Exploitation of Electromagnetic Radiation as a Foraging Cue by Conophagous Insects

by

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Abstract

Many insects exploit sections of the electromagnetic spectrum as foraging or attraction cues, detecting wavelengths in the ultraviolet (UV; ~ 300–400 nm), human visible (400–750 nm) and infrared (> 750 nm) range. Two distinct types of receptors are involved. Compound eyes in the head detect UV and human visible light, and IR receptors on the thorax or abdomen detect radiant IR which contrasts against the background and is therefore dectable. I investigated the potential use of electromagnetic foraging cues in three members of the conophagous insect guild: the diurnal Western conifer seed bug, *Leptoglossus occidentalis* Heidermann (Hemiptera: Coreidae), the nocturnal fir coneworm moth, *Dioryctria abietivorella* Groté (Lepidoptera: Pyralidae), and the diurnal Douglas-fir cone gall midge, *Contarinia oregonensis* Foote (Diptera: Cecidomyiidae). In electrophysiological recordings, two-choice laboratory bioassays, and field trapping experiments, I tested the hypotheses that during (cone) foraging *C. oregonensis*, *D. abietivorella* and *L. occidentalis* (1) rely on IR receptors that receive and respond to cone-derived radiant IR, (2) receive and respond to cone or plant-derived colour cues, and (3) integrate radiant IR and insect visible light cues. My data support these hypotheses, at least in part. Male and female *L. occidentalis* were more attracted to radiant IR from heat sources within but not outside the natural cone temperature range. Male and female *D. abietivorella* have IR receptors on their ventral prothorax which help them detect and discriminate between IR stimuli. Both *D. abietivorella* and *C. oregonensis* are attracted to warm objects with IR signatures resembling or corresponding to those of tree branches but not those of cones. No insects oriented towards cone-reflected light as a singular foraging cue, but mated female *L. occidentalis* preferred the complete light spectrum of conifer needles (their oviposition site) to a

narrow bandwidth of visible light. Visible (blue) light combined with radiant IR from a 40 ºC heat source were synergistic in attracting female *L. occidentalis,* indicating that the central nervous system of *L. occidentalis* is capable of processing and integrating information from compound eyes and IR receptors. Such integration of visible stimuli and IR was previously known only in viperid snakes.

Keywords: *Leptoglossus occidentalis*, *Dioryctria abietivorella*, *Contarinia oregonensis*, infrared (IR) receptor, compound eye, foraging cue

“A man who carries a cat by the tail learns something he can learn in no other way.”

- Mark Twain

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Table of Contents

List of Tables

List of Figures

1. Introduction

The ability of insects to forage on resources depends upon these resources having distinct attributes which the insects can sense. Resource-derived or -associated cues can be diverse and include sections of the electromagnetic spectrum, such as ultraviolet (UV) and human visible light, and infrared (IR) radiation.

1.1. Electromagnetic spectrum

The electromagnetic spectrum covers a wide range of wavelengths of photon energies, with differing quanta of energy for each wavelength. The compound eyes of insects contain visual pigments that are excited by high-energy frequencies of human visible light (henceforth “visible light”) (400-750 nm) (Schmitz & Bleckmann, 1997) and UV light (200-400 nm) (Briscoe & Chittka, 2001). Insects exploit visible and UV light to locate resources based on either or both hue and object shape (Briscoe & Chittka, 2001; Kelber et al., 2003). Infrared (IR) radiation (> 750 nm) has less energy than visible light, and is detected by specific IR receptors (Schmitz & Bleckmann, 1997) rather than visual pigments in compound eyes. The exact mechanism of IR detection remains unknown, and may differ between species (Vondran & Schmitz, 1995; Schmitz & Bleckmann, 1997; Schmitz et al., 2000, 2002).

1.2. Colour vision and response to specific wavelengths

The compound eyes of insects comprise ommatidia, each of which contains photosensitive visual pigments, supporting cells, and a crystalline lens (Nation, 2002). Visual pigments consist of a chromophore and a G-protein-coupled opsin (light-sensitive receptor) (Briscoe & Chittka, 2001). Both the chromophore and opsin protein (Briscoe & Chittka, 2001) and filtering pigments (Nation, 2002) determine the maximal spectral sensitivities of the photoreceptor. Insects are capable of detecting visible light in the human visible spectrum (400-750 nm) and in the ultraviolet range (< 400nm). The daylight available for the insect eye to receive comprises of both UV and visible light with blue light being the most represented and UV the least (Henderson, 1977)

True colour vision, unlike wavelength-specific behaviour, is demonstrated when insects both discriminate and choose between two colours independent of their intensity (Menzel, 1979; Cutler et al., 1995; Kelber, 1999; Nation, 2002). Only a few insect species have been shown unequivocally to be capable of true colour vision. In contrast, many insects have been reported to respond to specific wavelengths of light, which does not require a choice between colours (Menzel, 1979; Cutler et al., 1995; Kelber, 1999). To demonstrate responses to specific wavelengths of light (wavelength-specific behaviour), one must show (*i*) maximal spectral sensitivities (see below) of the eye to more than one wavelength, and (*ii*) behavioural responses to wavelengths of visible light.

Spectral sensitivities can be determined through intra- and extracellular optical recordings, microspectrophotometry (Briscoe & Chittka, 2001), or can be inferred from DNA sequences (Osorio & Vorobyev, 2008) or behavioural responses. Greater electrophysiological responses to a certain wavelength than to slightly shorter or longer

wavelengths indicate the potential presence of a visual pigment (Briscoe & Chittka, 2001).

Trichromatic vision with strong spectral responses to short (UV region), middle (violet/blue region), and long (green region) wavelengths of the electromagnetic spectrum is the most common pattern for insect vision (Höglund et al., 1973; Schecht, 1979; Bernard & Remington, 1991; Bennett et al., 1997; Kevan & Backhaus, 1998) Townson et al., 1998; Briscoe & Chittka, 2001; Kelber et al., 2003), but other plans exist (Briscoe & Chittka, 2001). However, other types of arrangement can occur. For example, the Japanese yellow swallowtail butterfly, *Papilio xuthus*, has eight receptor types corresponding to eight maximal spectral sensitivities including a receptor in the red (Koshitaka et al., 2008). Only a few insects have receptors responsive to wavelengths of red light. For example, the swallowtail butterfly *Papilio aegeus* has a spectral sensitivity in the red range, which is thought to aid in the discrimination of green foliage, allowing *P. aegeus* to find young shoots (Kelber, 1999). Similarly, the nymphalid butterfly *Heliconius erato* can discriminate light in the red region although it has only one long-wavelength-sensitive visual receptor. This may be possible due to filtering pigments in the eye (Zaccardi et al., 2006).

Wavelength-specific behaviour can be linked to certain resources. The trefoil seed chalcid, *Bruchophagus platypterus*, is more strongly attracted to yellow, the colour of its host flower, than to white, green or purple (Kamm et al., 1991). The onion fly, *Delia antiqua*, in contrast, prefers white or blue to yellow (Vernon & Bartel, 1985). Foraging butterflies visit yellow and pink flowers more frequently than red flowers (Yurtserver et al., 2010). In closely related congeners of *Lycanena* butterflies, spectral sensitivities are

related to conspecific wing patterns which mediate mate discrimination in sympatric species (Bernard & Remington, 1991).

The eyes of *Lycaena* butterflies are heterogenous with respect to spectral sensitivities (Bernard & Remington, 1991), with specific receptor types being concentrated in certain areas of the eye. This clustering of receptors may facilitate foraging for particular resources. For example, the eyes of male ruddy copper butterflies, *Lycaena rubidus*, are di- and trichromatic in the dorsal and ventral region, respectively, which aids in detecting conspecifics (Bernard & Remington, 1991). Eyes of female *L. rubidus*, in turn, are trichromatic in the dorsal region, with visual pigments maximally sensitive to wavelengths of light at 568 nm which aids females in locating red plants for oviposition (Bernard & Remington, 1991). Eyes of the tobacco hornworm moth, *Manduca sexta*, are dichromatic and mostly green-sensitive in the dorsal region, but trichromatic in the ventral region with a concentration of UV and blue receptors, the latter mediating feeding behavior (Bennett et al., 1997).

1.3. UV vision

The atmosphere allows wavelengths of UV light from 290–400 nm to reach earth's surface (Henderson, 1977). Insect vision is UV-shifted in that insects see well into the UV range which humans cannot (Kevan & Backhause, 1998; Land, 2003). UV is detected through the compound eye of insects. Each insect with spectral sensititivies studied is UV-sensitive (Briscoe & Chittka, 2001).

UV light has long been known as an effective stimulus for trapping moths (Roeder, 1967; Weissling & Knight, 1994; Roe et al., 2006; Whitehouse et al., 2011).

The dorsal and ventral rather than equatorial region of eyes of the almond moth, *Ephestia cautella,* have greatest UV efficiency which may aid in navigation (Gilburt & Anderson, 1996) or escape (Kevan et al., 2001).

There is relatively little UV light in daylight (Henderson, 1977) but it still provides a foraging cue for diurnal instects. Diurnal ants and bees are sensitive to UV light (Stark & Tan, 1982). For foraging bees, UV light is as important as blue and green lights to locate flowers (Chittka et al., 1993; Kevan et al., 2001). When bees were exposed to equal proportions of either UV and yellow light, or UV and blue light, the UV dominated the bees' field of vision to the exclusion of the yellow but not the blue light (Guldberg and Atsatt, 1975). However, UV light alone as a singular stimulus is not effective for honeybees foraging for flower resources. This is perhaps why flowers reflect UV and visible light wavelengths (Kevan et al., 2001). White flowers often have a yellow centre to provide contrast for the foraging pollinator (Kevan & Backhause, 1998).

1.4. IR receptors and IR detection

Radiant IR (not conductive or convective heat) with a > 750-nm wavelength is detected by specialized structures known as IR receptors or pit-organs. Conducive heat transfers engery from two touching objects, convection transfers energy from an object to the environment and radiant IR is the transfer of electromagnetic radiation. In vertebrates, true IR receptors (Vondran et al., 1995) are generally located in the head and measure heat not temperature. In invertebrates, IR receptors may take the form of modified mechanoreceptors (Vondran et al., 1995; Schmitz & Bleckmann, 1997) and can be found on the abdomen (Schmitz & Trenner, 2003; Takács et al., 2009) and thorax

(Evans, 1966; Schmitz et al., 2002; Schmitz et al., 2008). IR receptors differ from eyes in that they lack visual pigments and require a lower energy source for stimulation (Evans, 1966; Schmitz & Bleckmann, 1997). Vertebrates use IR receptors for different functions. Rattle snakes use facial pits during thermoregulation (Krochmal & Bakken, 2003), but Crotalinae snakes (Moiseenkóvà et al., 2003), *Python* snakes (Grace et al., 1999; Grace et al. 2001) and vampire bats (Kürten & Schmidt, 1982) use IR receptors to aid in the detection of warm-bodied prey.

Several insect species are known to have IR receptors. The blood-sucking bugs *Rhodnius prolixus* (Schmitz et al., 2000a) and *Triatoma infestans* (Lazzari & Núñez, 1989) exploit radiant IR to locate warm-bodied hosts. The conophagous Western conifer seed bug, *Leptoglossus occidentalis* Heidemann, orients to radiant IR from conifer cones which contrast well against the much cooler foliage (Takács et al., 2009). The pyrophilic beetles and bugs *Merimna atrata* (Evans, 1964; Evans 1966; Schmitz & Trenner, 2003), *Acanthocnemus nigricans*, *Aradus albicornis (*Schmitz *et al*., 2008) and *Melanophila acuminata* (Schmitz & Bleckmann, 1997, 1998; Schmitz et al., 1997) respond to radiant IR from burning forest fires. This behaviour is particularly well documented for the jewel beetle *M. acuminata* (Evans, 1964, 1966; Schmitz & Bleckmann, 1997, 1998; Schmitz et al., 1997; Gronenberg & Schmitz, 1999). Attracted to radiant IR from forest fires, the beetles form mating swarms while the fire is still burning (Vondran et al., 1995; Schmitz & Bleckmann, 1998). Post-mating, females lay their eggs under the bark of smoldering wood (Hammer et al., 2001) because neither adult beetles nor larvae can overcome the natural defenses of living trees (Schmitz & Bleckmann, 1998). The beetles' IR receptors detect radiant IR of 2.5-4 μm, but they are most sensitive to the 3-μm wavelength of IR (Evans, 1966) which emanates from forest fires burning at a temperature of 600-1000 °C

(Schmitz & Bleckmann, 1997). The receptors are so sensitive that they can detect a 10 hectare fire over the distance of 12 km (Schmitz & Bleckmann, 1998).

Thermal contrast between an IR source and its background can also play a role in IR detection. The thermal contrast (or IR signature) of an object depends on the physical properties of the substance, size, and time of day (Thánh et al., 2009). Small (< 100 µm) IR receptors, such as those of *L. occidentalis* (Takács et al., 2009), require a stark thermal contrast between the background and the target object for detection (Bakkan & Krochmal, 2007). The larger pit organs of snakes can be so sensitive that they neurologically (Bullock & Diecke, 1956) and behaviourally (Noble & Schmidt, 1937) detect temperature differentials of respectively 0.003 °C and 0.2 °C. Despite this sensitivity, diamondback rattlesnakes, *Crotalus atrox*, responded most strongly to freshly killed rats on cold background which provide a stronger contrast than warm background (Theodoratus et al., 1997). Similarly, other snakes chose to forage in sites which provided strong thermal contrast of prospective prey (Shine et al., 2002; Bakken & Krochmal, 2007).

1.5. Combinations of visible light and radiant IR

Integration of electromagnetic wavelengths in the infrared and visual range has been explored in snakes but to date not in insects. This integration requires the synthesis of information from two separate sense organs: the eye and IR receptor. There are neurons within the tectum of the brain of boid and viperid snakes that respond exclusively to visual light, radiant IR or combinations thereof (Hartline et al., 1979). The combined information then forms spatial (visual and thermal) images of the environment

(Hartline et al., 1978). Although binocularly occluded *Python* snakes hunting for mice exhibited strike angles and distances similar to non-occluded snakes, they had a lower strike success, indicating that precise targeting of prey depends to some degree also upon visual information (Grace et al., 2001).

1.6. Conophagous insects in seed orchards

During their development and/or as adults, obligate conophagous insects feed on the content of seeds of conifer cones. They exploit the monocultural crops of seed orchards and, in turn, can become pests (de Groot et al., 1994). My thesis focused on three conophagous insects that have caused economic damage in seed orchards: the Western conifer seed bug, *L. occidentalis* Heidermann (Hemiptera: Coreidae) (Strong et al., 2001; Bates et al., 2002), the fir coneworm moth, *Dioryctria abietivorella* Groté (Lepidoptera: Pyralidae) (Hedlin et al., 1980; Whitehouse et al, 2011), and the Douglas-fir cone gall midge, *Contarinia oregonensis* Foote (Diptera: Cecidomyiidae) (Miller, 1986).

1.7. Research species

1.7.1. Leptoglossus occidentalis

The life cycles of *L. occidentalis, D. abietivorella, and C. oregonensis* differ (Table 1.1), but all three species exploit the same resource (conifer cones), and may respond to similar cues to locate cones. These cues may include specific ranges of the electromagnetic spectrum, such as visible light, radiant IR, or both (see 1.8.1).

As an obligate conophyte, *Leptoglossus occidentalis* forages for cones on trees in the genera *Pinus*, *Abies*, *Pseudotguga,* and *Tsuga* (Krugman & Koerber, 1969; de Groot et al., 1994). *Leptoglossus occidentalis* was first found only in Western N. America, but its range expanded East (McPherson et al., 1990; Gall, 1992), possibly due to human transport (Gall, 1992; Taylor et al., 2001) or climate change (Musolin, 2007). Recently and inadvertently, *L. occidentalis* has been introduced to northern Italy and other parts of Europe (Taylor et al., 2001; Musolin, 2007), and has rapidly expanded its range in Europe.

In the northern part of its range in N. America, a single generation occurs per year. During spring and summer when cones are maturing, overwintered adults fly to host plants and consume seeds (Krugman & Koerber, 1969; Blatt & Borden, 1999; Strong et al., 2001). Females lay rows of eggs on the needles of trees (Bates & Borden, 2005). Neonate 1st instar nymphs consume the content of needles, whereas 2nd to 5th instars consume the content of seeds within cones (Krugman & Koerber, 1969). Newly eclosed adults continue feeding on cones through autumn, when they fly to overwintering sites. *Leptoglossus occidentalis* has been reported to respond to radiant infrared (IR) as a foraging cue from conifer cones (Takács et al., 2009).

1.7.2. Dioryctria abietivorella

Dioryctria abietivorella larvae feed on cones of conifer trees in the genera *Abies*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga* and *Tsuga* (de Groot et al., 1994). Multiple overlapping generations may lead to three peak adult flight times throughout the summer

(McEntire, 1996; Strong et al., 2007; Grant et al., 2009), with males emerging first (Whitehouse et al., 2011). When eclosed females are 3- to 4-day old, they mate (Trudel et al., 1995) up to 8 times (Whitehouse et al., 2011), and 24 h later seek suitable oviposition sites (Trudel et al., 1995). Females are attracted to plant cuttings (Jactel et al., 1994), and oviposit in response to monoterpenes (Shu et al., 1994). Neonate larvae bore into cones; all instars consume cone contents including seeds (Grant et al., 2009). Adults do not consume cones.

1.7.3. Contarinia oregonensis

In early spring, during and shortly after pollination (Johnson, 1963), female *Contarina oregonensis* oviposit in cones of *Pseudotsuga menziesii*, Douglas-fir (Miller, 1983), placing their eggs singly or in clusters near the base of cone scales. Neonate larvae tunnel into cone scales and induce the formation of galls, within which they develop (Hedlin, 1961). When conelets have assumed a pendant position, they are no longer susceptible to oviposition by *C. oregonensis*. The colour of immature conesavailable for oviposition is clonally-dependant (Coles, 1972). During this time, cone colours range from dark purple to green.

1.8. Hypotheses and chapters

I tested the hypotheses that during cone foraging *C. oregonensis*, *D. abietivorella* and *L. occidentalis* (1) rely on IR receptors that receive and respond to cone-derived radiant IR, (2) receive and respond to cone-derived colour cues, and (3) integrate radiant IR and insect-visible light during foraging.

For Chapter 2, I surveyed for the warmest part of Douglas-fir trees in early spring when *C. oregonensis* forages, and I experimentally tested the effect of plant-derived or plant-associated visible light and radiant IR, as well as the shape of objects warmer than the background, on attraction of *C. oregonensis*. In thermographs, branches were warmer than foliage and cones. *Contarinia oregonensis* did not discern between different types of cone colours, but did prefer warm branch-like objects to warm branch-unlike objects. Collectively, these data indicate that the shape or temperature of Douglas-fir branches could serve as long-range foraging cues for female *C. oregonensis*. Once within the canopy of a host tree, they may then orient towards branch tips to find cones.

In Chapter 3, I present evidence for an IR receptor in *D. abietivorella.* To the best of my knowledge, this is the first evidence for such a receptor in a species of the Lepidoptera. Environmental scanning electron micrographs revealed a candidate receptor on the prothorax of male and female moths. In electrophysiological recordings, IR receptors responded to radiant IR from heat sources kept at 40-60 °C. Thermographs of Douglas-fir and spruce trees showed strong temperature differentials between parts of trees and their surroundings, which could be exploited by moths during their nocturnal flight period (21:00-03:00 h). In laboratory and field experiments, males and mated females, but not virgin females, were more strongly attracted to high-frequency than to low-frequency radiant IR emanating respectively from hot or cold sources.

In Chapter 4, I present data showing that the compound eyes of *D. abietivorella* have a distinct spectral sensitivity in the green region, with a possible sensitity peak in the UV, but lack regional specialization. The spectral sensitivity in the green range does not however translate directly to attractiveness of wavelengths found in test stimuli.

