emission to dispersal or foraging flight coupled with a strong phototactic response. My conclusion that UV and violet light are orientation or navigation cues, rather than nectar guides in flowers, is supported by reports that IMMs rarely feed (Nansen et al., 2003; Olsson et al., 2005a,b).

Stronger orientation to blue than to green lights by both males and gravid females (Figure 4) contrasts with previous conclusions (Stremer, 1959) that green is more attractive than blue light. These contrasting conclusions may be due, in part, to contrasting experiment designs. While Stremer (1959) used no-choice experiments to test UV, green or violet light (404.7 nm) as singular test stimuli at 9 $\mu W/cm^2$, I tested light stimuli at 15 $\mu W/cm^2$ in 2- or 4-choice

experiments. In electroretinograms, green wavelengths elicited the strongest response but there was also suggestive evidence for a potential “blue receptor” sensitive to wavelengths of ~460 nm (Marzke et al., 1973). If the spectral sensitivity pattern of photoreceptors were indicative of the wavelengths needed for orientation, then green light should have been the most effective phototactic cue. Strong attraction to blue light instead (Figure 4) suggests that electroretinograms have limited value in predicting the wavelengths that play key roles during various behaviours of insects, including orientation behaviour. They provide information, however, as to which wavelength an insect can detect.

Known blue receptors in the Lepidoptera exhibit peak sensitivity near 460 nm (Eguchi et al., 1982; Briscoe and Chittka, 2001). Strong attraction of male and female IMMs to the 405-nm violet LED, and hardly any attraction to the 435-, 450- and 470-nm blue LEDs (Figure 5), indicates that the violet region of visible blue light is used for orientation. This peak response to violet-blue light is likely not mediated by a dominant response of a blue-light receptor. For example, blue photoreceptors of the tobacco hornworm, *Manduca sexta* L*,* induce feeding responses and are most sensitive to a “mid” 450-nm wavelength, with shorter or longer wavelengths eliciting weaker behavioural responses (Cutler et al., 1995). IMMs do not show such responses towards blue light.

There are two potential explanations for the profound attractiveness of the 405-nm violet LED. First, IMMs may detect violet light with the tail end of the UV receptotor’s sensitivity range. The stronger stimulation of the UV receptor compared with the blue receptor could explain why males, virgin and mated

females orient more strongly to the 350-nm UV LED than to the 405-nm violet LED (Figure 6). Second, the strong response to violet light (Figure 5) may be due, in part, to specific properties of the green photoreceptor. The main alpha-band and small beta-band of green receptors absorb wavelengths of green and UV light, respectively (Stavenga, 2006). Conceivably, weak but concurrent stimulation by violet light of both the green receptors' beta-band and specific UV receptors may trigger strong electrophysiological responses (Figure 9) and behavioural responses (Figure 5) to violet light. Increasing the intensity of attractive wavelengths of light resulted in correspondingly stronger attraction of IMMs (Stremer, 1959). These results present intriguing prospects for manipulating the behaviour of IMMs in pest management settings, and inspired the design of experiment 15 (Figure 8).

Considering that visible-light LEDs are capable of emitting higher light intensities, and are less expensive and less damaging to human eyes than UV LEDs, we offered gravid female moths a choice between two 350-nm LED and a combination of one 350-nm LED and one 405-nm LED with a greater combined light intensity. Stronger attraction of females to the LED combination (Figure 8, Exp. 15), equal attraction to equal-intensity 405-nm lights with or without UV component (Figure 8, Exp. 16), and stronger attraction to a 405-nm LED than to a 350-nm LED at maximum light intensities (Figure 8, Exp. 16) all indicate that deployment of 405-nm LEDs should be considered as one of several possible tactics (Svensson et al., 2003) within integrated management programs for IMMs.

4: Regional spectral responses in the eye of the Indian meal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae)

4.1 Introduction

Spectral responses of different eye regions have been documented in nocturnal moths with superposition eyes, diurnal butterflies with apposition eyes, or in insects with superposition eyes that are active during diurnal and nocturnal periods. The ventral region of the apposition eye of the diurnal, flower-feeding butterfly *Papilio xuthus* responds strongly to UV/violet light in electroretinograms (ERGs) and is thought to contain the most UV/violet light receptors to detect UV nectar guides in flowers (Arikawa et al., 1987). Other diurnal Lepidoptera have a specialized area in the dorsal region of the eye. This dorsal rim area (DRA) is sensitive to polarized UV light (Hämmerle and Kolb, 1996; Stalleicken et al., 2006; Stavenga and Arikawa, 2006). Gilburt and Anderson (1996) compared regional ERG responses of the eyes of the flower moth, *Ephestia cautella* (Walker), and report higher spectral efficacy in the reception of UV wavelengths in the dorsal and ventral regions than in the equatorial region. The authors conclude that these responses are likely the result of adaptations to facilitate navigation and nectar feeding. Based on rhodopsin identification, the DRA of the superposition eye of the tobacco hornworm, *Manduca sexta,* detects polarized

sky light in the UV range, and the ventral region is more sensitive in the blue region, likely to facilitate nectar feeding (White et al., 2003).

The Indian meal moth (IMM), *Plodia interpunctella,* is a crepuscular moth that is most active in the first 2 hours of scotophase (twilight conditions). It is a serious pest of a variety of stored products (Mohandas, 2007). IMMs exhibit a strong phototactic response to traps emitting UV and green light (Stremer, 1959; Kirkpatrick et al., 1970; Soderstrom, 1970; Sambaraju and Philips, 2008) or violet light (Cowan and Gries, 2009). However, the regional spectral response of the IMM eye has not been investigated nor is it known whether regional efficacy affects phototactic attraction of IMM.

Because IMMs can move over a large spatial scale (Campbell and Abogast, 2004) and the dorsal eye region typically receives navigational cues, I tested whether (*i*) the dorsal region of IMM eyes responds more strongly to UV and violet light than the equatorial and ventral regions, and (*ii*) occlusion of the dorsal region adversely affects phototactic orientation.