For Chapter 5, I tested whether cone temperatures vary with time of day, age, size and colour, and whether *L. occidentalis* prefers radiant IR from objects with cone-like temperature and surface. Mean and hot-spot temperatures of western white pine cones, *Pinus monticola*, ranged between 15 °C and 35 °C from 09:00 to 18:30 h, when *L. occidentalis* forages for cones. Colour, length or width of within-year cones did not affect cone temperature but second-year cones were warmer than the smaller first-year cones. When given a choice between radiant IR from heat sources well within (40 °C) and just outside (60 °C) the natural cone temperature range, males and females of *L. occidentalis* were attracted to the former, supporting the concept that radiant IR from conifer cones serves as a foraging cue for *L. occidentalis.* Whether or not the IR receptors of *L. occidentalis* are as capable as the pit organs of snakes to discern between minute temperature differentials of warm-bodied resources is yet to be determined.

For Chapter 6, I conducted electrophysiological recordings (retinograms) with the eyes of *L. occidentalis.* I found that eyes have at least two regions of spectral sensitivity and likely a third in the UV region. Areas of spectral sensitivity of the eye did not correspond with preferred wavelengths of visible light.

For Chapter 7, I investigate attraction of *L. occidentalis* to the visible light spectrum of conifer plant parts in the absence of other attributes like shape and chemicals. Results showed that both males and females had significantly stronger electrophysiological reponses to spectral profiles of conifer needles and bark than to cones or other vegetation. Females preferred the spectral profile of conifer needles to a narrow bandwidth of visual light. This may, in part, be due to the fact that females seek needles as oviposition sites.

Finally, for Chapter 8, I tested the hypothesis that combinations of wavelengths from the UV, visual and IR range are more effective in attracting *L. occidentalis* and *D. abietivorella* than is a single range of these wavelengths. My data do not support this hypothesis for *D. abietivorella.* However, results obtained in laboratory experiments with male and female *L. occidentalis* revealed a synergistic effect between blue light (433-nm LED) and high-frequency radiant IR (from a 40 °C heat source). These data support the conclusion that the central nervous system of *L. occidentalis* is capable of processing and integrating information from two types of sensory receptors, compound eyes on the head which are tuned to visual light, and IR receptors on the ventral abdomen which are tuned to radiant IR. Such integration was previously known only in snakes.

Table 1.1: Pertinent life history information of the conophagous insects studied in my thesis.

Species	Order	Development	Foraging period	Ovipositionsite	Conophagous stage
Leptoglossus occidentalis	Hemiptera	hemimetabolous	diurnal	needles[a]	2nd instar - adult[e]
Dioryctria abietivorella	Lepidoptera	holometabolous	nocturnal	cones, needles, twigs[bc]	larvae[f]
Contariania oregonensis	Diptera	holometabolous	diurnal	cones[d]	larvae[g]

[a](Bates & Borden, 2005)
[b](Shu et al., 1996)
[c](Whitehouse et al., 2011)
[d](Miller 1983)
[e](Krugman & Koerberg 1969)
[f](Grant et al., 2009)
[g](Hedlin, 1961)

1.9. Literature Cited

Bakken GS & Krochmal AR (2007) The imaging properties and sensitivity of the facial pits of pit vipers as determined by optical and heat-transfer analysis. The Journal of Experimental Biology 210: 2801-2810.

Bates SL, Lait CG, Borden JH & Kermode AR (2002) Measuring impact of *Leptoglossus occidentalis* (Heteroptera: Coreidae) on seed production in lodgepole pine using an antibody-based assay. Journal of Economic Entomology 95: 770-777.

Bates SL & Borden JH (2005) Life table for *Leptoglossus occidentalis* Heidermann (Heteroptera: Coreidae) and prediction of damage in lodgepole pine seed orchards. Agricultural and Forest Entomology 7: 145-151.

Bennett RR, White RH & Meadows J (1997) Regional specialization in the eye of the sphingid moth *Manduca sexta*: Blue sensitivity of the ventral retina. Visual Neuroscience 14: 523-526.

Bernard GD & Remington CL (1991) Color vision in *Lycanena* butterflies: spectral tuning of receptor arrays in relation to behavioural ecology. Proceedings of the National Academy of Science of the United States of America 88: 2783-2787.

Blatt SE & Borden JH (1999) Physical characteristics as potential host selection cues for *Leptoglossus occidentalis* (Heteroptera: Corediae). Environmental Entomology 28: 246–254.

Briscoe AD & Chittka L (2001) The evolution of colour vision in insects. Annual Review of Entomology 46: 471-510.

Chittka L, Shmida A, Troje N & Menzel R (1993) Ultraviolet as a component of flower reflections, and the colour perception of Hymenoptera. Vision Research 34: 1489-1508.

Copes DL (1972) Inheritance of megastrobili colors in Douglas fir (*Pseudotsuga menziesii*). Canadian Journal of Botany 50: 2045-2048.

Cutler D, Bennett R, Stevenson R & White R. 1995. Feeding behaviour in the nocturnal moth *Manduca sexta* is medicated mainly by blue receptors, but where are they located in the retina? Journal of Experimental Biology 198: 1909-1917.

de Groot P, Turgeon JT & Miller GE (1994) Status of cone and seed insect pest mangagement in Canadian seed orchards. The Forestry Chronicle 70: 745-761.

Evans WG (1964) Infrared sensing in *Melanophila acuminata* De Geer. Nature 202: 211.

Evans WG (1966) Perception of infrared radiation from forest fires by *Melanophila acuminata* De Greer (Buprestidae: Coleoptera). Ecology 47: 1061-1065.

Gall WK (1992) Further eastern range extension and host records for *Leptoglossus occidentalis* (Heteroptera: Coreidae): well-documented dispersal of a household nuisance. Great Lakes Entomologist 25: 159-171.

Gilbert HL & Anderson M (1996) The spectral efficiency of the eye of *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae). Journal of Stored Products Review 32: 285-291.

Grace MS, Church DR, Kelly CT, Lynn WF & Cooper TM (1999) The *Python* pit organ: imaging and immunocytochemical analysis of an extremely sensitive natural infrared detector. Biosensors and Bioelectronics 14: 53-59.

Grace MS, Woodward OM, Church DR & Calisch G (2001) Prey targeting by the infrared-imaging snake *Python*: effects of experimental and congenial visual deprivation. Behavioural Brain Research 119: 23-31.

Grant GG, Millar JG & Trudel R (2009) Pheromone identification of *Dioryctria abietivorella* (Lepidoptera: Pyralidae) from an eastern North American population: geographic variation in pheromone response. The Canadian Entomologist 141: 129-135.

Gronenberg, W & Schmitz H (1999) Afferent projections of infra-red sensitive sensilla in the beetle *Melanophila acuminata* (Coleoptera: Buprestidae). Cell Tissue Research 297: 311-318.

Guldberg LD & Atsatt PR (1975) Frequency of reflectance and absorbance of ultraviolet light in flowering plants. American Midland Naturalist 93: 35-43.

Hammer DX, Schmitz H, Schmitz A, Rylander HG & Welch AJ (2001) Sensitivity threshold and response characteristics of infrared detection in the beetle *Melanophila acuminata* (Coleoptera: Buprestidae). Comparative Biochemistry and Physiology Part A 128: 805-819.

Hedlin AF (1961) The life history and habits of a midge, *Contarinia oregonensis* Foote (Diptera: Cecidomyiidae) in Douglas-fir cones. The Canadian Entomologist 93: 953-967.

Hedlin AF, Yates HO, Tovar DC, Ebel BH, Koerber TW & Merkel EP (1980) Cone and seed insects of North American conifers. Environment Canada, Canadian Forestry Service, Ottawa, Canada.

Henderson ST (1977) Daylight and its spectrum. Bristol, UK: Adam Hilger Ltd.

Höglund G, Hamdorf K & Rosner G (1973) Trichromatic visual systems in an insect and its sensitivity control by blue light. Journal of Comparative Physiology 86: 265-279.Jactel J, Menassieu P & Raise G (1994) Infestation dynamics of *Dioryctria sylvestrella* (Ratz.) (Lepidoptera: Pyralidae) in pruned maritime pine (*Pinus pinaster* Act.) Forest Ecology Management 67: 11-22.

Johnson NE (1963) Time of attack of the Douglas-fir cone midge in relationship to cone development. Journal of Forestry 61: 350-355.

Kamm JA, Fairchild CE, Gavin WE & Cooper TM (1991) Influence of celestial light on visual and olfactory behaviour of seed chalcids (Hymenoptera: Eurytomidae). Journal of Insect Behaviour 5: 273-287.

Kelber A (1999) Ovipositing butterflies use a red receptor to see green. The Journal of Experimental Biology 202: 2619-1630.

Kelber A, Vorobyev M & Osorio D (2003) Animal colour vision: Behavioural tests and physiological concepts. Biological Reviews 78: 81-118.

Kevan PG & Backhaus WGK (1998) Color vision: Ecology and evolution in making the best of photic environment. In WGK Backhaus R Kliegl & JS Werner (Eds.) Color Vision: Perspectives from different disciplines. Berlin, Germany, Walter de Gruyter.

Kevan PG, Chittka L & Dyer G. (2001) Limits to the salience in ultraviolet: Lessons from colour vision in bees and birds. Journal of Experimental Biology 204: 2571-2580.

Kürten L & Schmidt U (1982) Thermoperception in the common vampire bad (*Desmodus rotundus*). Journal of Comparative Physiology A 146: 223-228.

Krochmal AR & Bakken GS (2003) Thermoregulation is the pits: use of thermal radiation for retreat site selection by rattlesnakes. The Journal of Experimental Biology 206: 2539-2545.

Krugman SL & Koerber TW (1969) Effects of cone feeding by *Leptoglossus occidentalis* on ponderosa pine seed development. Forest Science 16: 104-111.

Koshitaka H, Kinoshita M, Vorobyev M & Arikawa K (2008) Tetrachromacy in a butterfly that has eight varieties of spectral receptors. Proceedings of the Royal Society B 275: 947-954.

Lazzari CR & Núñez JA (1989) The response to radiant heat and the estimation of the temperature of distant sources in *Triatoma infestans*. Journal of Insect Physiology 35: 525-529.

Land MF (2003) Encyclopedia of insects. (Resh VH & Carde RT Eds.) Academic Press Elsevier Science. New York.

McEntire B (1996) Pheromone-based trapping and life history of the fir coneworm, *Dioryctria* abietivorella (Lepidoptera: Pyralidae). M.P.M. Thesis. Simon Fraser University.

McPherson JE, Packauskas RJ, Taylor SJ & O'Brien MF (1990) Eastern range expansion of *Leptoglossus occidentalis* with a key to *Leptoglossus* species of America north of Mexico. The Great Lakes Entomologist 23: 99-104.

Menzel R (1979) Spectral sensitivity and colour vision in invertebrates. Handbook of sensory physiology, vol. VII/6A (ed. H. Autrum). Berlin, Heidelberg, New York: Springer.

Miller GE (1983) Biology, sampling and control of the Douglas-fir cone gall midge, *Contarinia oregonensis* Foote (Diptera: Cecidomyiidae) in Douglas-fir seed orchards in British Columbia. Ph.D. thesis, Simon Fraser University, Burnaby, British Columbia.

Miller GE (1986) Damage predictions for Contarinia oregonensis Foote (Diptera: Cecidomyiidae) in Douglas-fir orchards. The Canadian Entomologist 118: 1297-1306.

Moiseenkóvà V, Bell B, Motamedi M, Wozniak E & Christensen B (2003) Wide-band spectral tuning of heat receptors in the pit organ of the copperhead snake (*Crotalinae*). American Journal of Physiology - Regulatory, Integrative and Comparative Physiology 284: 598-606.

Musolin DL (2007) Insects in a warmer world: ecological, physiological and life-history responses of true-bugs (Heteroptera) to climate change. Global Change Biology 13: 1565-1585.

Nation JL (2002) Insect physiology and biochemistry. CRC Press, Boca Raton, Florida, USA.

Reches A, Nester S & Gutfreund Y (2010) Interactions between stimulus specific adaptation and visual auditory integration in the forebrain of barn owl. The Journal of Neuroscience 30: 6991-6998.

Roeder KD (1967) Turning tendency of moths exposed to ultrasound while in stationary flight. Journal of Insect physiology 13: 873-880.

Schmitz H & Bleckmann H (1997) Fine structure and physiology of the infrared receptor of beetles of the genus *Melanophila* (Colepotera: Burprestidae). International Journal of Insect Morphology and Embryology 26: 205-215.

Schmitz H & Bleckmann H (1998) The photomechanic infrared receptor for the detection of forest fires in the beetle *Melanophila acuminata* (Coleoptera: Buprestidae). Journal of Comparative Physiology A 182: 647-657.

Schmitz H, Trenner S, Hofmann MH & Bleckmann H (2000a) The ability of *Rodnius prolixus* (Hemiptera: Reduviidae) to approach a thermal source solely by its infrared radiation. Journal of Insect Physiology 46: 745-751.

Schmitz H, Schmitz A & Bleckmann H (2000b) A new type of infrared organ in the Australian “fire-beetle” *Merimna atrata* (Coleoptera: Buprestidae). Naturwissenschaften 87: 542-545.

Schmitz H, Schmitz A, Trenner S & Bleckmann H (2002) A new type of insect infrared organ of low thermal mall. Naturwissenschaften 89: 226-229.

Schmitz H & Trenner S (2003) Electrophysiological characterization of the multipolar thermoreceptors in the “fire-beetle” *Merimna atrata* and comparison with the infrared sensilla of *Melanophia acuminata* (both Coleoptera, Buprestidae). Journal of Comparative Physiology A 189: 715-722.

Schmitz A, Gebhardt M & Schmitz H (2008) Microfluidic photomechanic infrared receptors in a pyrophilous flat bug. Naturwissenschaften 95: 455-460.

Schlecht P (1979) Colour discrimination in dim light: an analysis of the photoreceptor arrangements in the moth *Deilephila*. Journal of Comparative Physiology 129: 257-267.

Shine R, Sun LX, Kearney M & Fitzgerald M (2002) Why do juvenile Chinese pit-vipers (*Gloydius shedaoensis*) select arboreal ambush sites? Ethology 108: 897-910.

Shu S, Grant GG, Langevin DA, Lombardo DA & MacDonald L (1997) Oviposition and electroantennogram responses of *Dioryctria abietivorella* (Lepidoptera: Pyralidae) elicited by monoterpenes and enantiomers from eastern white pine. Journal of Chemical Ecology 23: 35-50.

Strong WB, Bates SL & Stoehr MU (2001) Feeding by *Leptoglossus occidentalis* Heidermann (Hemiptera: Coreidae) reduces seed set in lodgepole pine. The Canadian Entomologist 133: 857–865.

Takács S, Bottomley H, Andreller I, Zahradnik T, Schwarz J, Bennett R, Strong W & Gries G (2009) Infrared radiation from hot cones on cool conifers attracts seed-feeding insects. Proceedings of the Royal Society B 276: 649-655.

Taylor SJ, Tescari G & Villa M (2001) A Nearctic pest of Pinaceae accidently introduced into Europe: *Leptoglossus occidentalis* (Heteropteran: Coreidae) in northern Italy. Entomological News 112: 101-102.

Townson TM, Chang BSW, Salcedo E, Chadwell LV, Pierce NE & Britt SG (1998) Honeybee blue- and ultraviolet-sensative opsins: Cloning heterologous expression in *Drosophila* and physiological characterization. The Journal of Neuroscience 18: 2412-2422.

Trudel R, Bauce É, Cabana J & Guertin C (1995) Rearing technique for *Dioryctria abietivorella* (Lepidoptera: Pyralidae). Journal of Economic Entomology 88:640-643.

Vernon RS & Bartel DL (1985) Effect of hue, saturation, and intensity of colour selection by the onion fly, *Delia antiqua* (Meigen) (Diptera: Anthomyiidae) in the field. Environmental Entomology 14: 210-216.

Vondran T, Apel KH & Schmitz H (1995) The infrared receptor of *Melanophila acuminata* De Geer (Coleoptera: Buprestidae): ultrastructural studies of a unique insect thermoreceptor of a possible descent from a hair receptor. Tissue & Cell 27: 645-658.

Weissling TH & Knight AL (1994) Passive trap for monitoring codling moth (Lepidoptera: Tortricidae) flight activity. Journal of Economic Entomology 87: 102-107.

Whitehouse CM, Roe AD, Strong WB, Evenden, ML & Sperling FAH (2011) Biology and management of North American cone-feeding *Dioryctria* species. The Canadian Entomologist143: 1-34.

Yurtserver S, Okyar Z & Necmettin G (2010) What colour of flowers do Lepidoptera prefer for foraging? Biologia 65: 1049-1056.

Zaccardi G, Kelber A, Sison-Mangus MP & Briscoe AD (2006) Colour discrimination in the red range with only one long-wavelength sensitive opsin. The Journal of Experimental Biology 209: 1944-1955.

2. Douglas-fir cone gall midges respond to shape and infrared wavelength attributes characteristic of host tree branches[1]

2.1. Abstract

We[1] tested the hypothesis that the conophagous Douglas-fir cone gall midge, *Contarinia oregonensis* Foote (Diptera: Cecidomyiidae), responds to infrared (IR) radiation and other electromagnetic wavelengths associated with cones of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco (Pinaceae). Early-season (March-April) thermographic images showed that cone orientation (upright, horizontal, pendant) and cone colour (green, purple, green/purple) did not affect apparent cone temperature (inferred from thermographic images). Tree components significantly differed in apparent temperature, foliage being coolest and branches warmest. There was no significant

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difference in the number of larvae in cones of different colours, and adult midges were equally attracted to traps painted green or purple, suggesting that cone colour does not affect oviposition decisions by gravid females. Adult midges were more strongly attracted to warm traps with IR frequency emissions higher than those of the background than to cold traps with IR frequency emissions lower than those of the background. They were also more strongly attracted to warm branch-shaped traps than to warm upright cylindrical traps. Collectively, these data indicate that the shape and IR attributes of Douglas-fir branches may serve as foraging cues for *C. oregonensis*.

Keywords: conophagous insect guild; plant-derived cues; profiles; attraction cues

2.2. Introduction

Insects are capable of exploiting various ranges of the electromagnetic spectrum as foraging cues. Ultraviolet andvisible light (~ 300-700 nm wavelength) excite visual pigments in compound eyes (Schmitz and Bleckmann 1997), which aid in locating resources based on their hue and shape (Briscoe and Chittka 2001; Kelber *et al.* 2003). While a particular wavelength range may play a singular role during stages of the resource finding process, it has become apparent that visual cues can be complex and may entail any combination of plant colour, shape, size, pattern and texture (e.g., Prokopy 1968; Moericke *et al*. 1975; Roitberg 1985; Owens and Prokopy 1986; Allard and Papaj 1996; Kelber 1994).

Radiant infrared (IR) wavelengths of > 700 nm are received in some insects by specialized IR receptors. For example, IR reception aids Australian fire beetles, *Melanophila acuminata* De Geer (Coleoptera: Buprestidae), in locating mating swarms near burning forest fires, and finding oviposition sites in recently burnt trees (Evans 1964; Vondran *et al.* 1995; Schmitz and Bleckmann 1997; Schmitz *et al*. 1997).

The Western conifer seed bug, *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae), is attracted to radiant IR from cones (Takács *et al*. 2009) and visible light (400-750 nm; TZ *et al*., Chapter 5). Similarly, adult *Dioryctria abietivorella* Groté (Lepidoptera: Pyralidae) are attracted to specific wavelengths of radiant IR and to specific hues of visible light (Chapter 4).

Like *L. occidentalis* and *D. abietivorella*, the Douglas-fir cone gall midge, *Contarinia oregonensis* Foote (Diptera: Cecidomyiidae), is a member of the conophagous insect guild. *Contarinia oregonensis* larvae colonize cone scales of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco (Pinaceae) (Johnson 1963). In early spring, female *C. oregonensis* oviposit in female conelets that are open for pollination (Miller 1983), placing their eggs singly or in clusters near the base of cone scales. Neonate larvae tunnel into cone scales and induce the formation of galls, within which they develop (Hedlin 1961). When conelets have assumed a pendant position, they are no longer susceptible to oviposition by female *C. oregonensis*. This narrows the oviposition timeframe, and necessitates the efficient exploitation of host-finding cues. Cone and foliage colour, as well as radiant IR, may be among these cues.

Spectrometric profiles of conifer cones and foliage differ (Blatt and Borden 1999; Takács *et al*. 2009), and cone colour greatly varies between clones (Copes 1972). Purple cones in particular contrast strongly against green foliage. We predicted (Hypothesis 1) that cone colour is a foraging cue for female *C. oregonensis.* We further predicted (Hypothesis 2) that early-season Douglas-fir cones exhibit a thermographic contrast to needles and that female *C. oregonensis* exploit this contrast to locate cones.

2.3. Materials and methods

2.3.1. Surveys 1-3: Effect of cone colour, cone orientation, and tree components (cone, foliage, branch) on apparent temperatures

In thermographic surveys (S) 1-3, we compared the temperature of cones with different colour (S1) and orientation (S2), as well as the temperatures of different tree components (S3). Thermographic and corresponding photographic images were taken with a mid-range IR (3-5 µm) AGEMA Thermovision 550 camera (FLIR Systems Ltd., Burlington, ON, Canada) and a Kodak EasyShare C613 (Kodak, Rochester, NY, USA), respectively, at the Kalamalka Forestry Centre (B.C. Ministry of Forests and Range, Vernon, BC, 119° 16´ W, 50° 14´ N) on 22 April 2010 between 11:30 and 13:30 h. All thermographic images were analyzed, using ThermaCam Reporter 2000 Pro software (FLIR Systems Ltd., Burlington, ON, Canada) to calculate the mean temperature of target objects, taking into account the values of parameters including (*i*) emissivity of the object (the ratio of the radiation emitted by a surface of interest to the radiation emitted by a black body at the same temperature), (*ii*) distance from the object, (*iii*) atmospheric temperature, (*iv*) reflected ambient temperature (the calculated temperature of incident IR radation), and (*v*) relative humidity.

In our study, thermographic images were recorded from the east side of trees at a distance of 5 m from the objects. Cones shaded by branches were not imaged. Reflected ambient temperature was recorded within each image using the mean temperature across the surface of an aluminium disk placed near the target object to

calculate the object's temperature. Standardized analyses of recorded images set emissivity at 0.95 [the approximate value of the emissivity of vegetated areas (Jiang *et al.* 2006)]. Hourly atmospheric temperature and relative humidity data were obtained from Environment Canada Weather. Foliage temperature was determined using the calculated mean temperature of three randomly selected areas of foliage, using a grid and random number generator. We refer to apparent temperature of target objects because temperature was not measured. Software calculations were confirmed by some direct temperature measurements with a thermal couple (Takács, person. com.).

Within the orchard, all trees were ascribed to categories of bearing cones of specific colour (green, purple, purple/green) and orientation (upright, horizontal, pendant). In S1, 15 trees of each cone colour category were randomly selected, the apparent temperature of one cone per tree was recorded, and the mean temperature of 15 green, purple or purple/green cones was calculated. Images were taken on the East side of trees when all cones were in sunlight. In S2, 15 trees of each cone orientation category were randomly selected, the temperature of one cone per tree was recorded, and the mean temperature of 15 cones each with upright, horizontal, or pendant orientation was calculated. In S3, thermographic images of 25 randomly selected trees were recorded. Each image contained cones, branches and foliage. In each image, the temperature of one cone, one branch and three areas (each ~ 10 × 10 cm) of foliage (averaged) was recorded, and the mean temperature of 25 cones, 25 branches and 25 foliage areas was calculated. The mean apparent temperature of cone colour, cone orientation and tree component in thermographic images were compared with a one-way ANOVA and followed by a Tukey-Kramer test, (Zar, 1999) using JMP software (SAS®, Cary, NC, USA). In S3 the one-way ANOVA was blocked by tree.

2.3.2. Survey 4: Effect of cone colour on oviposition by female C. orengonensis

Survey 4 measured the effect of cone colour on oviposition by female *C. orengonensis*. On 24 May 2009, early in the season, while cones were in upright and horizontal positions, each cone-bearing tree in a Douglas-fir seed orchard near Saanichton (British Columbia, Canada; 123°43′ W, 49°27′ N) was assigned a code for its cone colour (purple, purple with green, equal purple and green, green with purple, and green). The colours of immature cones are consistent within trees and between years (Copes 1972). In July 2009, three randomly selected cones on the west side of trees, ~ 150 cm from the ground, were removed from 10 randomly-selected (with a random number generator) trees (all different clones) for each of the five colour codes. Cones were cut in half longitudinally and exposed *C. orengonensis* larvae were counted. Half-cut counts were averaged across the three harvested cones from each tree. The mean number of *C. oregonensis* larvae exposed per cone half-cut was compared for the five colour categories by one-way ANOVA and a post-hoc Tukey-Kramer test (Zar 1999) using JMP softare.

2.3.3. General design of trapping experiments

In the Saanichton Seed Orchard and Kalamalka Forestry Centre, *P. menziesii* trees bearing at least 50 cones were randomly selected Experimental traps were constructed of sections of PVC piping (5.1 cm × 12.7 cm) (IPEX, Toronto, ON, Canada) closed with a 5.1-cm PCV cap at the bottom and covered with a lid at the top (NIBCO Elkhart, IN, USA). Pairs of traps were deployed ~ 165 cm above ground on the east side of selected trees to allow traps to be warmed by the early-morning sun. Traps were suspended

vertically (unless otherwise noted) from tree branches with galvanized 20 GA wire. Traps were covered with Painter's Mate tape (Shurtape Technologies, Hickory, NC, USA), spray-painted with Rust-oleum Painter's Touch Grey spray paint (RPM International Inc., Medina, OH, USA), and coated with a thin layer of adhesive TangleFoot (Contech Enterprises, Victoria, BC, Canada). Spectrometric profiles and thermographic images taken with an HR4000 spectrometer (Ocean Optics, Duneline, FL, USA) and the AGEMA Thermovision camera confirmed that TangleFoot did not significantly alter the spectrometric profile of paint colour or the apparent temperature. The trapping period ran from approximately 10:00 h until shade started to cover traps, at about 17:00 h. Captured midges were identified on site using a hand-held 15X magnifier. On one day specifically, traps were checked continuously to be able to carefully inspect midges before they were completely entangled in the adhesive. Antennae and wing veination of midges deduced to be *C. oregonensis* were consistent with those reported in the literature. Sample specimens were removed, preserved in 70 % ethanol, and sent for taxonomic confirmation to Dr. Bradley J. Sinclair (Canadian National Collection of Insects, Ottawa Plant Laboratory – Entomology, CFIA). He concluded that we had captured male and female *C. oregonensis*. All other midges found in traps were about 50% smaller and could readily be distinguished from *C. oregonensis*.

2.3.4. Experiments 1-4: Effect of trap colour, temperature, and shape on attraction of C. oregonensis

Experiment 1 tested the effect of trap colour on attraction of *C. oregonensis*. This experiment was run at the Kalamalka Forestry Centre between 30 April and 6 May 2010. A set of paired traps (each trap 5 × 13 cm) was suspended ~ 120 cm apart from

25 randomly selected *P. menziesii* trees, each bearing at least 50 cones. One member of the pair was painted purple (Rust-oleum Satin Claret Wine), the other green (Rust-oleum Satin Green Apple) (Fig. 2.1). These paint colours were chosen because they represented the extremes of cone colour variation. Captured midges were recorded every 60 min, identified and removed. The AGEMA camera was used to confirm that the temperature of colour traps was similar.

Experiments 2 and 3 tested the effect of radiant IR on attraction of *C. oregonensis.* They were conducted at the Saanichton Seed Orchard between 23-24 May 2009 (Exp. 2) and at the Kalamalka Forestry Centre between 13-15 May 2010 (Exp. 3). In each experiment, paired grey-painted traps (see above) were suspended ~ 120 cm apart from 21 randomly selected trees of *P. menziessii*, each bearing at least 50 cones. Warm traps (with higher-frequency, higher-energy IR emissions than the background) were filled with 50 mL of water and allowed to heat up in the sun, whereas cold traps (with lower-frequency, lower-energy IR emissions than the background) were filled with 150 mL of ice water which maintained a cold surface (Fig. 2.1, b). Every 120-180 min, 75 mL of water were withdrawn from the cold trap by syringe and replaced by ice cubes. Lids were kept on both types of traps to maintain a similar relative humidity. Periodically throughout the experiment, the AGEMA camera (see above) was used to confirm that warm and cold traps maintained warmer and cooler temperatures than the background.

Experiment 4 tested the effect of trap shape [branch-like (2 × 43 cm) or upright cylindrical (6.5 × 13 cm); Fig. 2.1, c)] on attraction of *C. oregonensis.* Both types of traps were painted with Rust-oleum Gray Primer, had similar trapping surfaces and temperatures (see Results), and were warmer than the background (see Results).

Temperature data were obtained from thermographic images of foliage (see method for S3), four branch-like traps (long and thin), and four can-shaped traps (wide and short, not resembling branches), which were taken on 26 April 2010 at Timber West in Saanichton British Columbia, Canada (123°43′ W, 49°27′). Experiment 4 was run at the Kalamalka Forestry Centre between 1-7 May 2010. Paired branch-like or upright cylindrical traps were suspended ~ 60 cm apart from 18 randomly selected *P. menziesii* trees, each bearing at least 50 cones per tree. Upright cylindrical traps were suspended vertically and branch-like traps horizontally. Every hour, captured midges were recorded, removed and identified. In experiments 1-3, total numbers of *C. oregonensis* adults per trap were tallied and compared between test stimuli by a two-tailed Wilcoxon signed-rank test (Zar 1999) using JMP software.

In experiment 4, total number of *C. oregonensis* adults per trap were tallied and compared between test stimuli by a one-tailed Wilcoxon signed-rank test (Zar 1999) uing JMP software.

2.4. Results

There was no significant difference in apparent temperature between Douglas-fir cones of different colour (S1: $F_{2,42}$ = 1.25, p = 0.30; Fig. 2.2) or orientation (S2: $F_{2,42}$ = 1.87, p = 0.17; Fig. 2.2). There were significant temperature differentials between foliage, cones and branches (S3: $F_{2.24}$ = 97.5, p < 0.0001; Fig. 2.2). Branches were significantly warmer than cones and foliage (Tukey: cones, p < 0.0001; foliage, p < 0.0001), and cones were significantly warmer than foliage (Tukey: p < 0.0001). There

was no significant difference in the number of *C. oregonensis* larvae found in cones of different colour (S4: $F_{4,71}$ = 1.2, p = 0.32; Fig. 2.3).

Traps painted green or purple, representing the extremes of cone colour variation, captured similar numbers of adult *C. oregonensis* (Exp. 1: Z = -1.73, p = 0.082; Fig. 2.4). Warm traps attracted significantly more adult *C. oregonensis* than did cold traps in Sechelt (Exp. 2: Z = 2.59, p = 0.0095; Fig. 2.4) and in Kalamalka (Exp. 3: Z = 3.69, p = 0.0002; Fig. 2.4). Branch-like traps captured significantly more adult *C. oregonensis* than did upright cylindrical traps (Exp. 4: p = 0.0002; Fig. 2.4). Branch-like and upright cylindrical traps had mean apparent temperatures of 12.9 ˚C (± 0.38 ˚C), and 13.3 ˚C (± 0.77 ˚C), respectively, whereas the foliage had a mean temperature of 10.9 ˚C (± 0.61 ˚C). The mean temperature of the two trap types did not differ (Tukey-Kramer test: p = 0.88) but significantly exceeded that of foliage (Tukey-Kramer test: branch-like trap, p = 0.011; upright cylindrical trap, p = 0.03).

2.5. Discussion

Our data indicate that (*i*) Douglas-fir cones, branches and foliage in early spring differ in temperature, (*ii*) cone colour and cone orientation do not affect cone temperature, and (*iii*) female *C. oregonensis* show no oviposition preference for cones of particular colours, prefer warm traps to cold traps, but show no preference for green vs. purple traps.

Cones susceptible to oviposition by female *C. oregonensis* are held upright and horizontal. Because cone temperature did not change with cone orientation (S2; Fig. 2.2), we conclude that it cannot be used as a cue for female *C. oregonensis* to gauge

cone suitability for oviposition. However, cones were significantly warmer than surrounding foliage (S3; Fig. 2.2), suggesting that female *C. oregonensis* may utilize this contrast in radiant IR to locate cones. Contrasting radiant IR between the target object and background is used by vertebrates such as the Chinese pit-viper, *Gloydius shedaoensis* Zhao (Squamata: Viperidae) (Shine and Sun 2002), and invertebrates such as *L. occidentalis* (Takács *et al*. 2009) as a foraging cue. When given a choice between warm and cold traps, female *C. oregonensis* preferred the warm traps (Exp. 2; Fig. 2.4). Because they also preferred branch-like over upright cylindrical cues (Exp. 4; Fig. 2.4), and branches are the warmest part of early-season Douglas-fir trees (S3; Fig. 2.2), female *C. oregonensis* may exploit the shape and IR cue of branches, not cones, as a foraging cue. Having located the branches of a host tree, they may then use other cues to orient towards cones.

The design of our field experiments did not allow us to separate convective and conductive heat from radiant IR to demonstrate conclusively that *C. oregonensis* responded to radiant IR from warm, branch-like sources. However, the thermal gradient between a warm object and its external environment is steep (Baierlein 1999), making conductive heat perceptible over only a very short range. Thus, conductive or convective heat is not a suitable long-range foraging cue to pollinators or herbivores. Radiant IR, however, is effective over a long distance (Takács *et al*. 2009). Two other members of the conophagous insect guild, the heteropteran *L. occidentalis* (Takács *et al*. 2009) and the nocturnal lepidopteran *D. abietivorella* (Chapter 3), are known to have IR receptors and respond to radiant IR. Thus, it seems reasonable to predict that *C. oregonensis* has functional IR receptors.

The adaptive significance for responding to radiant IR from natural resources appears to differ between groups of insects. Adult *M. acuminata* exploit radiant IR from forest fires as an indirect cue to locate future sites for oviposition and larval development (Evans 1966; Schmitz and Bleckmann 1997; Schmitz and Trenner 2003). This may also apply to female *C. oregonensis* which may exploit branch-derived radiant IR as an indirect, or intermediate, cue to locate cones for oviposition and larval development. Adult *L. occidentalis,* in contrast, feed on the content of seeds (Krugman and Koerber 1969; Blatt and Borden 1999; Strong *et al.* 2001) and exploit cone-derived radiant IR as a direct cue to locate a food source.

We predicted that cone colour mediates foraging behaviour of *C. oregonensis.* Cone colour may provide contrast to surrounding foliage and modulate cone temperature. In white fir, *Abies concolor* (Gord. & Glend.) Lindl. ex Hildebr. (Pinaceae), the interior of purple cones is warmer than that of green cones (Sturgeon and Mitton 1980) and thus might be more conducive for the development of conophagous insect larvae. That female *C. oregonensis* showed no preference for green or purple traps (Exp. 1, Fig. 2.4), and no oviposition preference for cones of specific colour (Fig. 2.3), suggest that cones are not distinguished from each other based on colour.

Our findings have implications for monitoring *C. oregonensis* populations. Pheromone-baited traps attract males and provide only an indirect measure of female population abundance (Gries *et al*. 2002; Morewood *et al*. 2002). In our study, traps warmer than the background attracted females, offering new possibilities of population assessment. However, the mechanisms of retaining captured specimens must be improved so that sex identity can be more readily determined.

2.6. References

Allard, R.A., and Papaj, P.R. 1996. Learning of leaf shape by pipevine swallowtail butterfly: A test using artificial models. Journal of Insect Behaviour, **9**: 961–967.

Baierlein, R. 1999. Thermal physics. Cambridge University Press, Cambridge, United Kingdom.

Blatt, S.E., and Borden, J.H. 1999. Physical characteristics as potential host selection cues for *Leptoglossus occidentalis* (Heteroptera: Coreidae). Environmental Entomology, **28**: 246–254.

Briscoe, A.D., and Chittka, L. 2001. The evolution of colour vision in insects. Annual Review of Entomology, **46**: 471–510.

Copes, D.L. 1972. Inheritance of megastrobili colours in Douglas fir (*Pseudotsuga menziesii*). Canadian Journal of Botany, **50**: 2045–2048.

Evans, W.G. 1964. Infra-red receptors in *Melanophila acuminata* De Geer. Nature, **11**: 202–211.

Evans, W.G. 1966. Perception of infrared radiation from forest fires by *Melanophila acuminata* De Greer (Buprestidae: Coleoptera). Ecology, **47**: 1061–1065.

Gries, R., Khaskin, G., Gries, G., Bennett, R.G., King, G.G.S., Morewood, P., Slessor, K.N., and Morewood, W.D. 2002. (*Z*,*Z*)-4,7-Tridecadien-(*S*)-2-yl acetate: Sex pheromone of Douglas-fir cone gall midge, *Contarinia oregonensis*. Journal of Chemical Ecology, **28**: 2283–2297.

Hedlin, A.F. 1961. The life history and habits of a midge *Contarinia oregonensis* Foote (Diptera: Cecidomyiidae) in Douglas-fir cones. The Canadian Entomologist, **93:** 953–967.

Jiang, G., Li, Z., and Nerry, F. 2006. Land surface emissivity retrieval from combined mid-infrared and thermal infrared data of MSG-SEVIRI. Remote Sensing of Environment, **105**: 326–355.

Johnson, N.E. 1963. Time of attack of the Douglas-fir cone midge in relationship to cone development. Journal of Forestry, **61**: 350–355.

Kelber, A. 1994. Colour learning in the hawkmoth *Macroglossum stellatarum.* The Journal of Experimental Biology, **199**: 1127–1131.

Kelber, A., Balkenius, A., and Warrant, E.J. 2002. Scotopic colour vision in nocturnal hawkmoths. Nature, **419**: 922–925.

Krugman, S.L., and Koerber, T.W. 1969. Effects of cone feeding by *Leptoglossus occidentalis* on ponderosa pine seed development. Forest Science, **16**: 104–111.

Miller, G.E. 1983. Biology, sampling and control of the Douglas-fir cone gall midge, *Contarinia oregonensis* Foote (Diptera: Cecidomyiidae) in Douglas-fir seed orchards in British Columbia. Ph.D. thesis, Simon Fraser University, Burnaby, British Columbia.

Moericke, V., Prokopy, R.J., Berlocher, S., and Bush, G.L. 1975. Visual stimuli eliciting attraction of *Rhagoletis pomonella* (Diptera: Tephritidae) flies to trees. Entomologia Experimentalis et Applicata, **18**: 497–507.

Morewood, P., Morewood, D.W., Bennett, R.G., and Gries, G. 2002. Potential for pheromone-baited traps to predict seed loss caused by *Contarinia oregonensis* (Diptera: Cecidomyiidae). The Canadian Entomologist, **134**: 689–697.

Owens, E.D., and Prokopy, R.J. 1986. Relationship between reflectance spectra of host plant-surfaces and visual detection of host fruit by *Rhagoletis pomonella* flies. Physiological Entomology, **11**: 297–307.

Prokopy, R.J. 1968. Visual responses of apple maggot flies *Rhagoletis pomonella* (Diptera: Tephritidae) in orchard studies. Entomologia Experimentalis et Applicata, **11**: 403–422.

Roitberg, B.D. 1985. Search dynamics in fruit-parasitic insects. Journal of Insect Physiology, **31**: 865–872.

Schmitz, H., and Bleckmann, H. 1997. Fine structure and physiology of the infrared receptor of beetles in the genus *Melanophila* (Coleoptera: Buprestidae). International Journal of Insect Morphology and Embryology, **26**: 205–215.