4.2 Materials and methods

4.2.1 Origin and maintenance of IMM colony

Indian meal moth larvae were obtained from infested cereal bars collected from a processing plant. Larvae were reared at 25–27ºC at a photoperiod of L17:D7. The rearing diet was modified from Le Cato (1976), and consisted of whole wheat flour (27.5% by volume), yellow cornmeal (27.5%), Purina One dog food

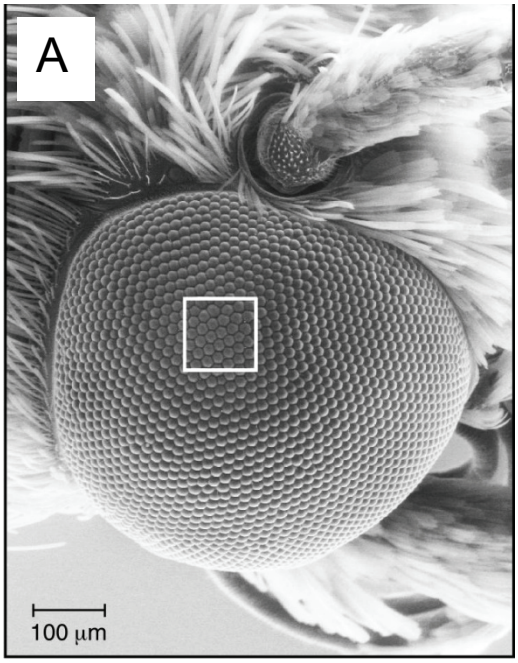
(13.5%), brewers yeast (6.9%), honey (6.9%), glycerine (6.9%; 96% pure), Quaker rolled oats (6.8%) and wheat germ (3.4%).

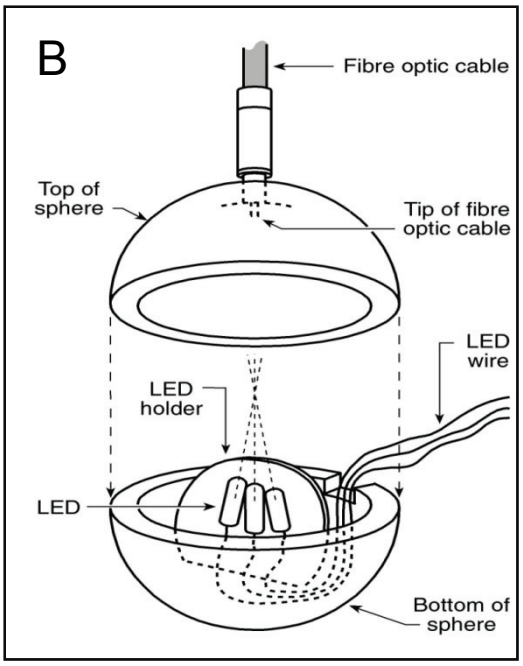
Fifth-instar larvae were separated by sex and placed into Petri dishes (10 cm diameter), in groups of 12–15 specimens containing corrugated cardboard as pupation sites. Eclosed adults were kept at both reversed and staggered photoperiods to allow experimentation throughout the entire day. To obtain gravid females, 2–3 virgin females and 3–4 virgin males were confined in small cages (10 × 10 × 10 cm) during the scotophase. The next day, females were assumed mated, and were used for colony rearing or laboratory experiments. All adult moths used in experiments were 2–5 days old.

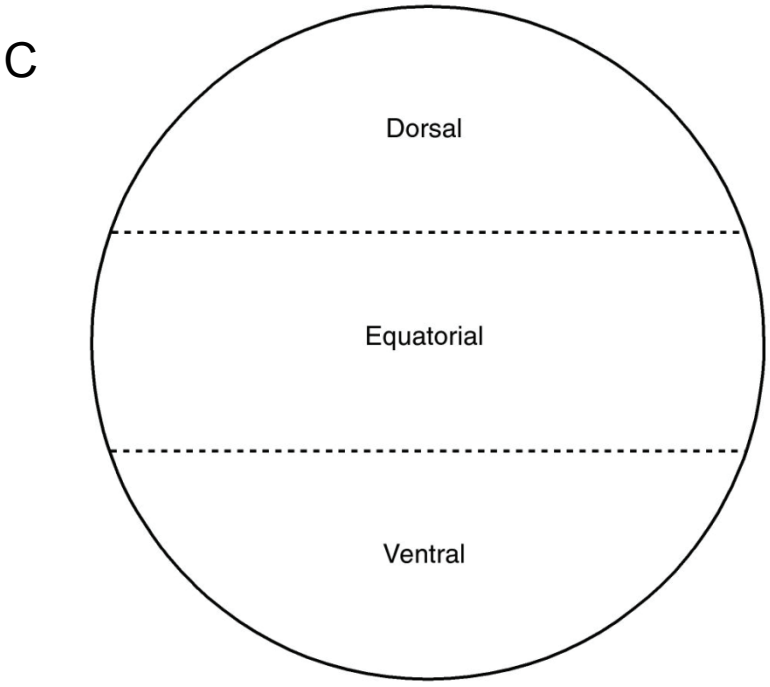
4.2.2 Scanning electron micrographs of compound eyes

To compare the number of facets in the dorsal, equatorial and ventral region of eyes, Environmental Scanning Electron Micrographs (ESEM) were taken by an FEI Quanta FEG 400 instrument (Shottky-type field-emission source; accelerating voltages: 0.2 to 30 keV; resolution: 3.5 nm at 3 kV in low-vacuum mode) at the Pulp and Paper Institute of Canada (Paprican). For counting facets, an area of 100 × 100 μm was superimposed over the micrograph (Image J, Wayne Rasband, National Institute of Mental Health, Bethesda, Maryland, USA) at the point of focus in each region (Figure 10A) of four eyes of female and male moths.

Figure 10 Environmental scanning electron micrograph of a *Plodia interpunctella* eye with a 100 × 100 μm area superimposed for counting facets; **(B)** Experimental design showing a sphere, light-emitting diodes and a fibre optic light guide deployed for exposure of eyes to light stimuli; **(C)** Schematic drawing depicting dorsal, equatorial and ventral regions of an eye.







Compound eye

4.2.3 Electroretinogram responses of eye regions to LED stimuli (experiments 1, 2)

Insects where immobilized with grey modelling clay (Sargent Art Inc., Hazleton, PA, USA) lateral-, ventral- or dorsal-side down on an adhesive-coated glass slide. Antennae and left wing were removed to allow access of electrodes. Eye sections were occluded with acrylic metal paint (Dazzling Metallics elegant finish rich espresso; DecoArt, Stanford, KY) applied with a single bristle of a paint brush. Grey clay was then placed around the entire head to immobilize it and further block light.

Electrically sharpened Tungsten electrodes (Cool et al., 1970) were used for all recordings. The indifferent electrode was micro-manipulated (Leitz micromanipulator M; Leitz, Vienna, Austria) into the thorax and the recording electrode into the left edge of the eye to standardize recordings and prevent light shadows. Electrical potentials from the eye in response to test stimuli were preamplified (Syntech Auto Spike; Syntech, Hilversum, The Netherlands) and recorded with an electroantennogram (EAG) oscilloscope program (Syntech). Based on the signal-to-noise ratio, potentials of > 5 mV were considered responses (Cowan and Gries, 2009).