Schmitz, H, and Trenner, S. 2003. Electrophysiological characterization of the multipolar thermoreceptors in the “fire-beetle” *Merimna atrata* and comparison with the infrared sensilla of *Melanophila acuminata* (both Coleoptera: Bupresitidae). Journal of Comparative Physiology A, **189**: 715–722

Schmitz, H., Trenner, S., Hofmann, M.H., and Bleckmann, H. 2000. The ability of *Rhodnius prolixus* (Hempitera: Reduviidae) to approach a thermal source solely by its infrared radiation. Journal of Insect Physiology, **46**: 745–751.

Schmitz, H., Bleckmann, H., and Mürtz, M. 1997. Infrared detection in a beetle. Nature, **386**: 773–774.

Shine, R., and Sun, L.X. 2002. Arboreal ambush site selection by pit-vipers *Gloydius shedaoensis*. Animal Behaviour, **63**: 565–576.

Sturgeon, K.B., and Mitton, J.B. 1980. Cone colour polymorphism associated with elevation in white fir, *Abies concolor*, in Southern Colorado. American Journal of Botany, **67**: 1040–1045.

Strong, W.B., Bates, S.L., and Stoehr, M.U. 2001. Feeding by *Leptoglossus occidentalis* Heidermann (Hemiptera: Coreidae) reduces seed set in lodgepole pine. The Canadian Entomologist, **133**: 857–865.

Takács, S., Bottomley, H., Andreller, I., Zahradnik, T., Schwarz, J., Bennett, R., Strong, W., and Gries, G. 2009. Infrared radiation from hot cones on cool conifers attracts seed-feeding insects. Proceedings of the Royal Society B, **276**: 649–655.

Vondran, T., Apel, K.H., and Schmitz, H. 1995. The infrared receptor of *Melanophila acuminata* De Geer (Coleoptera: Buprestidae): ultrastructural study of a unique insect thermoreceptor and its possible descent from a hair mechanoreceptor. Tissue Cell, **27**: 645–658.

Zar, J.H. 1999. Biostatistical analysis. 4th edition. Prentice Hall, Upper Saddle River, New Jersey.

2.7. Figure captions

Figure 2.1. Photo- and thermographic images of paired traps in experiments 1-4 that (a) represented the extremes (green or purple) of cone colour variation (Exp. 1), (b) represented temperatures warmer or colder than the background (Exps. 1, 2), and (c) had branch-like or upright cylindrical shape (Exp. 4). Inserts in (b) represent thermographic images of hot and cold traps.

Figure 2.2. Mean (+ SE) apparent temperature recorded in thermographic surveys (S) 1-3 of cones of different colour (S1) and position (S2), and of components of trees (S3). In each of surveys 1-2, there was no significant temperature difference between recorded objects (ANOVA; $p > 0.05$). In S3, branches were significantly warmer than foliage which was significantly cooler than cones (one-way ANOVA, $p < 0.05$).

Figure 2.3. Mean (+ SE) number of *Contarina oregonensis* larvae found in Douglas-fir cones halves (n=10) of various colours in survey 4. Cone colour had no significant effect on numbers of larvae present in cones (one-way ANOVA, $p > 0.05$).

Figure 2.4. Effect of trap colour (Exp. 1), trap apparent temperature (Exps. 2, 3) and trap shape (Exp. 4) on captures of adult *Contarina oregonensis*. In each of experiments 1-4, bars with an asterisk indicate a significant preference for the test stimulus (Exp. 1-3: two-talied Wilcoxon signed-rank test, $p < 0.05$; Exp. 4: one-tailed Wilcoxon signed-rank test, $p < 0.05$).

Figure 2.1

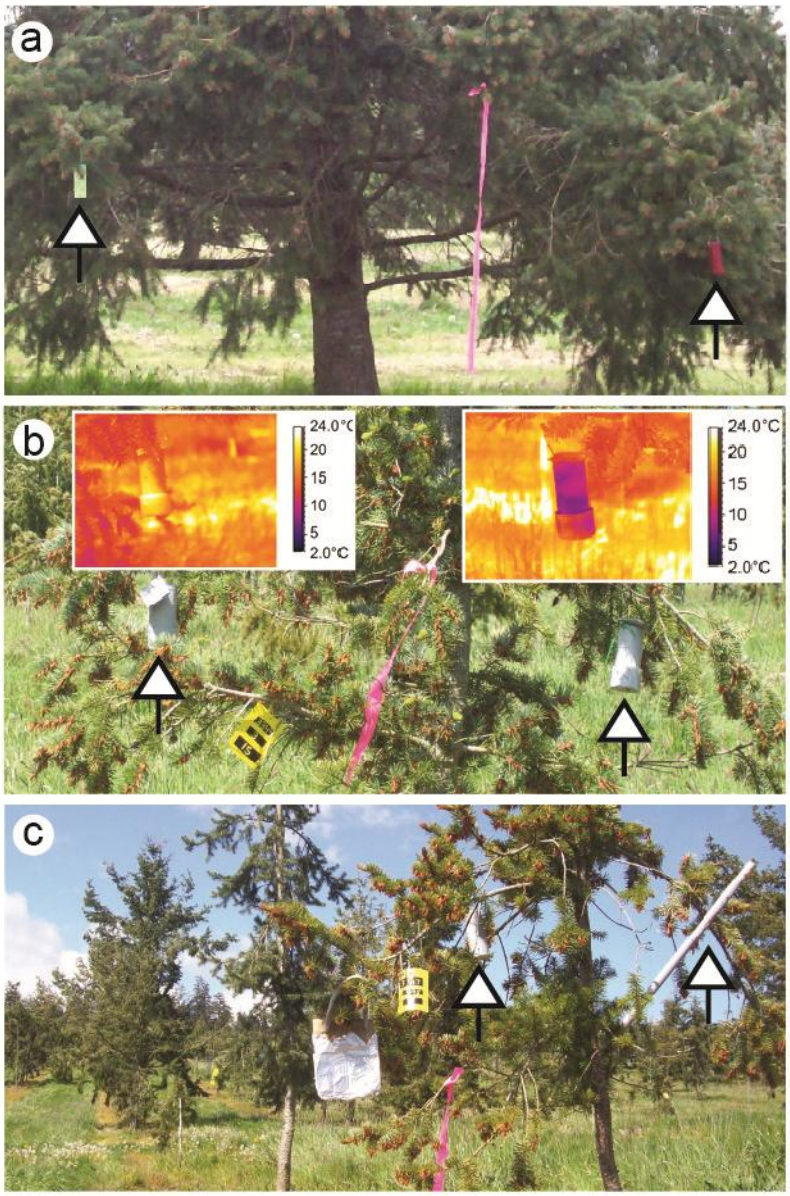


Figure 2.2

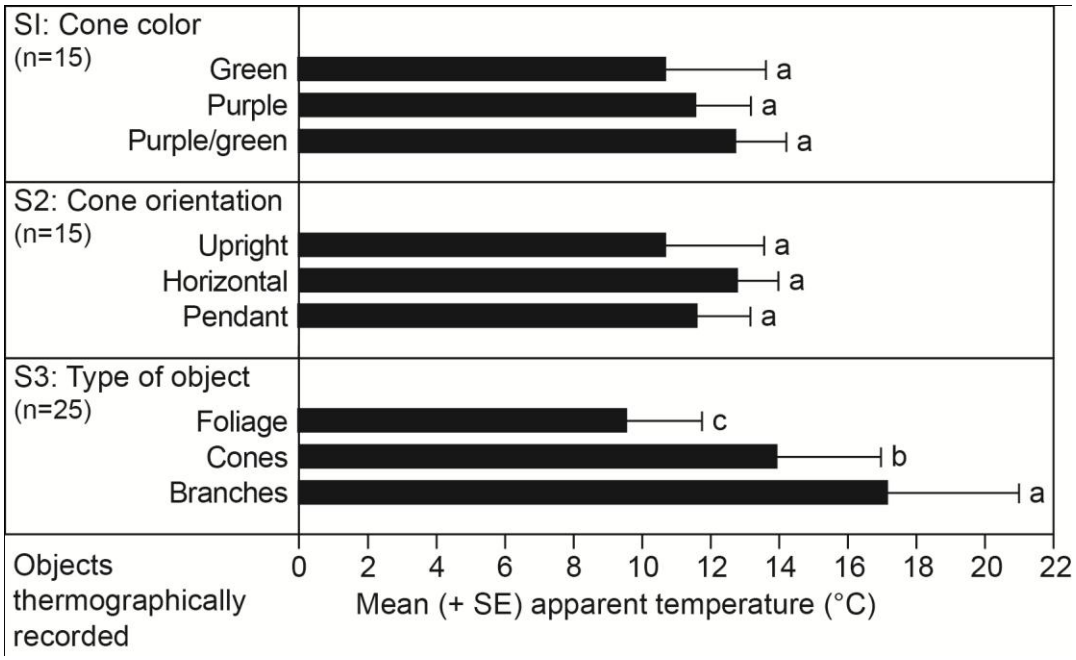

SI: Cone color
(n=15)
Green
Purple
Purple/green
a
a
a
S2: Cone orientation
(n=15)
Upright
Horizontal
Pendant
a
a
a
S3: Type of object
(n=25)
Foliage
Cones
Branches
c
b
a
Objects thermographically recorded
0 2 4 6 8 10 12 14 16 18 20 22
Mean (+ SE) apparent temperature (°C)

Figure 2.3

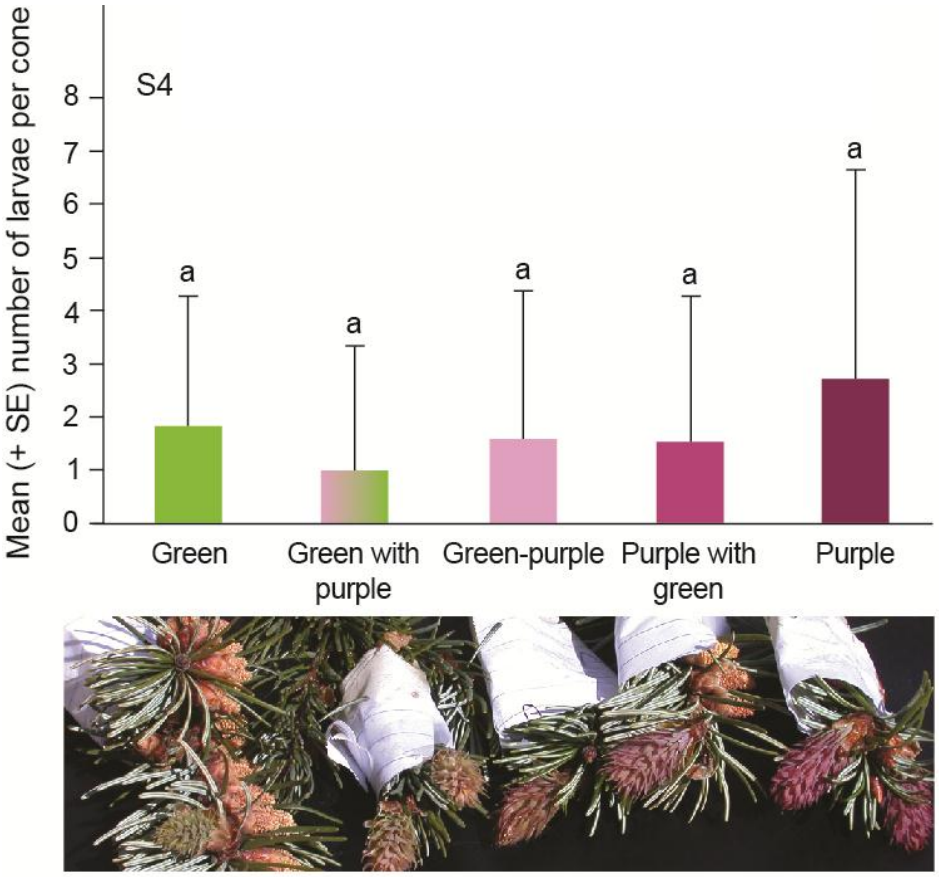
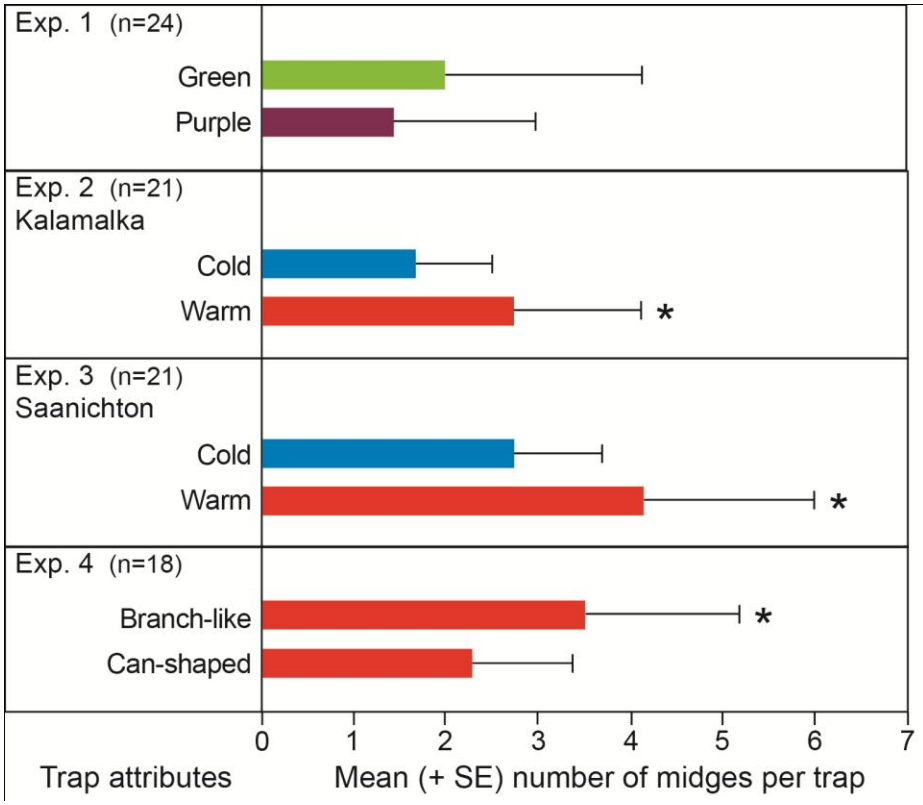

Mean (+ SE) number of larvae per cone
S4
0
1
2
3
4
5
6
7
8
a
a
a
a
a
Green
Green with purple
Green-purple
Purple with green
Purple

Figure 2.4


Exp. 1 (n=24)
Green
Purple
Exp. 2 (n=21)
Kalamalka
Cold
Warm
*
Exp. 3 (n=21)
Saanichton
Cold
Warm
*
Exp. 4 (n=18)
Branch-like
*
Can-shaped
0
1
2
3
4
5
6
7
Trap attributes
Mean (+ SE) number of midges per trap

3. Morphological, electrophysiological and behavioural evidence for an infrared receptor in the pyralid moth *Dioryctria abietivorella*[2]

3.1. Abstract

Some insects use infrared radiation (IR) sensing pit organ receptors (from here on "IR receptors") that aid in locating resources. Here, we present evidence for an IR receptor in the pyralid moth *Dioryctria abietivorella* (Groté). Environmental scanning electron micrographs revealed a candidate receptor on the prothorax of male and female moths. In electrophysiological recordings, IR receptors responded to (*i*) radian IR from heat sources kept at 40-60 °C, (*ii*) ambient temperature raised >38 ºC, and (*iii*) wavelengths of UV and visible light (250-950 nm). Thermographs of Douglas-fir (*Pseudotsuga menziesii*) and spruce (*Picea engelmanni* x *glauca*) trees taken hourly for 24 hours showed minor temperature differentials between cones and foliage at night, but strong differentials between other parts of trees and their surroundings, that could be

[2]Authors: Tracy Zahradnik, Audrie Labrie, Keiko Nabetani, Allison Gamble, Ward Strong, Gerhard Gries. This chapter has been formatted following the requirements of Naturwissenschaften.

exploited by moths during their nocturnal flight period (21:00-03:00 h). In laboratory and field experiments, males and mated females, but not virgin females, were more strongly attracted to high frequency than to low frequency radiant IR emanating respectively from hot or cold sources. Moths with their IR receptors occluded showed no such preference. Following the recent discovery that the Western conifer seed bug, *Leptoglossus occidentalis,* orients toward cone-derived radiant IR, it seems that *D. abietivorella,* and possibly other members of the conophagous insect guild, might also exploit plant-arrived radiant IR to locate resources.

Keywords: conophagous insect guild; infrared radiation; foraging; attraction cue

3.2. Introduction

IR receptors have been reported in diverse vertebrate and invertebrate taxa. In common vampire bats (Kürten and Schmidt 1982), *Python* snakes (Grace et al. 2001), and the blood-sucking bug *Rhodnius prolixus* (Schmitz et al. 2000), IR receptors aid in orientation towards warm-bodied food sources. The pyrophilic (fire-loving) jewel beetle, *Melanophila acuminata*, Australian flat bug, *Aradus albicornis,* and the Australian fire beetles *Merimna atrata* and *Acanthocnemus nigricans* have IR receptors on the prothorax or abdomen, and sense forest fires from afar. This cue helps them find oviposition sites in smouldering bark of burnt trees, where their offspring develop with minimal competition (Evans 1964; Schmitz et al. 1997, 2000a, 2002, 2008). The Western conifer seed bug, *Leptoglossus occidentalis* Heidemann (Hemiptera: Coreidae), a tissue specialist herbivore that forages during the photophase and feeds on the contents of seeds within the cones of many conifers (Blatt & Borden 1999; Strong et al. 2001), uses IR radiation from developing cones as a long-range foraging cue (Takács et al. 2009).

Whether or not nocturnal species of the conophagous insect guild orient towards radiant IR from conifer cones remains uncertain. Conceivably, such radiant IR may be present only at day time, when cones warm by absorbing solar radiation, and emit more IR than surrounding cool foliage (Takács et al. 2009). However, if there is a lag in cone cooling during the scotophase, or if cones are thermogenic due to high metabolic activity, then their IR radiation could be exploited by conophagous insects that forage at

night. Thermogenicity of cones, live flowers and inflorescences has been reported in several families of gymnosperm and angiosperm plants, including cycads, arum lilies, Dutchman's pipes, palms, custard apples, winter's bark and magnolias (Thien et al. 2000). Moreover, cycads use an intriguing heat- and odour-mediated strategy to direct the movement of insect pollinators between their cone-like male and female infloresences (Terry et al. 2007).

Radiant IR can be detected in the absence of visble light (Schmitz et al. 2000) and may serve conophagous nocturnal moths, such as *Dioryctria abietivorella* (Groté), as a foraging cue in addition to monoterpenes (Shu et al. 1997) or other plant semiochemicals (Jactel et al. 1994).

Adults of *D. abietivorella* are active throughout the summer (McEntire 1996; Strong et al. 2007; Grant et al. 2009). Virgin, 3- to 4-d old females engage in sexual communication starting ~ 2 h after sunset (Whitehouse et al. 2011). By pheromone they attract protandrous males and mate up to eight times (Whitehouse et al. 2011). One day post mating (Trudel et al. 1995), gravid females forage for conifer cones of many genera, including *Abies*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga* and *Tsuga* (de Groot et al. 1994).

In the laboratory and in seed orchards of Douglas-fir, *Pseudotsuga menziesii*, and Engelmann spruce, *Picea engelmannii,* we tested the hypotheses that there is a temperature differential between cones and needles at night, and that *D. abietivorella* responds to radiant IR from conifer cones to locate them.

3.3. Methods

3.3.1. Experimental insects

Dioryctria abietivorella pupae were obtained from Insect Production Services (Canadian Forest Service, Sault Ste Marie, ON, Canada). Eclosed moths were separated by sex, provided with a 10 % sugar water solution (Trudel et al. 1995), and kept in growth chambers at 60 % relative humidity, 25 °C, and a 16:8 (L:D) light regime. To obtain mated females, >2-day old virgin females were caged with males for at least two days, resulting in female mating success of 97 %, as indicated by the presence of a spermataphore (see Chapter 4, section 4.3.1).