Dorsal, equatorial and ventral sections (figure 10B) of female (experiment 1) and male (experiment 2) eyes were exposed to light-emitting diodes (LEDs; Roithner Lasertechnik, Vienna, Austria) with peak wavelengths of 350 nm, 405 nm, and 525 nm (Figure 3). LEDs were mounted in an array within a white-painted sphere (DecoArt no prep metal paint Deepcar Sheffield) that was connected to a fibre optic cable (Rocketfish™, Richfield, MN, USA) as a light

guide (Figure 10C). The 405- and 525-nm LEDs were at a 40^{o} angle relative to the centre 350-nm LED. Each LED emitted a light intensity of 20 μW/cm^2 calibrated with a HR4000 high-resolution spectrometer (Ocean Optics, Dunedin, FL, USA) fitted with a cosine corrector at the detector (Cowan and Gries, 2009). A light intensity of 20 μW/cm^2 was chosen because it was within the eyes' linear ERG response to wavelengths tested at 5–100 μW/cm^2, with the highest intensity saturating receptors. For calibration, the lens of the fibre optic cable was positioned 2 mm from the spectrometer's detector, with the light spot focused at the middle of the detector surface. For electro-retinograms, the fibre optic cable was positioned directly over, and 2 mm from, the insect eye. Eyes were dark-adapted for 40 min before they were exposed at random order and 20-s intervals to a 0.5-s flash of each wavelength. For each eye (n = 30) only one region was tested.

4.2.4 Attraction of IMMs with partially occluded eyes to a 405-nm LED (experiments 3, 4)

Experiments 3 and 4 were designed to determine whether IMMs, with part of their visual field occluded, can still orient to a light source. Males (experiment 3, n = 10) and mated females (experiment 4, n = 10) were CO_2-anesthetized to occlude with paint (see above) the dorsal, equatorial or ventral surface of their eyes (treatments 1–3). A fourth treatment group consisted of moths whose eyes were painted with water as a positive control. All moths were allowed 12 hr to recover from the treatment prior to bioassays ensuring that the water placed on the eyes of the control insects had evaporated and would not distort the eye's

optics. Gravid females were produced by allowing eye-occluded virgin females to mate (which they readily did) during the 12-h recovery time. Each experimental replicate tested the response of 20 moths, five of each of the four treatment groups.

Experiments were conducted in a modified wind tunnel (1.10 × 1.10 × 3.30 m long) with air entry and exit sections covered by mesh screens and black paper to minimize light reflections. For each replicate, two Petri dishes with 10 insects each were placed on a 50-cm tall, black felt-covered platform (23 × 30 cm) in the centre of the tunnel. For each replicate, one 405-nm LED calibrated to emit 400 μW/cm^2 (see above) was placed inside an adhesive-lined green delta trap (Cowan and Gries, 2009) that was positioned by random assignment at one end of the wind tunnel, altering the position in subsequent replicates. All experiments were conducted in the first 2 h of the 7-h dark phase.

An experimental replicate was initiated by lifting the lid of each Petri dish, and terminated by scoring the number of moths trap-captured 2 h later. All moths not responding were removed from the wind tunnel prior to initiating a new replicate. After each set of four replicates, the wind tunnel was wiped with 70% ethanol and left to “aerate” overnight.

4.2.5 Statistical Analyses

Differences in response between male and female eyes in their dorsal, equatorial or ventral region when exposed to a 350-, 405-, or 525-nm LED were analyzed by Student’s t-test. Numbers of facets in dorsal, equatorial and ventral regions of

male and female eyes, gender-combined ERG responses in experiments 1 and 2 within or between eye regions exposed to a 350-, 405-, or 525-nm LED, were log-transformed prior to analyses to ensure normality of data and compared using ANOVA followed by the Tukey's HSD test. In each replicate of behavioural experiments 3 and 4, the total number of insects trap-captured was counted and the number of insects in the four treatment groups converted to proportion of total trap catch, so that only responding insects would be included in statistical analyses. These proportional data were arcsine-transformed and subjected to the Kruskal-Wallis test for non-parametric data followed by the Student-Newman-Keuls' analog for multi-comparison of means (Zar, 1999). All data analyses employed JMP software (SAS®, Cary, NC, USA).

4.3 Results

4.3.1 Scanning electron micrographs of compound eyes

In each of the dorsal, equatorial or ventral eye region, there were no differences between the number of facets in the eyes of females and males (Student's t-test, $P > 0.05$), which justified pooling of data for each region. There were significantly more gender-combined facets in the dorsal eye region than in the equatorial or ventral eye region ($F_{2,5} = 9.0580$, $P < 0.0013$; Figure 11). There was no difference in response between male or female eyes in their dorsal, equatorial or ventral region when the region was exposed to a 350-, 405-,or 525-nm LED (Student's t tests, $P > 0.05$). These results justified pooling data of region-specific

responses of male and female eyes. Combined-gender responses of the equatorial eye region to either a 350-, 405-, or 525-nm LED were significantly stronger than those of the ventral region but not those of the dorsal region (350-nm LED: $F_{2,57} = 3.2353$, $P < 0.05$; 405-nm LED: $F_{2,57} = 4.1295$, $P < 0.05$; 525-nm LED: $F_{2,57} = 3.2291$, $P < 0.05$; Figure 12). Combined-gender responses of the dorsal, equatorial or ventral region were strongest to the 525-nm LED (dorsal region: $F_{2,57} = 11.2348$, $P < 0.001$; equatorial region: $F_{2,57} = 10.6569$, $P < 0.001$; ventral region: $F_{2,57} = 9.2271$, $P < 0.003$; Figure 12). When combined-gender and region-specific responses to the 350- or 405-nm LED were expressed as percent of the strongest response (set 100%) to the 525-nm LED, there were no significant differences between regions (350-nm LED: $F_{2,57} = 1.0619$, $P > 0.05$; 405-nm LED: $F_{2,57} = 1.0425$, $P > 0.05$; 525-nm; Figure 13).

4.3.2 Attraction of IMMs with partially occluded eyes to a 405-nm LED (experiments 3, 4)

There was no significant difference in captures of females (experiment 3: df = 3, $\chi^2 = 5.6846$, $P > 0.05$) or males (experiment 4: $\chi^2 = 1.2698$, $P > 0.05$) in traps baited with a 405-nm LED irrespective of whether or not a specific region of the eye was experimentally occluded (Figure 14).