3.3.2. Environmental scanning electron microscopy

Environmental scanning electron micrographs (ESEMs) were taken at the Pulp and Paper Institute of Canada (Paprican, Vancouver, BC, Canada), using a FEI Quanta FEG 400 Environmental Scanning Electron Microscope (FEI Company, Hilsbro, OR, USA). The head, thorax and abdomen of male and female moths were secured on a metal stub, sputter-coated with gold/palladium, and imaged. A candidate IR receptor was identified based on its morphological resemblance with IR receptors reported in the pyrophilous beetles *Melanophila acuminata* (Evans 1966; Schmitz and Vondran et al. 1995; Bleckmann 1997) and *Acanthocnemus nigricans* (Schmitz et al. 2002).

3.3.3. Electrophysiology

The candidate IR receptor of males, mated females, and virgin females (each n = 3) was subjected to various stimuli in electrophysiological recordings. After descaling moths by air forced through a pipette tip, they were mounted dorsal side up with wings glued onto a glass slide using plasticine and TangleFoot adhesive (Contec Enterprises Inc., Victoria, BC, Canada). Preperations were placed on a brass platform inside a Faraday cage to reduce electrical noise (Cowan and Gries 2009). An electrically sharpened (Cools et al. 1970) tungsten recording electrode (0.2 mm diam; A-M Systems Inc., Carlsborg, WA, USA) was inserted by a Leitz micromanipulator (Leica Inc., Vienna, Austria) into the center of the candidate IR receptor while an indifferent electrode was inserted into tissue adjacent to the IR receptor. Electrical responses from the IR receptor were pre-amplified using Syntech Auto Spike (Syntech Inc., Hilversum, The Netherlands), processed with an IDAC signal interface box (Syntech), and analyzed in EAG Ver. 2.4 software (Syntech).

Radiant IR emitted from a metal rod heated to 40 °C (R. Holland, Science Technical Centre, Simon Fraser University, Burnaby, BC, Canada) was separated from conductive or convective heat with a gold-coated, first-face BK7 mirror (10.2 × 10.2 cm) (Tempotec Optics Co. Ltd., Fuzhou, Fujian, China). The mirror reflected ~ 96 % of wavelengths between 700 – 20,000 nm. The mirror was equidistant (30 cm) from the insect and the heat source, and positioned to reflect IR at a 90 ° angle onto the putative IR receptor (Fig 3.1a). A programmable, custom-built electronic camera shutter (R. Holland, Science Technical Centre, Simon Fraser University, Burnaby, BC, Canada), positioned between the mirror and insect, continuously intercepted the IR beam, except for intermittent 500 ms intervals every 10 s during which putative IR receptors were

exposed to radiant IR. For each moth, the IR source was heated to 40 °C and the moth was subjected to 5-15 cycles of 500-ms exposure of the stimulus. Physiological controls were carried out by leaving the electrodes in place and removing test stimuli, or by inserting the recording electrode into tissue adjacent to the IR receptor, and then subjecting the receptor to IR test stimuli as described above. Receptor potentials following exposure to test stimuli were considered responses when the response amplitude exceeded the the background noise three fold.

3.3.4. Thermographs

Temperatures of conifer cones and other components of the seed orchard environment were measured with IR and visible light photography. Mid-range IR (3-5 µm), and long-range IR (8-20 µm) were taken with an AGEMA Thermovision 550 camera (FLIR Systems, Burlington, ON, Canada), and a Fluke TI-20 thermal image camera (Fluke, Everett, WA, USA). The recording distance was 90-150 cm from the cones, and emissivity (the ratio of the radiation emitted by a surface to the radiation emitted by a black body at the same temperature) was set to 0.95 [the approximate value of the emissivity of vegetated areas (Jiang et al. 2006)]. Aluminum foil was used to measure absolute reflectance. Relative humidity and ambient temperature were recorded from Environment Canada Weather (Vernon Auto weather station). These measurements are required for ThermaCam Reporter 2000 software. Cone images were analyzed in ThermaCam Reporter 2000 Pro (FLIR Systems) for mean and hot spot temperatures (the spot on the cone with the highest temperature). If several cones were visible in an image, their mean temperature was calculated. Foliage temperatures were measured at three points around cones and averaged.

Temperatures of *in-situ* Douglas-fir cones on the east side of trees and spruce cones were measured hourly or every second hour in mid-range thermographs (see above). Thermographic images of Douglas-fir cones were taken at the Sechelt Seed Orchard (Canadian Forestry Products, Vancouver, BC, Canada; Sechelt, BC, Canada, 123°43′W, 49°27′N) on 06 August 2009. Thermographs of spruce cones were taken at the Kalamalka Seed Orchard (British Columbia Ministry of Forests and Range, Vernon, BC, Canada, 119°16′W, 50°14′N) on 08 July 2010. Foliage temperature was determined from three random spots (using a grid and random number generator) of foliage and averaged. Temperature differences between cone and foliage were determined by subtracting the temperature of the entire cone from the mean foliage temperature.

On 05 July 2010, between 00:30 and 01:30 h at the Kalamalka Seed Orchard, 10 thermographs were taken of Douglas-fir cones, foliage, branches, trunks, ground and sky. A one-way ANOVA (with Tukey's post hoc test) (Zar 1999) was used to compare the mean temperatures of tree parts and backgrounds in JMP (SAS®, Cary, NC, USA).

3.3.5. Behavioural responses of moths in laboratory and field experiments 1-8

Two-choice laboratory experiments 1-3 tested the response of > 4-day old males (Exp. 1), virgin females (Exp. 2) and mated females (Exp. 3) to a stimulus complex of conducive and radiant heat. The moths were bioassayed during their natural nocturnal flight period (see Chapter 4), within the 2nd to 6th h of the scotophase.

In each replicate, a moth was placed through a central hole (~ 2 cm) into a 60-cm long disposable bioassay device made from two 1-litre milk cartons (Fig. 3.1b). The inner distal 5-cm sections were coated with adhesive Tanglefoot to capture bioassay insects. Hot or cold test stimuli were randomly assigned (with a coin flip) to the triangular opening at either end of the bioassay device. They consisted of a 1000-mL Pyrex flask filled with heated (100 ± 2 °C) or cooled (2 ± 2 °C) water, which was maintained at these temperatures throughout each replicate. Insects that were captured on the adhesive within 1 h were considered responders. Each experiment was terminated when 20 responders had been obtained. Bioassay devices were discarded after each replicate. The number of responders to each stimulus was compared using a Fisher's exact two-sided test (Zar 1999) using JMP software.

Two-choice laboratory experiments 4-8 tested attraction of males (Exp. 4), virgin females (Exp. 5), mated females (Exp. 6), and of males and mated females with occluded IR receptors (Exps. 7-8) to radiant IR. IR receptors were occluded by applying an IR-opaque suspension of silica gel and acrylic paint (1.33 g and 59 ml; Craft Smart White, Plaid Enterprises Inc., Norcross, GA 30091-7600, USA) (Takács et al. 2009) on the receptor. All experiments used a cooled chamber designed to eliminate external thermal cues (Fig. 3.1b) (Takács et al. 2009). The chamber consisted of a glass aquarium (outside dimensions: 50.5 × 26.7 × 33.0 cm) covered with a glass lid (50.5 × 26.7 cm) and nested on 5-cm tall rubber stoppers inside a larger aquarium (outside dimensions: 61.0 × 33.0 × 41.0 cm), with cooled water (16 ± 2 °C) between them. A black PVC tube (7 cm inside diameter; IPEX, Toronto, ON, Canada) was sealed with aquarium grade black silica (All Glass Aquarium, Franklin, WI, USA) between the two

aquaria in each of the two end sections to exclude water, allowing external radiant IR to enter the inner aquarium.

High- and low-frequency radiant IR was generated from Pyrex glass flasks (1000 ml) containing heated (30 ± 2 °C) or ice-cooled (2 ± 2 °C) water (Fig. 3.1c). Flasks were randomly assigned (with a coin flip) to either end section of the chamber in each replicate, and placed 60 cm diagonal from the tube openings. Mirrors (see above) reflected a beam of radiant IR (but not conductive or convective or conducive heat) from the IR sources at 90 ° angles into the inner aquarium. To ensure that the moth inside the glass T-tube (see below) within the inner aquarium could perceive the radiant IR, a horizontal laser was used to properly position the mirrors. The Agema thermographic camera (see above) confirmed the temperature of 30 ± 2 °C or 2 ± 2 °C that was reflected by the mirrors.

Moths were contained for 30-60 min to a glass holding tube (2.5 × 6 cm) to acclimate to experimental conditions. The tube was then opened and inserted into the stem of a glass T-tube (for dimensions see Fig. 3.1c), which had been fitted with strips of paper towel to provide traction for the walking insects. The T-tube was then mounted on a stub in the center of the inner aquarium and held in place with black plasticine. Moths that approached within 2.5 cm of the distal end of one of the side arms within 60 min were considered responders. Each experiment was terminated when 20 responders had been obtained. After each replicate, the T-tube was baked overnight at 120 °C and the inside of the inner aquarium and the lid was wiped with 70 % ethanol to remove any chemical residues (Cowan & Gries 2009). The number of responders to each stimulus was compared using a Fisher's exact two-sided test (Zar 1999) using JMP software.

3.3.6. Field experiments 9-10

Field experiments 9-10 were designed to test the responses of moths to paired traps (Fig. 3.1d) emitting high- and low-frequency IR, respectively. Experiments 9 and 10 were run between 19-21 August 2009, and between 24 June to 31 July 2010, respectively, at the Kalamalka Forestry Centre. Traps (5 cm inside diameter × 13 cm) were made of a PVC pipe (IPEX, Toronto, ON, Canada), capped on one end with a 5-cm PVC lid (NIBCO Elkhart, IN, USA), covered with Painter's Mate tape (Shurtape Technologies, Hickory, NC, USA) and spray painted black using Rust-oleum Painter's Touch Black spray paint (RPM International Inc., Medina, OH, USA). The painter's tape provided a surface to which the spray paint adhered. To emit high- or low-frequency radiant IR, traps were filled with boiling water that was replaced every 20-30 min, or filled with ice water, 75 mL of which was withdrawn by syringe and replaced by ice cubes every 120-180 min. Trap temperatures were monitored with the AGEMA thermographic camera (above). Two traps were suspended from a rope (diameter ~ 0.5 cm) strung between each of 21 pairs of spruce trees bearing at least 50 cones per tree. Members of spruce tree pairs were no more than 25 m apart from each other. Traps were hung 30-40 cm apart from one another and 240-270 cm above ground, with high- or low-frequency radiant IR randomly assigned (with a coin flip) to each position. During experiment 9, but not 10, the outer surface of traps was thinly covered with adhesive Tanglefoot. In experiment 10, moths which had entered traps (and were floating in the water) were collected throughout the night (20:00 to 6:00 h) at 30-min intervals, and preserved in 70 % ethanol. Moths were identified to species group (*D. abietivorella* or *D. schuetzeella* group) and sex based on descriptions by Sopow et al. (1996). Females were dissected to check for the presence of a spermataphore in the bursa copulatrix to

determine mating status. In experiments 9-10, the total numbers of moths per trap were tallied and compared between test stimuli by a two-tailed Wilcoxon signed-rank test (Zar 1999) using JMP software.

3.4. Results

3.4.1. Environmental scanning electron microscopy

ESEMs revealed a candidate site for an IR receptor on the ventral prosternum anterior to the coxa of each foreleg of both male and female moths (Fig. 3.2).

3.4.2. Electrophysiology

In electrophysiological recordings, candidate IR receptors of male, virgin female, and mated female *D. abietivorella* responded to radiant IR from a metal rod heated to 40 °C (Fig. 3.3). They did not respond in control recordings when the shutter of the stimulus delivery system opened but radiant IR was absent, or when radiant IR was present but the recording electrode was inserted adjacent to, instead of into, the IR receptor.

3.4.3. Thermographs

Cones of Douglas-fir and spruce were warmer than foliage during the day but were slightly cooler than foliage during the night (Fig. 3.4). Between 00:30 to 01:30 h, the peak flight period of *D. abietivorella* (see below), there were significant differences in apparent temperature between some parts of Douglas-fir trees [one-way ANOVA; $F_{(4,35)}$

= 76.5; p < 0.0001]. Trunks were significantly warmer than foliage (Tukey's test; p < 0.0001) and the ground (Tukey's test; p < 0.0001) (Fig. 3.4). Foliage was significantly warmer than the sky (Tukey's test; p < 0.0001) but not than cones (Tukey's test; p = 0.32) (Fig. 3.4).

3.4.4. Behavioural responses of moths in laboratory and field experiments 1-10

In laboratory experiments 1-3, males (Exp. 1), virgin females (Exp. 2) and mated females (Exp. 3) did not show a preference for either hot or cold temperatures (Fisher's exact two-sided test; Exps. 1, 2: p = 0.75 each; Exp. 3: p = 0.19) (Fig 4). In experiments 4-6, males (Exp. 4) and mated females (Exp. 6), but not virgin females (Exp. 5), preferred high-frequency over low-frequency radiant IR (Fisher's exact two-sided test; Exp. 4: p = 0.04, Exp. 5: p = 1.00, Exp. 6: p = 0.0033) (Fig 3.5). When males (Exp. 7) and mated females (Exp. 8) were tested with their IR receptors occluded, neither showed a preference for high- or low-frequency radiant IR (Fisher's exact two-sided test; Exp. 7: p = 0.75, Exp. 8: p = 0.53) (Fig 3.5).

In field experiment 9 (2009), males and mated females of the *D. schuetzeella* group were more strongly attracted to traps with high-frequency radiant IR than to traps with low-frequency radiant IR (two-tailed Wilcoxon signed-rank test; males: Z = 2.59; p = 0.0095; females: Z = 4.83; p < 0.0001) (Fig. 3.6). Similarly, in field experiment 10 (2010), mated female *D. abietivorella* were more strongly attracted to traps with high-frequency radiant IR than to traps with low-frequency radiant IR (two-tailed Wilcoxon signed-rank test; Z = 3.37; p = 0.0007) (Fig 3.6). Male *D. abietivorella* were captured in insufficient numbers to analyze data statistically.

Peak flight of male and female *D. abietivorella* recorded in experiment 10, and in experiments 10 and 18-20 reported in Chapters 4 and 7, occurred between 23:00 and 02:00 h and between 22:00 and 02:00 h, respectively (Fig 3.7).

3.5. Discussion

We present morphological, electrophysiological and behavioural evidence for the presence of an IR receptor in *D. abietivorella*. The IR receptor found in SEMs has a surface texture which differs from that of the surrounding integument, and which resembles that of IR receptors in (*i*) the pyrophilic beetles *M. acuminata* and *A. canthocnemus nigricans* (Evans 1966; Vondran et al. 1995; Schmitz and Bleckmann 1997; Schmitz et al. 2002), (*ii*) *L. occidentalis* seed bugs (Takács et al. 2009), and (*iii*) *Agkistrondon contortix* copperhead snakes (Moiseenkóvà *et al.* 2002).

Our data do not support the hypothesis that the IR signatures of conifer cones and needles contrast at night, but do support the hypothesis that male and female *D. abietivorella* possess IR receptors and may respond to radiant IR as a foraging cue. This radiant IR, however, appears to emanate from tree parts other than cones. At night cones are colder than foliage (Fig 3.4a). Using IR to discern objects in the environment requires a thermal contrast (or IR signature), the extent of which depends upon the physical substrate properties and size of the object, and the time of day (Thánh et al. 2009). The diurnally active *L. occidentalis* uses the thermal contrast between needles and cones to locate cones (Takács et al. 2009). During the nocturnal foraging flight of *D. abietivorella* (22:00 and 02:00 h), the thermal contrast between cones and foliage is less than 3 ^{o}C (Fig. 3.4), and cones are cooler than foliage, rendering cone-derived radiant IR

an unlikely foraging cue, particularly in light of findings that female *D. abietivorella* are attracted to radiant IR from warm sources (Fig. 3.4). Cones of Douglas-fir and spruce may not be sufficiently large to store solar heat throughout the day and to retain it at night. However, tree trunks and foliage of conifer trees contrast well in thermographs against the ground and sky, and their radiant IR may provide reliable long-range foraging cues for *D. abietivorella.* Indeed, gravid female *D. abietivorella* often oviposit on twigs (Shu et al. 1996) and needles (Whitehouse et al 2011) of trees and may be guided by their IR cues.

In electrophysiological recordings, IR receptors of male, virgin female and mate female *D. abietivorella* responded to radiant IR from 40 °C heat sources (Fig. 3.3) which predominantly emit IR wavelengths of ~ 10 µm (Schmitz and Bleckmann 1997).

Behavioural attraction of *D. abietivorella* to high-frequency IR became apparent in the laboratory and field. The behavioural response to IR cues disappeared when IR receptors were occluded suggesting that radiant IR rather than convective or conducive heat are used in host-finding. Attraction of males, and of mated females of *D. schuetzeella* group which do not oviposit on cones, to high-frequency IR cues indicates that these moths may exploit radiant IR to forage for host resources other than cones. Radiant IR as a foraging cue may thus be widespread among nocturnal moths. The relatively high number of non-responding insects in the laboaratory could be due to the fact that foraging insects typically respond to a cue complex and that radiant IR as a single cue was less effective in elicting a response. Alternatively, the temperature in the cooled bioassay device was suboptimal for foraging.

High-frequency IR cues elicited electrophysiological responses from male, virgin and mated female *D. abietivorella*, but behavioural responses only from mated females and males. Mating may trigger a behavioural switch to a responsive state. Virgin, pheromone-emitting females are sedentary (Whitehouse et al. 2011), and have no need to find oviposition sites. Following mating, gravid females forage for suitable oviposition sites, which may be facilitated by the activation of IR responsiveness.In conclusion, there is evidence now that both diurnal (Takács et al. 2009) and nocturnal (this study) members of the conophagous insect guild respond to high-frequency IR cues, and that different parts of trees appear to provide these cues at day and night.

3.6. References

de Groot P, Turgeon JJ, Miller GE (1994) Status of cone and seed insect pest management in Canadian seed orchards. For Chron 70:745-761.

Cowan T, Gries G (2009) Ultraviolet and violet light: attractive orientation cues for the Indian meal moth, *Plodia interpunctella*. Entomol Exp Appl 131: 148–158.

Evans W (1966) Morphology of the infrared sense organs of *Melanophila acuminatae* (Buprestidae: Coleoptera). Ann Entomol Soc Am 59:873-877.

Grace MS, Woodward OM, Church DR, Calisch G (2001) Prey targeting by the infrared-imaging snake *Python*: effects of experimental and congenial visual deprivation. Behav Brain Res 119:23-31.

Grant GG, Millar JG, Trudel R (2009) Pheromone identification of *Dioryctria abietivorella* (Lepidoptera: Pyralidae) from an eastern North American population: geographic variation in pheromone responses. Can Entomol 141:129-135.

Jactel J, Menassieu P, Raise G (1994) Infestation dynamics of *Dioryctria sylvestrella* (Ratz.) (Lepidoptera: Pyralidae) in pruned maritime pine (*Pinus pinaster* Act.) For Ecol Manage 67:11-22.

Jiang G, Li Z, Nerry F. (2006) Land surface emissivity retrieval from combined mid-infrared and thermal infrared data of MSG-SEVIRI. Remote Sens Environ, 105: 326–355.

Kürten L, Schmidt U (1982) Thermoperception in the common vampire bat (*Desmondus rotundus*). J Comp Physiol 146:223-228.

McEntire B (1996) Pheromone-based trapping and life history of the fir coneworm, *Dioryctria* abietivorella (Lepidoptera: Pyralidae). M.P.M. Thesis. Simon Fraser University.

Moiseenkóvà V, Bell B, Motamedi M, Wozniak E, Christensen B (2003) Wide-band spectral tuning of heat receptors in the pit organ of the copperhead snake (*Crotalinae*). Am J Physiol Regul Integr Comp Physiol 284:598-606.

Schmitz H, Bleckmann H (1997) Fine structure and physiology of the infrared receptor of beetles of the genus *Melanophila* (Coleoptera: Buprestidae). Int J Insect Morphol Embryol 26:205-215.

Schmitz H, Bleckmann H (1998) The photomechanic infrared receptor for the detection of forest fires in the beetle *Melanophila acuminata* (Coleoptera: Buprestidae). J Comp Physiol A 182:647-657.

Schmitz H, Schmitz A, Bleckmann H (2000) A new type of infrared organ in the Australian “fire-beetle” *Merimna atrata* (Coleoptera: Buprestidae). Naturwissenschaften 87:542-545.

Schmitz H, Trenner S, Hofmann MH, Bleckmann H (2000) The ability of *Rodnius prolixus* (Hemiptera: Reduviidae) to approach a thermal source solely by its infrared radiation. J. Insect Physiol 46:745-751.

Schmitz H, Schmitz A, Trenner S, Bleckmann H (2002) A new type of insect infrared organ of low thermal mass. Naturwissenschaften 89:226-229.

Schmitz H, Trenner S (2003) Electrophysiological characterization of the multipolar thermoreceptors in the “fire-beetle” *Merimna atrata* and comparison with the infrared sensilla of *Melanophia acuminata* (both Coleoptera, Buprestidae). J Comp Physiol A 189:715-722.

Schmitz A, Gebhardt M, Schmitz H (2008) Microfluidic photomechanic infrared receptors in a pyrophilous flat bug. Naturwissenschaften 95:455-460.

Shu S, Grant GG, Lagevin D, Lombardo DA, MacDonald L (1997) Oviposition and electroantennogram responses of *Dioryctria abietivorella* (Lepidoptera: Pyralidae) elicited by monoterpenes and enantiomers from eastern white pine. J Chem Ecol 23:35-45.

Sopow SL, Bennett RG, Landry J, Landry B (1996) Identification of the “grey” *Dioryctria* species of British Columbia (Lepidoptera, Pyralidae). J Entomol Soc Brit Columbia 93:75-92.

Strong WB, Millar JG, Grant GG, Moreira JA, Chong HM, Rudolph C (2007) Optimization of pheromone lure and trap design for monitoring fir coneworm, Dioryctria abietivorella. Entomol Exp Appl 126:67-77.

Takács S, Bottomley H, Andreller I, Zahradnik T, Schwarz J, Bennett R, Strong W, Gries G (2009) Infrared radiation from hot cones on cool conifers attracts seed-feeding insects. Proc R Soc B 276:649-655.

Thánh NT, Háo DN, Sahli H (2009) Infrared thermography for land mine detection. In: Hammond RI (ed) Augmented vision perception in infrared: Algorithms and applied systems, advanced pattern recognition. Springer London, London, pp 3-36.

Thien LB, Azuma H, Kawano S (2000). New perspectives on the pollination biology of basal angiosperms. Int. J. Plant Sci, 161: S225-S235.

Trudel R, Bauce É, Cabana J, Guertin C (1995) Rearing technique for *Dioryctria abietivorella* (Lepidoptera: Pyralidae). J Econ Entomol 88:640-643.

Vondran T, Pel KH, Schmitz H (1995) The infrared receptor of *Melanophila acuminata* De Geer (Coleoptera: Buprestidae): ultrastructural study of a unique insect thermoreceptor and its possible descent from a hair mechanoreceptor. Tissue Cell 27:645-658.

3.7. Figure captions

Figure 3.1. Illustrations of the experimental design used in electrophysiologial recordings (a), laboratory experiments 1-3 (b) and 4-8 (c); and photographs and thermographs of paired (hot or cold) traps tested in field experiments 9 and 10 (d); see methods for details.

Figure 3.2. (a) Drawing depicting locations of IR receptors on the ventral prothorax of a female *Dioryctria abietivorella;* (b-c) environmental scanning electron micrographs of the IR receptor (encircled).

Figure 3.3. Representative recordings of electrophysiological responses from an infrared (IR) receptor (see Fig. 3.2) of a female *Dioryctria abietivorella* (a) exposed for 500ms every 10 s to a radiant IR beam from a heated (40 °C) metal rod, and (b) when the recording electrode had been inserted adjacent to the receptor.

Figure 3.4. (a) Temperature differentials recorded in mid-range (3-5 µm wavelength) thermographs of *in situ* cones and foliage of Douglas-fir and spruce trees, calculated by subtracting the mean cone temperature from the mean foliage temperature; (b) mean (+ SE) temperature of trunks, cones and foliage of Douglas-fir trees, as well as ground and sky, obtained between 00:30 and 01:30 h on 05 July 2010. Data for respectively Douglas-fir and spruce trees were obtained every hour for 24 h on 06 August 2009 and 06 July 2010.

Figure 3.5. Numbers of male, virgin female, and mated female *Dioryctria abietivorella* responding in two-choice laboratory experiments 1-8 to test stimuli consisting of (a) heat and radiant IR in combination (Exps. 1-3), and (b) radiant IR emitted from water flasks kept at 30 °C (high-frequency IR) or 2 °C (low-frequency IR) (Exps. 4-8). In experiments 7-8, IR receptors of bioassayed moths were occluded. In each experiment, bars with an asterisk (*) indicate a significant preference for the test stimulus (Fisher's exact two-sided test; $p < 0.05$). nr = non-responding insects.

Figure 3.6. Mean (+ SE) number of males and mated females of *Dioryctria schuetzeella* group and *Dioryctria abietivorell*a captured in field experiments 9 (19-21 August 2009) and 10 (24 June to 31 July 2010), in traps emitting high- and low-frequency radiant IR. Within pairs of bars, an asterisk (*) indicates a significant difference (two-tailed Wilcoxon signed rank test, $p < 0.05$, n=21). Note: No virgin females of either species were captured.

Figure 3.7. Percent of total male (22) and female (41) *Dioryctria abietivorella* captured in traps during each hour of the scotophase in experiment 10 (see caption of Fig. 3.5) (n = 21), and in Experiments 10 and 18-20 (n = 6 total, June to August 2010) reported in Chapters 4 and 7.

Figure 3.1

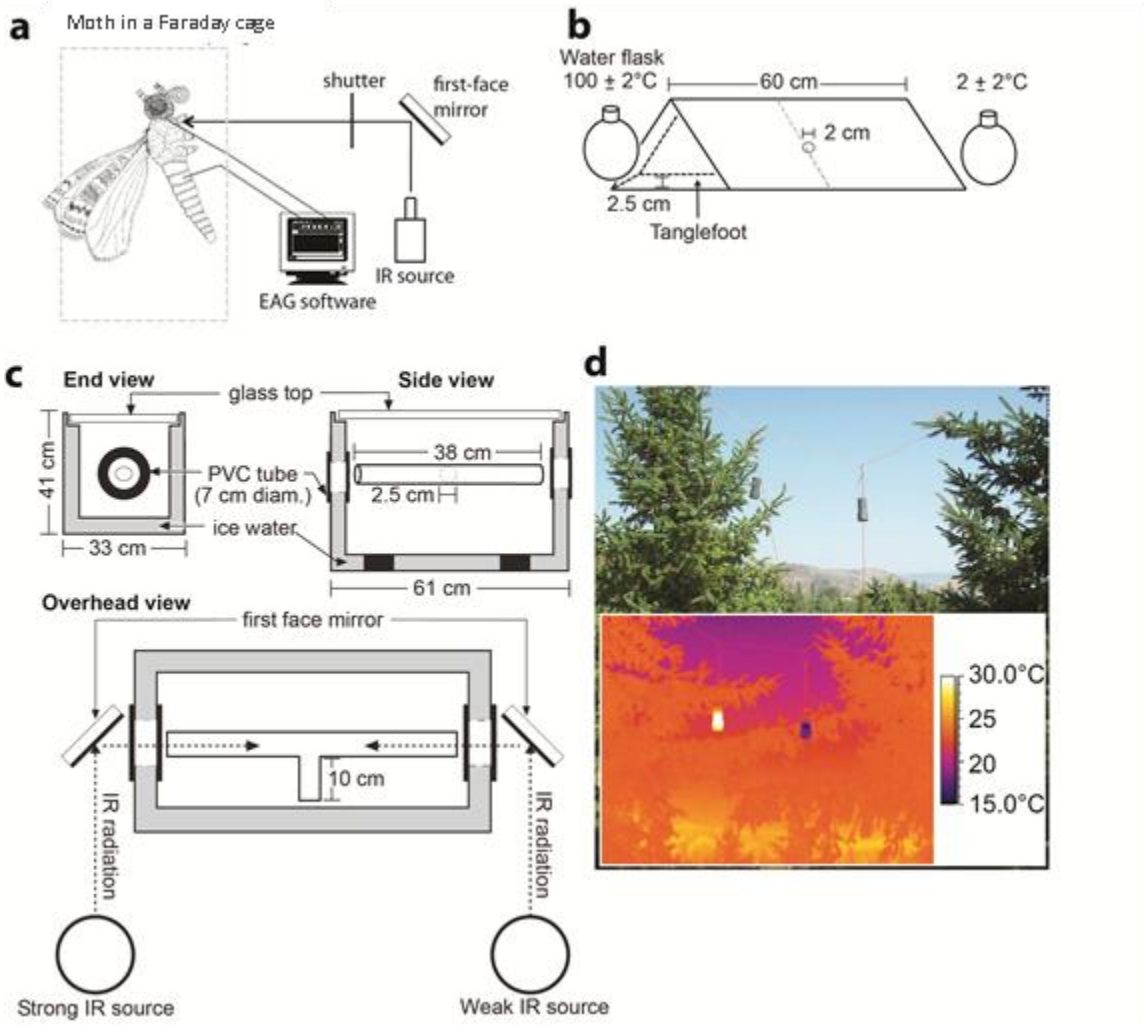

a
Moth in a Faraday cage
shutter
first-face mirror
IR source
EAG software
b
Water flask
100 ± 2°C
60 cm
2 ± 2°C
2 cm
2.5 cm
Tanglefoot
c
End view
glass top
Side view
41 cm
PVC tube
(7 cm diam.)
38 cm
2.5 cm
ice water
33 cm
61 cm
Overhead view
first face mirror
10 cm
IR radiation
IR radiation
Strong IR source
Weak IR source
d
30.0°C
25
20
15.0°C

Figure 3.2

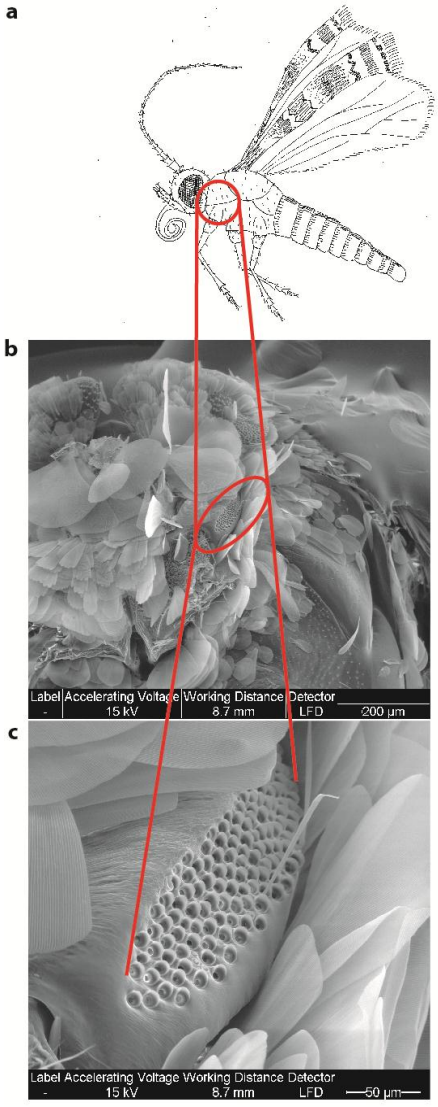

a
b
Label Accelerating Voltage Working Distance Detector
- 15 kV 8.7 mm LFD 200 µm
c
Label Accelerating Voltage Working Distance Detector
- 15 kV 8.7 mm LFD 50 µm

Figure 3.3

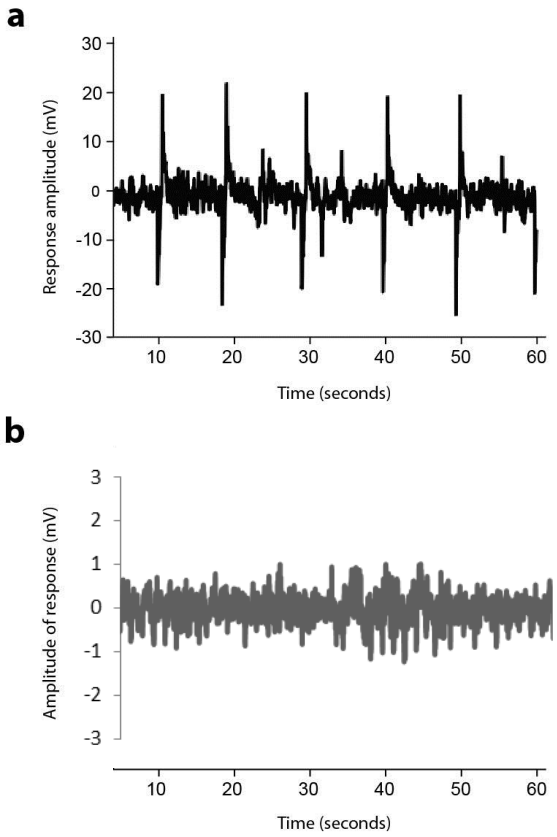

a
Response amplitude (mV)
30
20
10
0
-10
-20
-30
10
20
30
40
50
60
Time (seconds)
b
Amplitude of response (mV)
3
2
1
0
-1
-2
-3
10
20
30
40
50
60
Time (seconds)

Figure 3.4

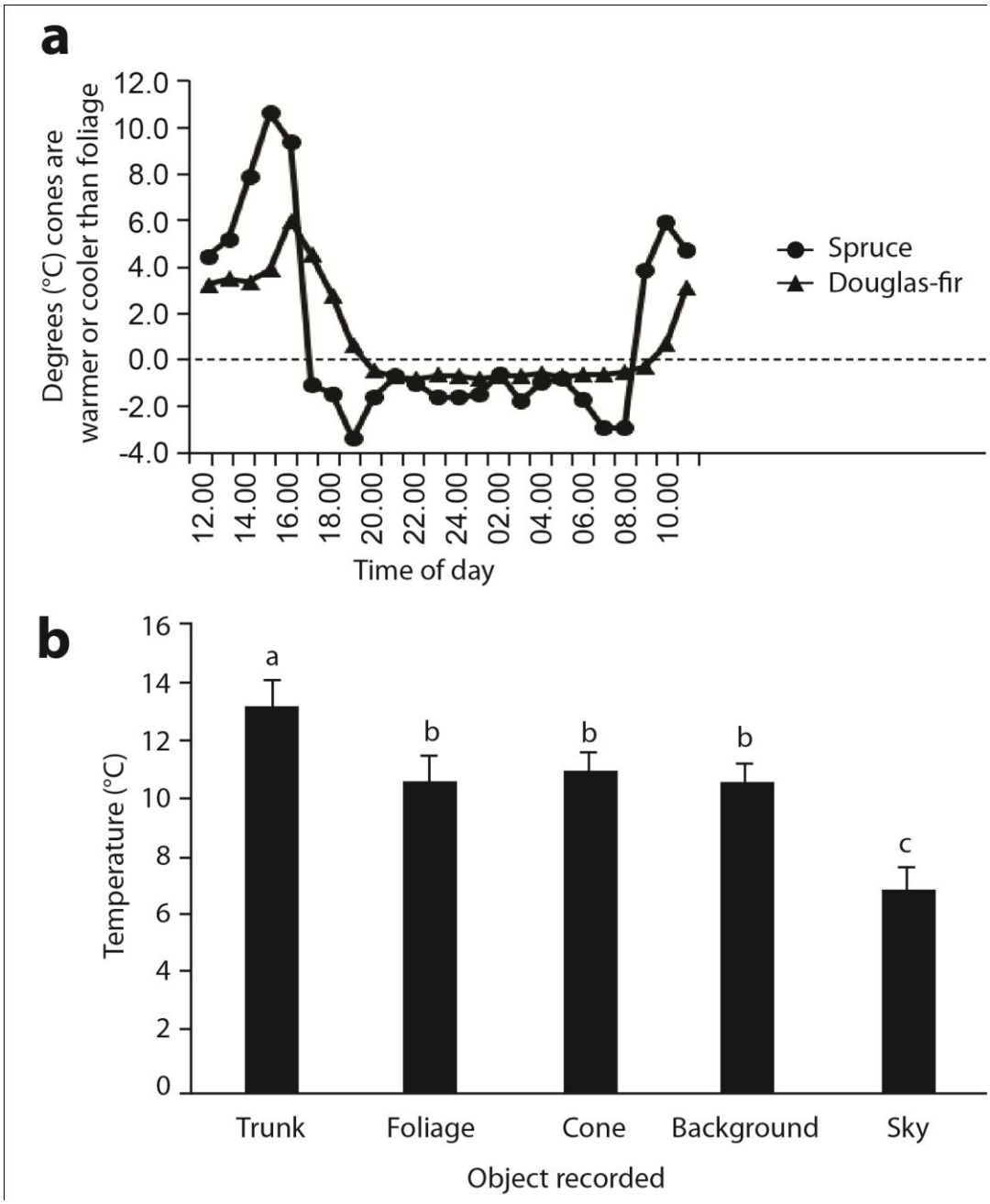

a
Degrees (°C) cones are warmer or cooler than foliage
12.0
10.0
8.0
6.0
4.0
2.0
0.0
-2.0
-4.0
12.00
14.00
16.00
18.00
20.00
22.00
24.00
02.00
04.00
06.00
08.00
10.00
Time of day
Spruce
Douglas-fir
b
Temperature (°C)
16
14
12
10
8
6
4
2
0
a
b
b
b
c
Trunk
Foliage
Cone
Background
Sky
Object recorded

Figure 3.5

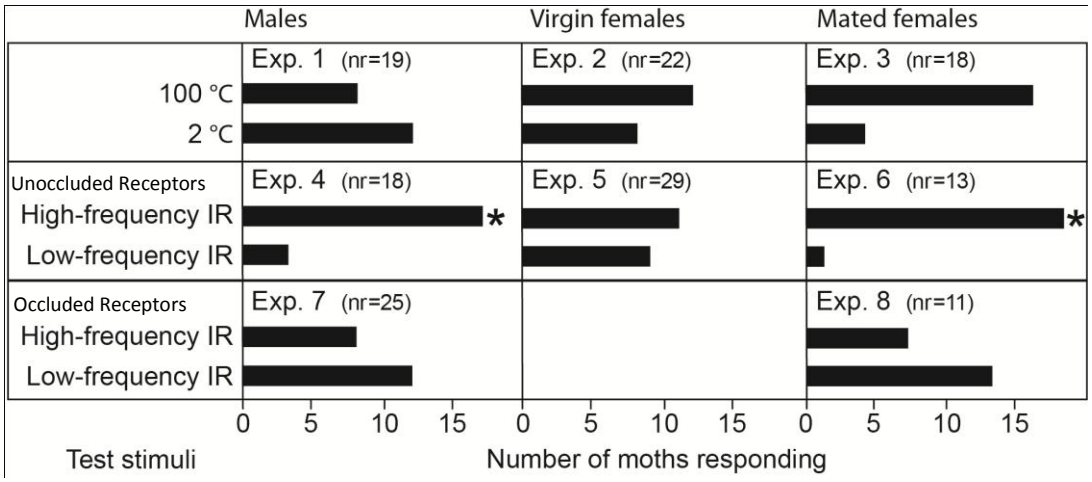

Males
Virgin females
Mated females
Exp. 1 (nr=19)
Exp. 2 (nr=22)
Exp. 3 (nr=18)
100 °C
2 °C
Unoccluded Receptors
Exp. 4 (nr=18)
Exp. 5 (nr=29)
Exp. 6 (nr=13)
High-frequency IR
Low-frequency IR
*
*
Occluded Receptors
Exp. 7 (nr=25)
Exp. 8 (nr=11)
High-frequency IR
Low-frequency IR
0 5 10 15 0 5 10 15 0 5 10 15
Test stimuli
Number of moths responding