Figure 11 Mean (+ SE) number of facets in dorsal, equatorial and ventral regions of eyes of female and male *Plodia interpunctella* (1-way ANOVA, $P < 0.05$, followed by Tukey's HSD test, $P < 0.05$).

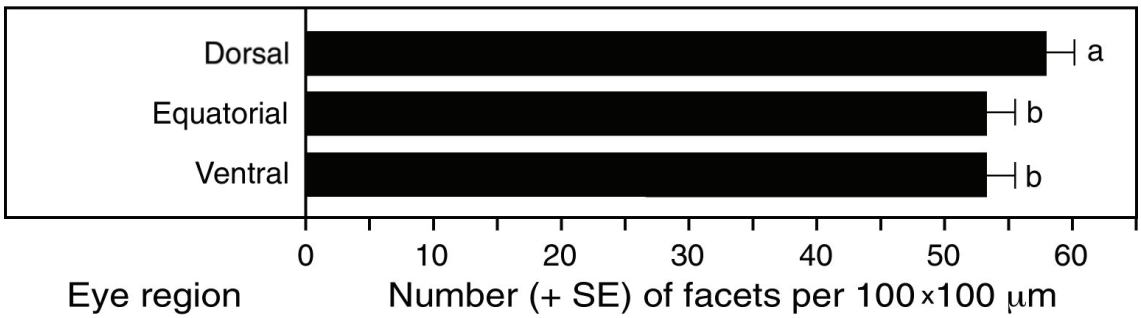
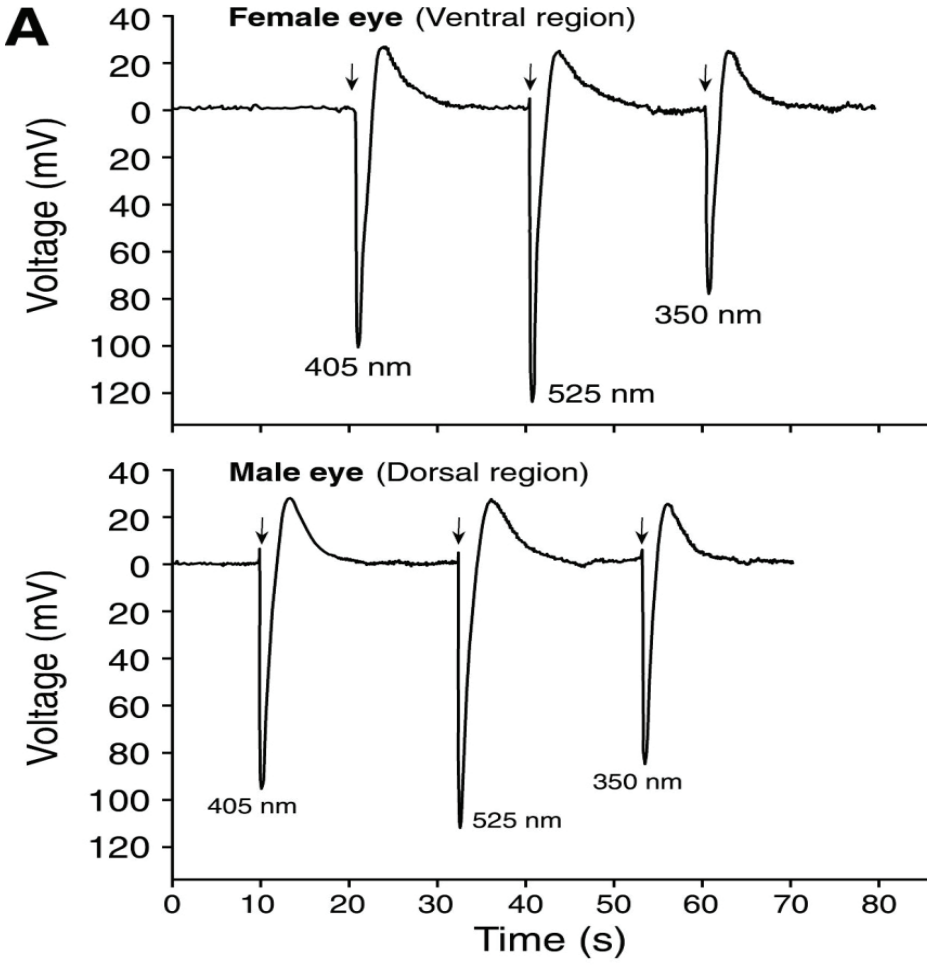

Dorsal
Equatorial
Ventral
a
b
b
0
10
20
30
40
50
60
Eye region
Number (+ SE) of facets per 100 x 100 μm

Figure 12 **(A)** Representative electroretinograms (n = 10) of dark-adapted eyes of male and female *Plodia interpunctella* responding to light-emitting diodes (LED) with peak wavelength of 350, 405 or 525 nm at 20 μW/cm^2 each (arrows indicate the onset of a 0.5-s light stimulus); **(B)** Combined-gender responses of dorsal, equatorial or ventral eye regions to a 350-, 405-, or 525-nm LED. Within each of three encircled data sets, means associated with different capital letters are significantly different (1-way ANOVA, $P < 0.05$, followed by Tukey's HSD test; $P < 0.05$); within each eye region, responses associated with different lower case letters are significantly different from each other (1-way ANOVA, $P > 0.05$).


A
Female eye (Ventral region)
Male eye (Dorsal region)
Voltage (mV)
405 nm
525 nm
350 nm
Time (s)

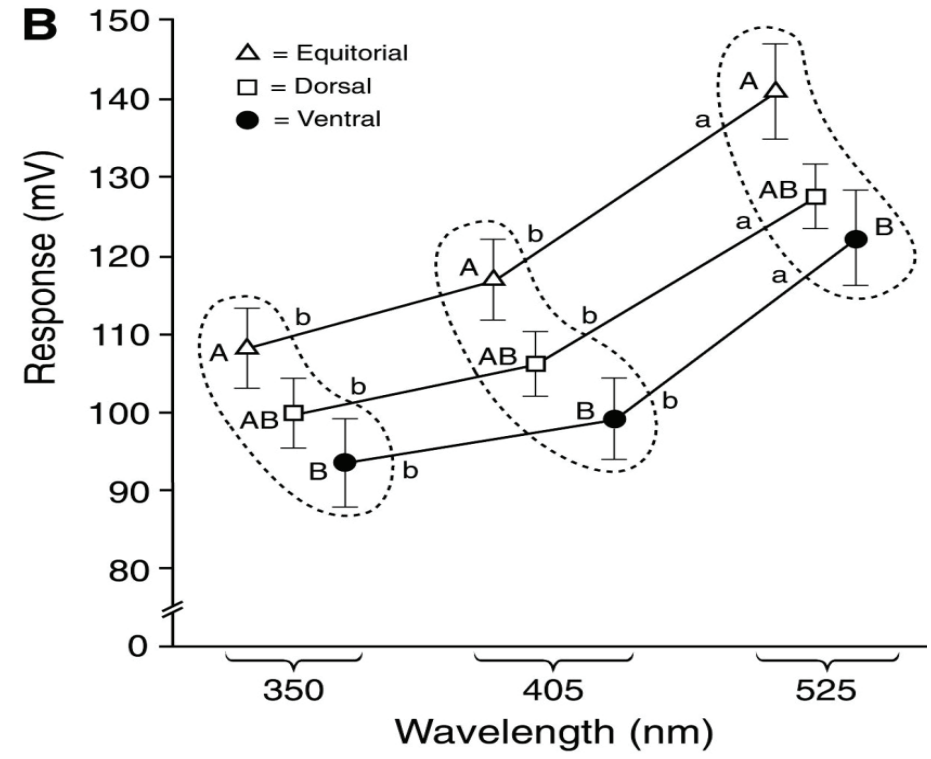

B
= Equitorial
= Dorsal
= Ventral
Response (mV)
Wavelength (nm)
350
405
525

Figure 13 Standardized combined-gender responses of dark-adapted eyes of female and male *Plodia interpunctella* to light stimuli. Within each of the two encircled data sets, means associated with the same capital letter are not significantly different (1-way ANOVA, $P < 0.05$, followed by Tukey's HSD test; $P < 0.05$).

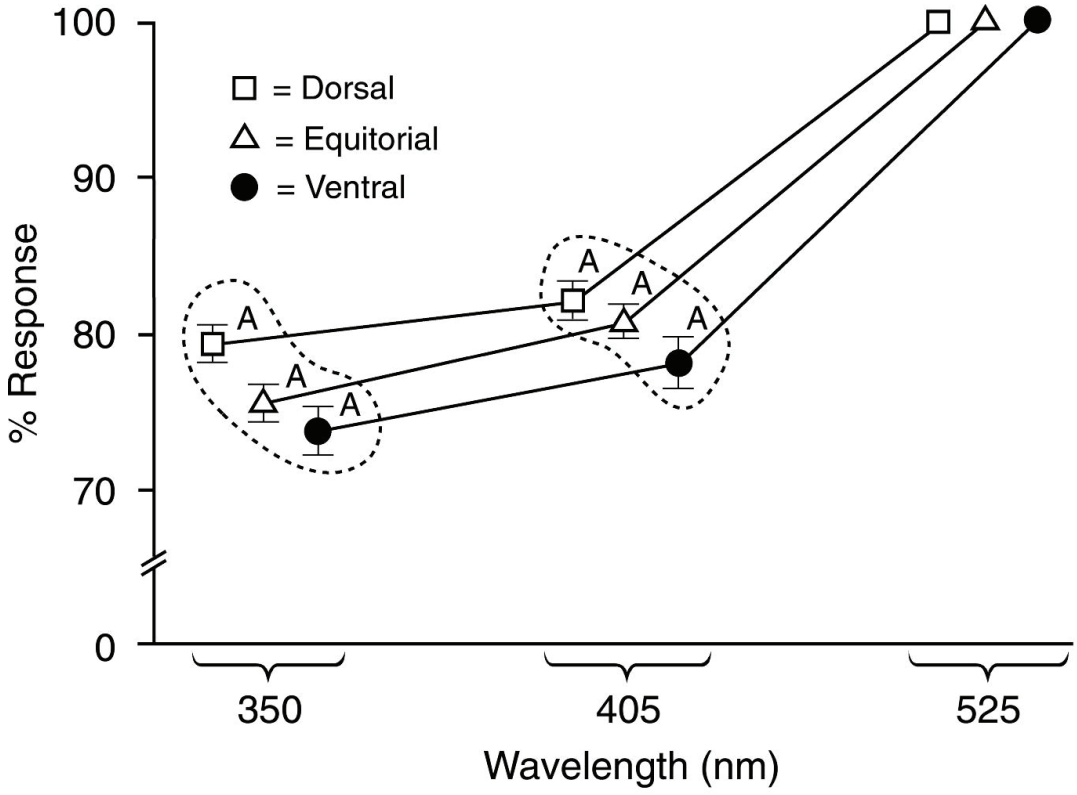
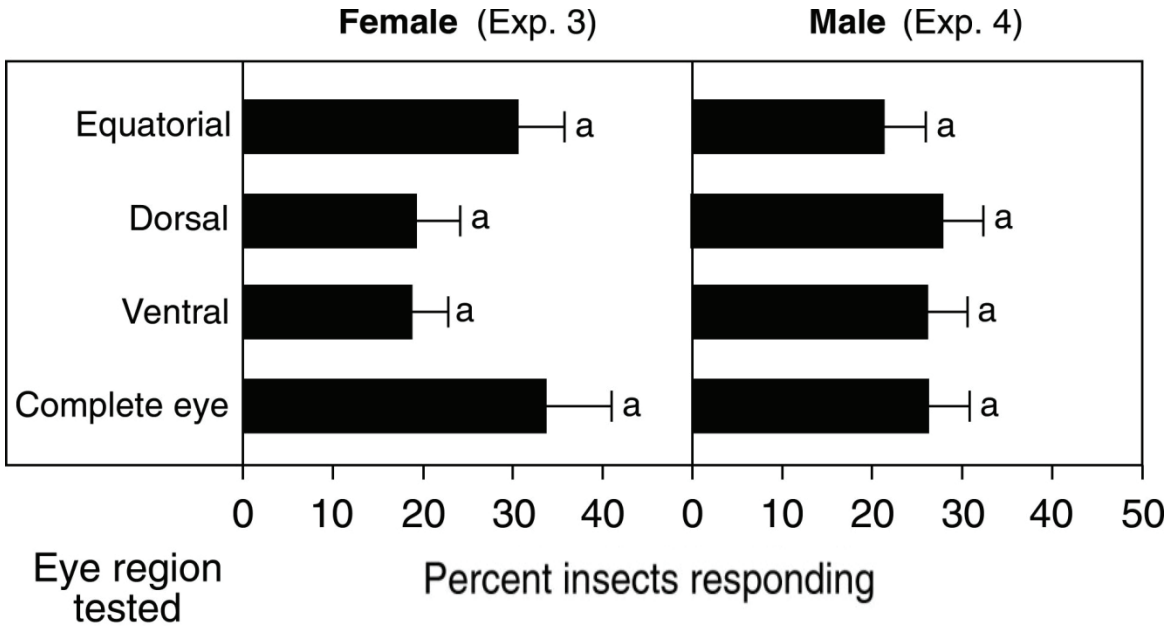