Figure 3.6

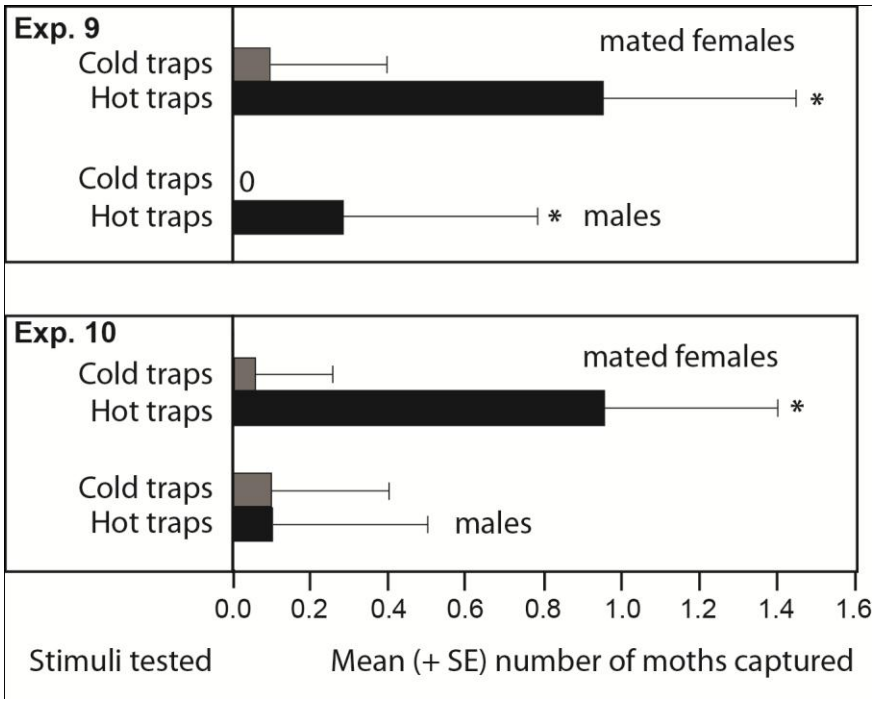
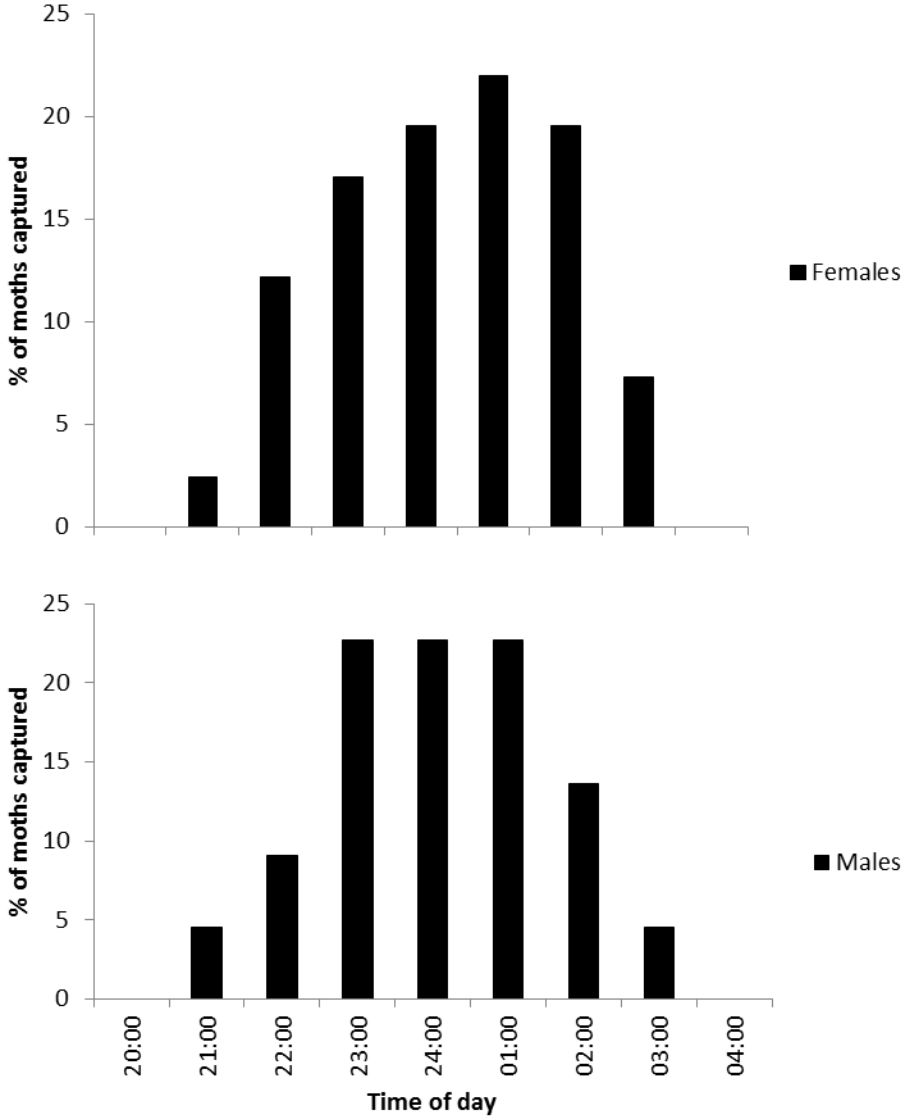

Exp. 9
mated females
Cold traps
Hot traps
*
Cold traps
0
Hot traps
* males
Exp. 10
mated females
Cold traps
Hot traps
*
Cold traps
Hot traps
males
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
Stimuli tested
Mean (+ SE) number of moths captured

Figure 3.7


25
20
15
10
5
0
% of moths captured
Females
25
20
15
10
5
0
% of moths captured
Males
20:00
21:00
22:00
23:00
24:00
01:00
02:00
03:00
04:00
Time of day

4. Spectral sensitivity and attraction of Douglas-fir cone worm moth, *Dioryctria abietivorella,* to specific wavelengths of UV and visible light[3]

4.1. Abstract

Trichromatic [ultraviolet (UV), blue, green] vision is common within the class Insecta. In electroretinograms, with light-emitting diodes (LEDs) and monochromatic light as test stimuli, we explored spectral sensitivities of the eyes of Douglas-fir cone worm, *Dioryctria abietivorella.* Eyes showed peak spectral sensitivity in the green to -yellow region and possible spectral sensitivity in the UV light range, deviating from the trichromatic sensitivity. There was no evidence for regional specialization of eyes. In two-choice laboratory experiments, we tested attraction of *D. abietivorella* to

[3]Authors: Tracy Zahradnik, Allison Gamble, Keiko Nabetani, Ward Strong, Gerhard Gries. This Chapter has been formatted using the requirements of Environmental Entomology.

wavelengths within the insects' green to green-yellow peak spectral sensitivity range and to blue wavelengths outside that range. In the field, we tested the ability of *D. abietivorella* to discriminate between similar wavelengths of UV (350 nm or 373 nm) light. When both light stimuli were in the green (505 nm) or green-yellow (573 nm) range, they were equally effective in attracting moths. When light stimuli were either in the green (505 nm) or blue ([433 nm) range, the latter was significantly more effective in attracting moths. *Dioryctria abietivorella* showed no preference for either UV wavelength in the field. Thus, spectral sensitivity of eyes to specific wavelengths did not correlate with wavelength attractiveness.

Keywords: wavelength-mediated behaviour; trichromatic vision**;** regional specialization of eyes; electroretinograms

4.2. Introduction

Nocturnal moths can use visual colour cues even at light intensities as low as starlight, which is not sufficient for human colour vision (Schlecht 1978; Kelber *et al.* 2003). Trichromatic maximum spectral sensitivities to ultraviolet (UV), blue and green wavelengths are the most commonly reported in insect vision (Kevan and Backhaus 1998, Briscoe and Chittka 2001).

Methods for determining spectral sensitivity in insects include intracellular optical recordings, electroretinograms (ERGs) and microspectrophotometry (Briscoe and Chittka 2001). The experimental methods has bearing on the results, as exemplified in experiments with the green peach aphid, *Myzus periscae*, which is trichromatic. Simple electroretinograms with dark-adapted aphid eyes revealed only monochromatic vision with a peak sensitivity in the green (530 nm) range (Kirchner *et al.* 2005). For example, spectral sensitivities in the UV (300-340 nm) range were revealed only when the eye was white-adapted, and a third spectral sensitivity at 470 nm was revealed when eyes were yellow-adapted, causing weakening of response to wavelengths of light at 530 nm (Kirchner *et al.* 2005). Alternatively, this may be explained by the Bezold-Brücke effect where the perception of the hue of a wavelength is altered due to an increase or decrease in stimulus intensity (Backhaus 1992).

In some insects, spectral sensitivities can be linked to important resources in their environment (Wallace 1878, Kevan and Backhaus 1998). For example, species-

specific spectral arrangements of visual pigments in the eyes of sympatric species of *Lycanea* butterflies are thought to help in mate distinction (Bernard and Remington 1991). In the swallowtail butterfly *Papilio aegeus* there is an indirect link between spectral sensitivities and visual characteristics of plant leaves, with receptors being spectrally tuned to see red, aiding discrimination between foliage with different types of green leaves (Kelber 1999). The behavioural responses of insects and the peak of spectral sensitivities of their photoreceptors do not overlap, as shown in the butterfly *Pieris rapae* (Scherer and Kolb 1987, Shimohigashi and Tominaga 1991, Skorupski and Chittka 2011). It indicates that the wavelength-dependent behaviour of feeding, egg-laying and open space location exhibited by *P. rapae* cannot be accounted for by a single photoreceptor (Skorupski and Chittka 2011). Instead, wavelength discrimination may be explained by the extent to which the photoreceptive range of two distinct yet overlapping photoreceptors are being excited by photons of light within the overlapping range (Skorupski and Chittka 2011). A single photoreceptor is colour blind (Skorupski and Chittka 2011). The extent to which two different wavelengths of the same intensity can be distingued is the wavelength discrimination function (Goldsmith et al. 1981).

The Douglas-fir cone worm, *Dioryctria abietivorella* Groté, is an obligate conophagous nocturnal moth. During the summer, females oviposit on cones and needles (Whitehouse *et al.* 2011) or twigs (Shu *et al*. 1996) of conifer trees. Larvae bore through cones, consuming cone contents and seeds (Grant *et al*. 2009). As such, they are important pests of conifer seed orchards in Canada (Hedlin *et al.* 1980). Understanding the spectral sensitivities of *D. abietivorella* eyes might help in understanding host relationships or design of traps, leading to improved pest management in commercial seed orchards.

Herein we investigated the eyes of male, virgin and mated female *D. abietivorella* for spectral sensitivities. We tested attraction of moths in laboratory and field experiments to selected wavelengths in or outside of the spectrally most sensitive area of *D. abietivorella* vision.

4.3. Methods and materials

4.3.1. Experimental insects

All experimental insects were provided by Insect Production Services (Canadian Forest Service, Sault Ste-Marie, ON, Canada). They were kept at 25 °C, 60 % relative humidity and a photoperiod of 16:8 (L:D) (Trudel *et al.* 1995). Four day old males, virgin females and mated females were bioassayed. To obtain mated females, > 2 day old virgin females were retained with 2 day old males for > 2 days. After experiments, 30 mated females were dissected to check for the presence of a spermataphore in the bursa copulatrix as an indicator of successful mating. At least one spermataphore was found in 29 of the 30 females.

4.3.2. Electrophysiology

For electrophysiological recordings, moths were restrained laterally to glass slides using plasticine and adhesive (TangleFoot, Contec Enterprises, Victoria, BC, Canada). Legs and antennae were amputated from the insects. An electrically sharpened (Cools et al. 1970) bare tungsten wire electrode (0.2 mm cm diam., A-M Systems Inc., Carlsborg, WA, USA) was inserted with a micromanipulator (Leitz, Leica, Vienna, Austria) into the central region of the equatorial section of an eye facing

upwards. Another tungsten electrode was inserted into the lateral side of the second abdominal segment. Electrodes were connected to the measurement equipment described below (Fig. 4.1A). Eyes were dark-adapted by keeping insects in total darkness for 45 min before recordings. All recordings weremade in a Faraday cage completely covered in black cloth to exclude both electrical noise and any light other than that of test stimuli (see below). Electrical responses from the eye were pre-amplified (Syntech Auto Spike, Syntech, Hilversum, The Netherlands), processed (IDAC signal interface box, Syntech), and analyzed for amplitude with oscilloscope software (EAG, Version 2.4, Syntech).

4.3.3. Spectral sensitivity of eyes

To determine spectral sensitivities of the eyes of males and females we used two methods. The first method used 22 light-emitting diodes (LEDs) of the following peak wavelengths (nm): 350, 372, 385, 390, 400, 433, 452, 470, 505, 527, 567, 573, 592, 610, 628, 641, 654, 672, 688, 702, 730, and 752 (Roithner LaserTechnic GmbH, Vienna, Austria). LEDs were calibrated at a distance of 2.5 cm from the point source to an intensity of 5.0×10^{13} photons/cm^2/s, using a spectrometer with cosine corrector (HR4000, Ocean Optics, Dunedin, FL, USA) and SpectaSuite (Ocean Optics) software.

LEDs were shone into a 1000-µm single fiber optic cable (fused silica solarized UV resistant patch, Multimode Fiber Optics, Hackettstown, NJ, USA) fitted with a collimator assembly attached to a sub multi assembly (SMA) terminus (LC-4U-THD, Multimode Fiber Optics). A second cable was fitted with a collimator assembly at one terminus and an SMA-only terminus at the other, the latter held ca. 5 mm above the insertion site of the electrode in the insect's eye. Located between the two collimator

termini of these cables was a programmable shutter (R. Holland, Science Technical Centre, Simon Fraser University, Burnaby, BC, Canada) which continuously intercepted any light, except for intermittent 500-ms intervals every 9.5 s, during which the eye was exposed to the light stimulus. The insect's dark adapted eye can recover in < 2 s. Each of the 22 LEDs was tested in random order (assigned by a random number generator). During the 9.5 s intervals of total darkness, LEDs were switched, and eyes recovered in darkness from the previous stimulus. Eyes of 10 males, 10 virgin females and 10 mated females were tested.

The second method for determining spectral sensitivities used a 35-watt Xenon light (Mikropak GmbH, Ostfildern, Germany) and a fiber optic scanning monochrometer (Monoscan2000, Ocean Optics, Dunedin, FL, USA) to produce the test stimuli. They consisted of 31 10-nm bandwidths consecutively-tested at 10-nm increments from 345 nm to 655 nm. Technical set-up of the monochrometer required testing of consecutive wavelength bandwidths. A 600-µm optical fibre (premium-grade solarized-resistant assembly, Ocean Optics) attached to the monochrometer transmitted light to a 0-2 stop circular variable neutral density wheel [Fused Silica (200 nm to 2500 nm), Reynard, Calle Sombra, San Clemente, California, USA] directly in front of a 70:30 beam splitter ("polka dot" 4-7001, Optometrics, Ayer, MA, USA). Thirty percent of a light beam was transmitted to the spectrometer, and calibrated such that each increment had an intensity of 3.0×10^{12} photons/cm^2/s at a distance of 0.5 cm from the point source using a spectrometer with SpectraSuite software. The remaining light beam (70% or 7.0×10^{12} photons/cm^2/s) of the test stimulus was delivered to an eye as described above. For each wavelength tested, data were standardized using the ratio to the highest response per eye, and then averaged between eyes (Kirchner *et al.* 2005).

Standardized responses of the eye were compared for each wavelengnth with a one-way ANOVA and post-hoc Tukey-Kramer test blocked by insect (Zar 1999) using JMP software (SAS®, Cary, NC, USA).

4.3.4. Light attraction of moths in two-choice laboratory experiments 1-9

Two-choice experiments were designed to test attraction of moths to wavelengths within or outside of the green to green-yellow peak of spectral sensitivities. Experiments were run during the moths' natural nocturnal flight (Chapter 3), within the 2^{nd} to 6^{th} h of the scotophase. Because *D. abietivorella* responds to infrared (IR) radiation (see Chapter 3), we used a cooled chamber designed to eliminate external thermal cues (Takács *et al.* 2009; Fig. 4.1B). The chamber consisted of a glass aquarium (outside dimensions: 50.5 × 26.7 × 33.0 cm) covered with a glass lid (50.5 × 26.7 cm) and nested (on 5-cm tall rubber stoppers) inside a larger aquarium (outside dimensions: 61.0 × 33.0 × 41.0 cm), with cooled water (16 ± 2 °C) between them. A black PVC tube (7 cm i.d.; IPEX, Toronto, ON, Canada) was sealed with aquarium grade black silica (All Glass Aquarium, Franklin, WI, USA) between the two aquaria in each of the two end sections to exclude water, allowing light stimuli to enter the inner aquarium.

A single LED run by a custom-built LED driver (Pavel Kowalski, Science Technical Centre, SFU) was placed 5 cm from each of the two end sections of the outer aquarium behind a piece of glass which excluded LED-derived radiant IR (Fig. 4.1B). LEDs were calibrated to an intensity of 1.0×10^{15} photons/cm^2/s at a distance of 1 cm from the source, using a spectrometer with cosine corrector and SpectaSuite software.

For each replicate, a moth was acclimated for 30-60 min in darkness in a glass holding tube (2.5 × 7 cm) before the tube was inserted into the stem of a T-tube (Fig. 4.1B) which was then mounted on a stub inside the inner aquarium. Each tube was fitted with paper towel to provide traction for the walking moths during locomotion. Moths that approached within 2.5 cm of the distal end orifice of one of the T-tube's side arms within 2-60 min were considered responders. Each experiment was terminated when 10 responders had been obtained. Non-responders were recorded and are reported in the results.

In experiments 1-3, we explored a potential side bias in the experimental set-up by testing the choice of males (Exp. 1), virgin females (Exp. 2), and mated females (Exp. 3) between one white LED (white sun LED, B5-43SUN-JD, Roithner Laser Technic) at either end section of the outer aquarium. Experiments 4-6 tested the choice of males (Exp. 4), virgin females (Exp. 5), and mated females (Exp. 6) between two LEDs with peak wavelength 505 nm or 573 nm, both within the most spectrally sensitive green to yellow-green region, as determined in the ERGs with LEDs as test stimuli. Experiments 7-9 tested the choice of males (Exp. 7), virgin females (Exp. 8), and mated females (Exp. 9) to one LED with peak wavelength of 505 nm and one blue LED with peak wavelength 433 nm. In each experiment, results recorded as the number of moths responding to test stimuli were analyzed with Pearson's chi square test (Zar 1999) using JMP software.