100
90
80
70
0
% Response
□ = Dorsal
△ = Equitorial
● = Ventral
A
A
A
A
A
A
350
405
525
Wavelength (nm)

Figure14 Mean (+ SE) percent of female (experiment 3) or male (experiment 4) *Plodia interpunctella* captured in traps fitted with a 405-nm LED. In each experiment, there was no difference in the number of moths captured irrespective of whether or not specific regions of the eye was experimentally occluded (Kruskal-Wallis test; $P > 0.05$).


Female (Exp. 3)
Male (Exp. 4)
Equatorial
Dorsal
Ventral
Complete eye
a
a
a
a
a
a
a
a
0 10 20 30 40
0 10 20 30 40 50
Eye region tested
Percent insects responding

4.4 Discussion

My electroretinogram data demonstrate that the equatorial region of female and male eyes is more sensitive to light stimuli than the ventral region, with the dorsal region being intermediate in light sensitivity. These findings contrast with those obtained for eyes of *E. cautella* where dorsal and ventral regions elicited stronger responses to UV and violet light than the equatorial region (Gilburt and Anderson, 1992). The sensitivity of the equatorial region of IMM eyes could not be attributed to (*i*) a larger surface area that was stimulus-exposed, or (*ii*) a greater number of facets per area. Great care was taken to ensure that the area of exposure was comparable between eye regions, and scanning electron micrographs of eyes revealed more facets per area in the dorsal region than in the equatorial or ventral region. Thus, equatorial ommatidia must either be more numerous with smaller inter-ommatidial angles or more efficient in photon-gathering or processing than their counterparts in the ventral eye region. A large eye curvature, long rhabdoms and a wide clear zone coupled with small inter-ommatidial angles improve resolution in the superposition eye of nocturnal male winter moth, *Operophtera brumata* (L.) (Lepidoptera: Geometridae) (Meyer-Rochow and Lue, 2008). Such increased resolution requires more receptors intercepting the same number of photons, thus decreasing—instead of increasing—the sensitivity of the eye (Land, 1997). In the ERG experiments, light levels exceeded those of crepuscular conditions and provided sufficient numbers of photons to activate all receptors. As the response ratio to UV, violet or green

light appears to remain constant in each of the dorsal, equatorial and ventral region of the IMM eye, greater sensitivity in the equatorial region is likely due to the presence of more ommatidia with smaller inter-ommatidial angles.

The fact that the dorsal but not ventral region of the eye is statistically as light-sensitive as the equatorial region may be explained by a phenomenon other than the region-specific abundance of ommatidia. There were significantly more facets in the dorsal than in the ventral region. These additional facets in the dorsal eye could improve the effectiveness of light gathering and focusing onto ommatidia. Assuming that light-sensitivity translates into visual acuity or improved object detectability, the frontal equatorial region of the IMM eye would be as adept as the dorsal region, and more adept than the ventral region, to detect obstacles in the flight path. Insects with apposition eyes that inhabit and maneuver in complex environments have highest acuity in the equatorial region because objects move too quickly across the field of view in the dorsal and ventral regions, rendering them unsuitable for high visual acuity (Land, 2003). Visual acuity or light sensitivity particularly of the equatorial region of IMM eyes would be adaptive because IMMs fly at low-intensity lights and seek mates and resources in complex environments. Both females and males engage in enduring foraging bouts with forward flight and intermittent horizontal and vertical casting movements. Not once in many hours of observations have we seen a single moth miss an obstacle in its flight path. Enhanced sensitivity also of the dorsal eye region would be adaptive for females and males that exploit navigational cues from the crepuscular sky during long-distance flights. It would also be

adaptive to males that tend to search (with their head lifted upward) for females beneath poorly illuminated overhangs (TC, unpublished observations).

In light of my ERG data (Figures 12, 13), the results of the behavioural experiments (Figure 14) seem surprising. Occluding the equatorial eye region did not significantly affect the insects' behavioural response to the 405-nm LED. There are, however, several possible explanations. The insects may have engaged in behaviour that compensated for the impaired equatorial eye region. By lifting or lowering their head relative to the longitudinal body axis, or by engaging in more pronounced vertical castings during forward flight, they may have effectively exposed dorsal and ventral eye region to the test stimulus. With each region of the eye capable of responding to each wavelength stimulus tested in experiments 1 and 2, dorsal and ventral eye regions could have become "back-up" systems for stimulus perception and processing. Alternatively, the recording of trap-captured moths was an inadequate proxy for comparing the relative visual acuity or sensitivity of various eye regions. Recording instead the flight path toward, or the time to reach the light stimulus might be more indicative of effects caused by an impaired equatorial eye region. Such recordings, however, would require elaborate tracking and timing of the flight of individual insects.

In conclusion, IMMs exhibit enduring flights in highly complex settings, like supermarkets, pet food stores and warehouses, and skilfully "negotiate" very narrow spaces often at very low light intensities. Greater sensitivity of the equatorial than the ventral region of IMM eyes and greater number of facets in

the dorsal region may have evolved as an adaptation to avoid obstacles in the flight path, perceive celestial navigational cues during long-distance flights, or to help recognize visual cues indicative of mates. Further evaluation of the acute zones for regional specialization as well molecular studies to identify and characterize the type and distribution of photoreceptors in the IMM eye is needed to gain a more complete understanding of the visual system of this insect.

5: Concluding summary

My data allow the following conclusions to be drawn:

1. Males and females are more strongly attracted to blue light than to green, red or orange light.
2. Virgin females show no significant preference for blue, green, red or orange light.
3. Males, virgin and mated females are significantly more strongly attracted to LEDs emitting violet light at peak wavelengths of 405 nm than to LEDs emitting blue light at peak wavelengths of 435 nm, 450 nm or 470 nm.
4. When tested at identical light intensities, two 405-nm LEDs are as attractive as one 405-nm LED in attracting male IMMs.
5. When tested at identical light intensities (700 $\mu W/cm^2$), two 405-nm LEDs are as effective as one 405-nm LED (600 $\mu W/cm^2$) and one 350-nm LED (100 $\mu W/cm^2$) in attracting mated female IMMs.
6. In electroretinograms, eyes of females and males responded more strongly to the wavelength stimulus of 405 nm than to the 350 nm stimulus.

7. There were significantly more gender-combined facets in the dorsal eye region than in the equatorial or ventral region.
8. Gender-combined responses of the equatorial eye region to either a 350-, 405-, or 525-nm LED were significantly stronger than those of the ventral region but not those of the dorsal region, implying greater visual acuity in the dorsal and equatorial region, which may aid maneuvering in complex environments.
9. Gender-combined responses of the dorsal, equatorial or ventral region were strongest to the 525-nm LED.
10. There was no significant difference in captures of female or male IMMs in traps baited with a 405-nm LED irrespective of whether or not a specific region of the eye was experimentally occluded.

Reference list

Arikawa K (2003) Spectral organization of the eye of a butterfly, *Papilio*. Journal of Comparative Physiology. 189: 791–800.

Arikawa K, Inokuma K and Eguchi E (1987) Pentachromatic visual system in a butterfly. Naturwissenschaften 74: 297–298.

Baker RR and Sadovy R (1978) The distance and nature of light-trap response of moths. Nature 276: 818–821.

Bennet GW, Owens JM and Corrigan RM. (1997) Truman's Scientific guide to pest control operations 5th edition. Advanstar Communications, Cleveland Ohio, USA.

Barrer PM (1977) Influence of airborne stimuli from con-specific adults on site of oviposition of Ephestia cautella (Lepidoptera-Phycitidae). Entomologia Experimentalis et Applicata 22: 13–22.

Barrer PM, Jay EG (1980) Laboratory observations on the ability of *Esphestia cautella* (Walker) (Lepidoptera, Phycitedae) to locate and to oviposit in response to a source of grain odor. Journal of Stored Product Research 16: 1–7.

Briggs CG, Sait SM, Begon M, Thompson DJ and Godfray HCG (2000) What causes generation cycles in populations of stored-product moths? Journal of Animal Ecology 69: 352–366.

Briscoe AD and Chittka, L (2001) The evolution of color vision in insects. Annual Review of Entomology 46: 471–510.

Campbell JF and Arbogast RT (2004) Stored-product insects in a flour mill: population dynamics and response to fumigation treatments. Entomologia Experimentalis et Applicata 112: 217–225.

Cool SJ Crawford MLJ and Scheer IJ (1970) Tungsten microelectrode preparations for CNS recording. Review of Scientific Instruments 41: 1506–1507.

Cowan T and Gries G (2009) Ultraviolet and violet light: attractive orientation cues for the Indian meal moth, *Plodia interpunctella*. Entomologia Experimentalis et Applicata 138: 148–158.

Cronin TW, Warrant EJ and Greiner B (2006) Celestial polarization patterns during twilight. Applied Optics 45: 5582-5589.

Cutler DE, Bennett RR, Stevenson RD and White RH (1995) Feeding behavior in the nocturnal moth *Manduca sexta* is mediated mainly by blue receptors, but where are they located in the retina? Journal of Experimental Biology 198: 1909–1917.

Dacke M, Byrne MJ, Scholtz C and Warrant EJ (2004) Lunar orientation in a beetle. Proceedings of the Royal Society of London B 271: 361–365.

Delisle J, West RJ and Bowers WW (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*. Entomologia Experimentalis et Applicata 89: 87–98.

Doud CW and Phillips TW (2000) Activity of *Plodia interpunctella* Lepidoptera: Pyralidae) in and around flour mills. Journal of Economic Entomology 93: 1842–1847.

Eguchi E, Watanabe K, Hariyama T and Yamamoto K (1982) A comparison of electrophysiologically determined spectral responses in 35 species of Lepidoptera. Journal of Insect Physiology, 28: 675–682.

Foster SP and Harris MO (1997) Behavioral manipulation methods for insect pest-management. Annual Review of Entomology 42: 123–46.

Gilburt H and Anderson M (1992) The spectral efficacy of the eye of *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae). Journal of Stored Product Research. 32: 285–291.

Greiner B (2006) Adaptations for nocturnal vision in insect apposition eyes. International review of cytoltoy – A Survey of Cell Biology: Elsevier Academic Pres, California, USA.

Hagstrum DW and Davis LR Jr. (1980) Mate-seeking behavior of *Ephestia cautella.* Environmental Entomology 9: 589–592.

Hardie RC (2006) Phototransduction in invertebrate photoreceptors. Invertebrate vision (ed. by E Warrant and DE Nilsson) Cambridge University Press, New York, USA, pp. 43–82.

Henze MJ and Labhart T (2007) Haze, clouds and limited sky visibility: polarotactic orientation of crickets under difficult stimulus conditions. The Journal of Experimental Biology 210: 3266–3276.

Hironaka M, Tojo S and Haariyam T (2007) Light compass in the provisioning navigation of the subsocial shield bug, *Parastrachia japonensis* (Heteroptera: Parastrachiidae). Applied Entomology and Zoology 42: 473–478.

Homberg U (2004) In search of the sky compass in the insect brain. Naturwissenschaften 91: 199–208.

Kelber A, Balkenius A and Warrant EJ (2003) Colour vision in diurnal and nocturnal Hawkmoths. Integrative and Comparative *Biology* 3: 571–579.

Johnsen S, Kelber A, Warrant E, Sweeney AM, Widder EA, Lee RL Jr and Hernández-Andrés J (2006) Crepuscular and nocturnal illumination and its effects on color perception by the nocturnal hawkmoth *Deilephila elpenor*. Journal of Experimental Biology 209: 789–800.

Kirkpatrick RL, Yangey DL and Marzke FO (1970) Effectiveness of green and ultraviolet light in attracting stored-product insects to traps. Journal of Economic Entomology 63: 1853–1855.

Klotz JH and Reid BL (1993) Nocturnal orientation in the black carpenter ant *Camponotus pennsylvanicus* (DeGeer) (Hymenoptera: Formicidae). Insectes Sociaux 40: 95–106.

Kriska G, Horváth G and Andrikovics S (1998) Why do mayflies lay their eggs en masse on dry asphalt roads? Water-imitation polarized light reflected from asphalt attracts Ephemoerptera. The Journal of Experimental Biology 201: 2273–2286.

Land MF (1997) Visual acuity in insects. Annual Review of Entomolgy 42: 147–77.

Land MF (2003) Eyes and Vision. Enclopedia of insects. (ed. by VH Resh andRT Cardé) Accedemic Press Elsevier New York, USA, pp. 393–406

Land MF and Nilsson DE (2006) General-purpose and special-purpose visual systems. Invertebrate vision (ed. by E Warrant and DE Nilsson) Cambridge University Press, New York, USA, pp.167–210.

Larsson ML and Svensson GP (2005) Methods in insect sensory ecology. Methods in insect sensory neuroscience (ed. by TA Chistensen) CRC Press Boca Raton, Florida, USA.

LeCato GL (1976) Yield, development, and weight of *Cadra cautella* (Walker) and *Plodia interpunctella* (Hübner) on twenty-one diets derived from natural products. Journal of Stored Products Research 12: 43–47.

Marzke FO, Street MW, Mullen MA and McCray TL (1973) Spectral responses of six species of stored-product insects to visible light. Journal of the Georgia Entomological Society 8: 195–200.

Mohandass S, Arthur FH, Zhu KY and Throne JE. (2007) Biology and management of Plodia interpunctella (Lepidoptera: Pyralidae) in stored products. Journal of Stored Products Research. 43: 302–311.

Meyer-Rochow VB and Lue TF (2008) Sexual dimorphism in the compound eye of the moth *Operophtera brumata* (Lepidoptera, Geometridae). Invertebrate Biology, 127: 201–216.

Nansen C and Phillips TW (2003). Ovipositional responses of the Indianmeal moth, *Plodia interpunctella* (Hubner) (Lepidoptera : Pyralidae) to oils. Annals of the Entolmological Society of America. 96: 524–531.

Nansen C, Phillips TW and Dillwith JW (2003) Oviposition response of the Indian meal moth *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) to food oil constituents. Advances in stored product protection (ed. by PF Credland, DM Armitage, CH Bell, PM Cogan and E Highley), pp. 300–305. CABI Publishing, New York, USA.

Olsson, P-OC, Anderbrant O and Löfstedt, C (2005a). Flight and oviposition behavior of *Ephestia cautella* and *Plodia interpunctella* in response to odors of different chocolate products. Journal of Insect Behavior 18: 363–380.

Olsson P-OC, Anderbrant O and Löfstedt C (2005b) Experience influences oviposition behaviour in two pyralid moths, *Ephestia cautella* and *Plodia interpunctella*. Animal Behaviour 72: 545–551.

Pfeiffer K and Homberg U (2007) Coding of azimuthal directions via time-compensated combination of celestial compass cues. Current Biology 17: 960–965.

Robertson GW (1966) The light composition of solar and sky spectra available to plants. Ecology 47: 640–643.

Sambaraju KR and Phillips TW (2008) Responses of adult *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) to light and combinations of attractants and light. Journal of Insect Behaviour 21: 422–439.

Shashar N, Sabbah S and Aharoni N. (2005) Migrating locusts can detect polarized reflections to avoid flying over the sea. Biology Letters 1: 472–475.

Soderstrom EL (1970) Effectiveness of green electroluminescent lamps for attracting stored-product insects. Journal of Economic Entomology 63: 726–731.

Stalleicken J, Labhart T, Mouritsen H (2006) Physiological characterization of the compound eye in monarch butterflies with focus on the dorsal rim area. Journal of Comparative Physiology. 192: 321–331.

Stavenga DG (2006) Invertebrate photoreceptor optics. Invertebrate vision (ed. by E Warrant and DE Nilsson) Cambridge University Press, New York, USA, pp. 1–42.

Stavenga DG and Arikawa K (2006) Evolution of color and vision of butterflies. Arthropod Structure and Development. 35: 307–318

Stremer RA (1959) Spectral response of certain stored-product insects to electromagnetic radiation. Journal of Economic Entomology 52: 888–892.

Svensson GP, Skals N and Löfstedt C (2003) Disruption of the odour-mediated mating behaviour of *Plodia interpunctella* using high-frequency sound. Entomologia Experimentalis et Applicata 106: 187–192.

Soderstrom EL (1970) Effectiveness of green electroluminescent lamps for attracting stored-product insects. Journal of Economic Entomology 63: 726–731.

Theobald JC, Coates MM, Wcislo WT and Warrant EJ (2007) Flight performance in night-flying sweat bees suffers at low light levels. *Journal of Experimental Biology* 210: 4034–4042.

Warrant E (2004) Vision in the dimmest habitats on earth. Journal of Comparative Physiology. 190: 765–789.

Warrant E (2006) Invertebrate vision in dim light. Invertebrate vision (ed. by E Warrant and DE) Cambridge University Press, New York, USA, pp. 43–82.

Warrant E, Bartsch K, and Gunther C (1999) Physiolgoical optics in the hummingbird hawk moth: a compound eye without ommatidia. The Journal of Experimental Biology 202, 497–511

Warrant E, Kelber A, Gislén A, Greiner B, Ribi W and Wcislo, W (2004) Nocturnal vision and landmark orientation in a tropical halictid bee. Current Biology 14: 1309–1318.

Wehner R (1984) Astronavigation in insects. Annual Review of Entomology 29: 277–298.

Wehner R and Labhart T (2006) Polarisation vision. Invertebrate vision (ed. by E Warrant and DE Nilsson) Cambridge University Press, New York, USA, pp. 291–348.

Wehner R and Muller M (2006) The significance of direct sunlight and polarized skylight in the ant's celestial system of navigation. Proceedings of the National Academy of Sciences of the United States of America 103: 12575–12579.

White RH, Xu H, Münch TA, Bennett RR and Grable EA (2003) The retina of *Manduca sexta*: rhodopsin expression, the mosaic of green-, blue and UV-sensitive photoreceptors, and regional specialization. Journal of Experimental Biology 206: 3337–3348.

Williams CG (1964) Life-History of Indian meal-moth *Plodia interpuctella* (Hubner) (Lep.: Pycitidae) in warehouse in Britain and on different foods. Annals of Applied Biology 53: 459.

Yong Jung K, Shafqat S and Marie Jose, D (2003) Control of *Plodia interpunctella* (Lepidoptera: Pyralidae), a pest in *Bombus terrestris* (Hymenoptera: Apidae) colonies. The Canadian Entomologist 135: 893–902.

Zar HJ (1999) Biostatistical Analysis. 4th edn. Prentice-Hall Inc., Upper Saddle River, New Jersey, USA.

Zhu J, Ryne C, Unelius CR, Valeur PG and Löfstedt C (1999) Re-identification of the female sex pheromone of the Indian meal moth, *Plodia interpunctella*: evidence for a four-component pheromone blend. Entomologia Experimentalis et Applicata 92: 137–146.