4.3.5. Light attraction of moths in field experiment: 10

Field experiment 10 was designed to test how well moths discern between LEDs with different peak wavelengths (350 nm or 373 nm) both in the ultraviolet range. The

experiment was run at the Kalamalka Forestry Centre (British Columbia Ministry of Forests and Range) in Vernon, BC, Canada (N 50.26, W 119.27) between 21-26 August 2010. Each of 12 traps (Fig. 4.1C) consisted of an inverted, clear plastic, 473-mL drinking glass, thinly coated with an adhesive (TangleFoot, Contech Enterprises) on the outside, and suspended by galvanized wire (20 GA, Corfil Products, Montreal, QC, Canada) ca. 2 m above ground between 12 randomly selected (assigned by random number generator) pairs of spruce (*Picea engelmanni* x *glauca*) trees, each tree bearing at least 50 cones. Each trap was baited with a light stimulus comprising four LEDs (1.0×10^{15} photons/cm^2/s per LED for a total intensity of 4.0×10^{15} photons/cm^2/s per trap) which were taped to the inside of the drinking glass (Fig. 4.1C), controlled by a custom-built 4- or 8-channel LED driver (P. Kowalski, Science Technical Centre, SFU), and powered by a 9-v battery. The intensity of each LED was calibrated at a dinstance of 2.5 cm from the point source to an intensity of 1.0×10^{15} photons/cm^2/s using a spectrometer with cosine collector run on SpectraSuite software. Traps were randomly assigned to 350 nm or 372 nm LEDs. LEDs were activated at 20:00 hours. In the morning at 06:00 hours, captured moths were collected, preserved in 70 % ethanol, and later dissected to determine their taxonomic identity and sex (Sopow et al. 1996). Mean number of captured male and mated female *D. abietivorella* and *D. schuetzeella* group in each trap type were compared with two-tailed Wilcoxon signed-rank test (Zar 1999) using JMP software.

4.4. Results

4.4.1. Spectral sensitivity of eyes

Electroretinograms with LEDs or monochromatic light as test stimuli gave similar results (Fig. 4.2). Wavelengths between 350-610 nm elicited strong electrical potentials (ratio to highest amplitude ≥ 0.6) in *D. abietivorella* eyes. Wavelengths between 610-750 nm induced progressively lower potentials approaching zero at 750 nm. Wavelengths between 475- 575 nm elicited the strongest potentials (Fig. 4.2). The LED with peak wavelength 505 nm elicited stronger responses than did the LED with 433-nm or 573-nm peak wavelength (Tukey: 433 nm, $p < 0.0001$; 573 nm, $p = 0.015$). The LED with peak wavelength 372 nm elicited a stronger response than the LED with 350 nm peak wavelength (Tukey, $p = 0.0088$).

4.4.2. Light attraction of moths in two-choice laboratory experiments 1-9

In experiments 1-3, males, neither virgin females nor mated females showed a preference for either side of the T-tube assembly (Pearson's chi-square test; Exp. 1: $\chi^2 = 0.20$, $p = 0.65$; Exp. 2: $\chi^2 = 0.00$, $p = 1.00$; Exp. 3: $\chi^2 = 0.20$, $p = 0.65$; Fig. 4.4). In experiments 4-6, males (Exp.4. 3), virgin females (Exp. 5), and mated females (Exp. 6) were equally attracted to LEDs with peak wavelengths 573 nm or 505 nm (Pearson's chi-square test; Exp. 1: $\chi^2 = 0.83$, $p = 0.36$; Exp. 2: $\chi^2 = 0.20$, $p = 0.65$; Exp. 3: $\chi^2 = 0.83$, $p = 0.36$; Fig. 4.3). In experiments 7-9, males (Exp. 7), virgin females (Exp. 8), and mated females (Exp. 9) preferred the LED with peak wavelength 433 nm over the LED

with peak wavelength 505 nm (Pearson's chi-square test; Exp. 7: $\chi^2 = 3.81$, $p = 0.051$; Exp. 8: $\chi^2 = 6.67$, $p = 0.0098$; Exp. 9: $\chi^2 = 3.81$, $p = 0.051$; Fig. 4.3).

4.4.3. Light attraction of moths in field experiment 10

LEDs with peak wavelengths 350 nm or 373 nm were equally effective in attracting males or mated females of *D. abietivorella* (two-tailed Wilcoxon signed-rank test; males: $Z = 0.47$, $p = 0.64$; mated females: $Z = 1.31$, $p = 0.19$; Fig. 4.5) or males and females of *D. schuetzeella* group (two-tailed Wilcoxon signed-rank test; males: $Z = -1.36$, $p = 0.17$; mated females: $Z = -0.74$, $p = 0.46$; Fig. 4.4). No virgin females were captured.

4.5. Discussion

Our data indicate that eyes of *D. abietivorella* are sensitive to wavelengths from 350 – 750 nm with an increased sensitivity in the green and yellow-green range. This sensitivity to green (505 nm) does not correlate with wavelength attractiveness and it likely has a different function (Kevan and Backhaus 1998). In behavioural experiments, the 505-nm wavelength was not more attractive to *D. abietivorella* than were wavelengths (573 nm and 433 nm) to which eyes were not as sensitive in ERGs.

Electroretinograms with *D. abietivorella* revealed increased sensitivity in the UV (360-370 nm) and green (505-510 nm) range, a slight yet statistically not significant sensitivity increase in the yellow-green (560-580) range, and little sentivitiy in the blue region (Fig. 4.2). Spectral sensitivity to wavelengths of green light (510-530 nm) has been reported in other phytophagous insects including the Douglas-fir beetle, *Dendroctonus pseudotsugae* (Groberman and Borden 1980), and the Western conifer

seed bug, *Leptoglossus occidentlis.* Sensitivity to green light may help insect herbivores locate host plants and oviposition sites (Gilburt and Anderson 1996). Gravid female *D. abietivorella* may respond to green light as a foraging cue, when they seek conifer needles as oviposition sites (Whitehouse *et al.* 2011). Green reception in honeybees,in contrast, facilates detection of motion, shape recognition and depth perception (Giurfa *et al.* 1999, Kevan *et al.* 2001).

The eyes of *D. abietivorella* (Fig. 4.2) exhibited strong sensitivity in the yellow-green (560-575 nm) range. As with *E. cautella,* which is spectrally sensitive and attracted to yellow-green (546 nm) lights (Gilbert and Anderson 1996), *D. abietivorella* may be attracted to yellow-green (573 nm) light. However, it is not more strongly attracted to yellow-green than to green (505 nm) light (Fig. 4.3, Exps. 4-6). Analogous to Swallowtail butterflies which have a red receptor that allows them to distinguish leaf age (Kelber 1999), *D. abietivorella* may use its yellow-green sensitivityto select the green conifer needles most suitable for oviposition. Results of behavioural experiments 7-9 (Fig. 4.3) imply that *D. abietivorella* eyes can detect blue (433 nm) light, and that this blue is more attractive than green (505 nm).

Slight differences existed between the spectral sensitivity curves obtained using LEDs and the monochrometer (Fig. 4.2). Using LEDs which have a much larger bandwidth than the monochrometer (10 nm), may have caused a weakening or bleaching of the strong spectral sensitivity in the green region (505-510nm). Eyes of *D. abietivorella* were dark adapted in our experiments. The use of LEDs may produce results more similar to yellow or white adapted eyes (Kirchner *et al.* 2005) revealing spectral sensitivities not apparent in dark adapted eyes. The peak in the UV of the LED curve is more pronounced than the gradual slope of the UV region in the monochrometer

curve (Fig. 4.2). Additionally, the LED curve shows a non-significant increase in sensitivity at 573 nm. This may be similar to the wild type *Frankliniella occidentalis* (Matteson *et al.* 1992) which Kirchner *et al.* (2005) speculate may have a spectral sensitity that does not appear in electroretinograms. If in future research the eyes of *D. abietivorella* were blue-green adapted prior to electroretinograms, thus weakening the response to 505-510 nm wavelengths, spectral sensitivity in the yellow-green region may be revealed. Alternatively, the apparent spectral sensitivity in the yellow-green region was due to different intensities of light exposure from the two types of light sources, and may be an artifact of the Bezold-Brücke effect (Backhaus 1992).

Electroretinograms revealed statistically significant spectral sensitivity of eyes only in the green region (Fig. 4.2, top). However, behavioural experiments 7-9 revealed a significant preference of males, mated females and virgin females to blue wavelengths over green wavelengths. Moreover, in field experiment 10, traps fitted with UV LEDs captured male and mated female *Dioryctria* spp., indicating that these moths are capable of receiving UV light. The single spectral sensitivity in the green region is not likely to account for these behavioural responses in laboratory and field experiments. Additional electrophysiological experiments are needed to determine second or third spectral sensitivity maxima.

Like other *Dioryctria* spp. (Roe *et al.* 2006, Whitehouse *et al.* 2011), *D. abietivorella* is sensitive to UV light (Figs. 4.2), which plays a role in the navigation of nocturnal moths (Gilburt and Anderson 1996) and during mate selection (Kevan and Backhaus 1998). As males and mated females of *D. abietivorella* and *D. schuetzeella* group were equally attracted to UV light with peak wavelength of 372 nm or 350 nm (Fig. 4.5), it seems that the particular range of UV light is not important. It is possible that UV

below 350 nm could induce a different behavioural response by moths. That UV wavelengths failed to attract any virgin female moths in field experiment 10 is likely linked to the females' life history. Virgin, pheromone-emitting females are sedentary and thus facilitate orientation of mate-seeking males toward them. Following mating, gravid females are more active and may use UV for orientation (see Chapter 3).

The relatively low numbers of moths responding in laboratory experiments 1-9 (Fig. 4.3) was likely due to the single-component nature of the test stimulus. Other foraging cues of importance could include stimulus size and shape (e.g., Moericke *et al*. 1975, Jermy *et al.* 1988), semiochemicals (e.g., Renwick and Chew 1994, Reeves *et al.* 2009), and sound (e.g., Rowland *et al.* 2011). Complex cues can be much more attractive than single-component cues, as shown with the webbing clothes moth, *Tineola bisselliella* (Takács *et al.* 2002).

4.6. Literature cited

Backhaus, W. 1992. The Bezold-Brücke effect in the color vision system of honeybees. Vis. Research 32: 1425-1431.

Bernard, G.D., and C.L. Remington. 1991. Color vision in Lycanena butterflies: spectral tuning of receptor arrays in relation to behavioural ecology. Proc. Natl. Acad. Sci. USA. 88: 2783-2787.**Briscoe, A.D., and L. Chittka. 2001.** The evolution of color vision in insects. Ann. Rev. Entomol. 46: 471-510.

Chittka, L. 1996. Optimal sets of receptors and color opponent systems for coding of natural objects in insect vision. J. Theor. Biol. 181: 179–196.

Cool, S.J., Crawford, M.L.J., and I.J. Scheer. 1970. Tungsten microelectrode preparations for CNS recording. Rev.Sci. Instrum. 41: 1506-1507.

Giurfa, M., Zaccardi, G., and M. Vorobyev. 1999. How bees detect coloured targets using different regions of the compound eye. J. Comp. physiol. A. 185: 591-600.

Goldsmith, T.H., J.S. Collins, and D.L. Perlman. 1981. A wavelength discrimination function for the hummingbird *Archilochus alexandri*. J. Comp. Physiol. A 143: 103-110.

Groberman, L.J., and J.H. Borden. 1980. Electrophysiology response of *Dendroctonus pseudotsguae* and *Ips paraconfusus* (Coleoptera: scolytidae) to selected wavelength regions of the visible spectrum. Can. J. Zoo. 60: 2180-2189.

Hedlin, A.F., H.O. Yates, D.C. Tovar, B.H. Ebel, T.W. Koerber, and E.P. Merkel. 1980. Cone and seed insects of North American conifers. Environment Canada, Canadian Forestry Service, Ottawa, Canada.

Kelber, A. 1999. Ovipositioning butterflies use a red receptor to see green. J. Exp. Biol. 202: 2619-2630.

Kevan, P.G. and W.G.K. Backhaus. 1998. Color vision: Ecology and evolution in making the best of photic environment. In WGK Backhaus R Kliegl & JS Werner (Eds.) Color Vision: Perspectives from different disciplines. Berlin, Germany, Walter de Gruyter.

Kevan, PG, Chittka, L, and Dyer G. 2001. Limits to the salience in ultraviolet: Lessons from colour vision in bees and birds. J. Exp. Biol. 204: 2571-2580.

Kirchner, S.M., Döring, T.F. and H. Saucke. 2005. Evidence for trichromacy in green peach aphid, *Myzus Periscae* (Sulz.) (Hempitera: Aphidae). J.Insect Physiol. 51: 1255-1260.

Matteson, N., Terri, I., Ascoli-Christensen, A. and C. Gilbert. 1992. Spectral efficiency of western flower thrips, *Frankliniella occidentlis*. J. Insect Physiol. 38: 453-459.

Moericke, V., Prokopy, R.J., Berlocher, S., and G.L. Bush. 1975. Visual stimuli eliciting attraction of *Rhagoletis pomonella* (Diptera: Tephritidae) flies to trees. Entomol. Exp. Applic. **18**: 497–507.

Reeves, J.L., Lorch, P.D., and M.W. Kershner. 2009. Vision is important for plant location by phytophagous aquatic specialists *Euhrychiopsis lecontei* Dietz (Coleoptera: Curculionidae). J Insect Behav. 22: 54–64.

Renwick, J. A. A. and F.S. Chew. 1994. Oviposition behaviour in Lepidoptera. Annu. Rev. Entomol. 39: 377-400.

Roe, A.D., Stein, J.D., Gillette, N.E. and F.A.H. Sperling. 2006. Identification of Dioryctria (Lepidoptera: Pyralidae) in a seed orchard at Chico, California. Ann. Entomol. Soc. Am. 99: 433-448.

Rowland, E., P. Schaefer, P. Belton, and G. Gries. 2011. Evidence for short-range sonic communication in lymatriine moths. J. Insect Physiol. 57: 292-299.

Scherer, C. and G. Kolb. 1987. Behavioral experiments on the visual processing of color stimuli in *Pieris brassica* L. (Lepidoptera). J. Comp. Physiol. A. 160: 645-656.

Schlecht, P. 1979. Colour discrimination in dim light: an analysis of the photoreceptor arrangements in the moth *Deilephila*. J. Comp. Physiol. 129: 257-267.

Shimohigashi, M. and Y. Tominaga. 1991. Identification of UV, green and red receptors, and their projection to lamina in the cabbage butterfly, *Pieris rapae*. Cell Tissue Res. 263: 49-59.

Shu, S., Grant, G.G., Langevin, D.A., Lombardo, D.A. and L. MacDonald. 1997. Oviposition and electroantennogram responses of *Dioryctria abietivorella* (Lepidoptera: pyralidae) elicited by monoterpenes and enantiomers from eastern white pine. J. Chem. Ecol. 23: 35-50.

Skorupski, P. and L. Chittka. 2011. Is colour cognitive? Opt. Laser Technol. 43: 251-260.

Sopow, S.L., Bennett, R.G., Landry, J., and B. Landry. 1997. Identification of the "grey" *Dioryctria* species of British Columbia (Lepidoptera, Pyralidae). J. Entomol. Soc. B. C. 93: 75-92.

Takács, S., Gries, G., and R. Gries. 2002. Where to find a mate? Resource-based sexual communication of webbing clothes moth. Naturwissenschaften 89: 57-59.

Trudel, R., Bauce, É., Cabana, J., and C. Guertin. 1995. Rearing technique for *Dioryctria abietivorella* (Lepidoptera: Pyralidae). J. Econ. Entomol. 88: 640-643.

Whitehouse, C.M., Roe, A.D., Strong, W.B., Evenden, M.L. and F.A.H. Sperling. 2011. Biology and management of North American cone-feeding *Dioryctria* species. Can. Entomol.143: 1-34.

Wallace, A.R. 1878. Tropical nature and other essays. London, UK: Macmillan and Company.

Zar, J.H. 1999. Biostatistical Analysis, 4th ed. Prentice Hall, Upper Saddle River, NJ.

4.7. Figure captions

Figure 4.1. Drawings of (A) the set-up for electrophysiological recordings (B) the experimental design used in laboratory experiments 1-9, and (C) the LED light stimulus field tested in experiment 10; see methods for details.

Figure 4.2. Mean (+ SE) spectral responses standardized to ratio to highest response for each eye) obtained in electroretinograms from eyes of male, virgin female and mated female *Dioryctria abietivorella* (n = 10 each), using light-emitting diodes (top) or monochromatic light (bottom) as test stimuli.

Figure 4.3 Number of male, virgin female, and mated female *Dioryctria abietivorella* responding in two-choice laboratory experiments to various light stimuli. In each experiment, bars with an asterisk indicate a significant preference for the test stimulus (Chi-square test, $p < 0.05$). nr denotes the number of non-responding moths.

Figure 4.4. Mean (+ SE) number of male and mated female *Dioryctria abietivorella* and *D. schuetzeella* group captured in experiment 10 in traps (n = 6) baited with a UV light stimulus (peak wavelength of 350 nm or 372 nm). Note: Virgin females were not captured. Data were analyzed with a two-tailed Wilcoxon signed-rank test ($p < 0.05$).

Figure 4.1

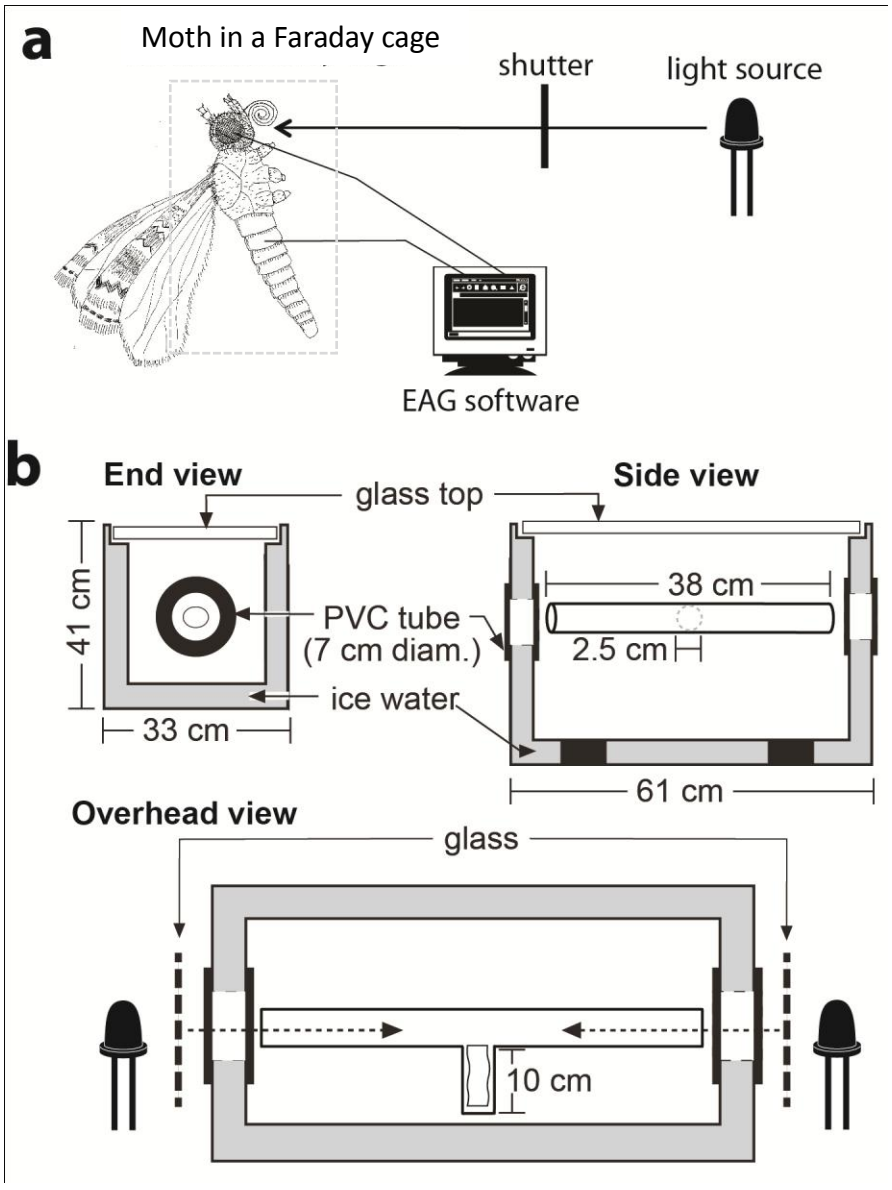

a
Moth in a Faraday cage
shutter
light source
EAG software
b
End view
glass top
Side view
41 cm
PVC tube
(7 cm diam.)
ice water
33 cm
38 cm
2.5 cm
61 cm
Overhead view
glass
10 cm

Figure 4.2

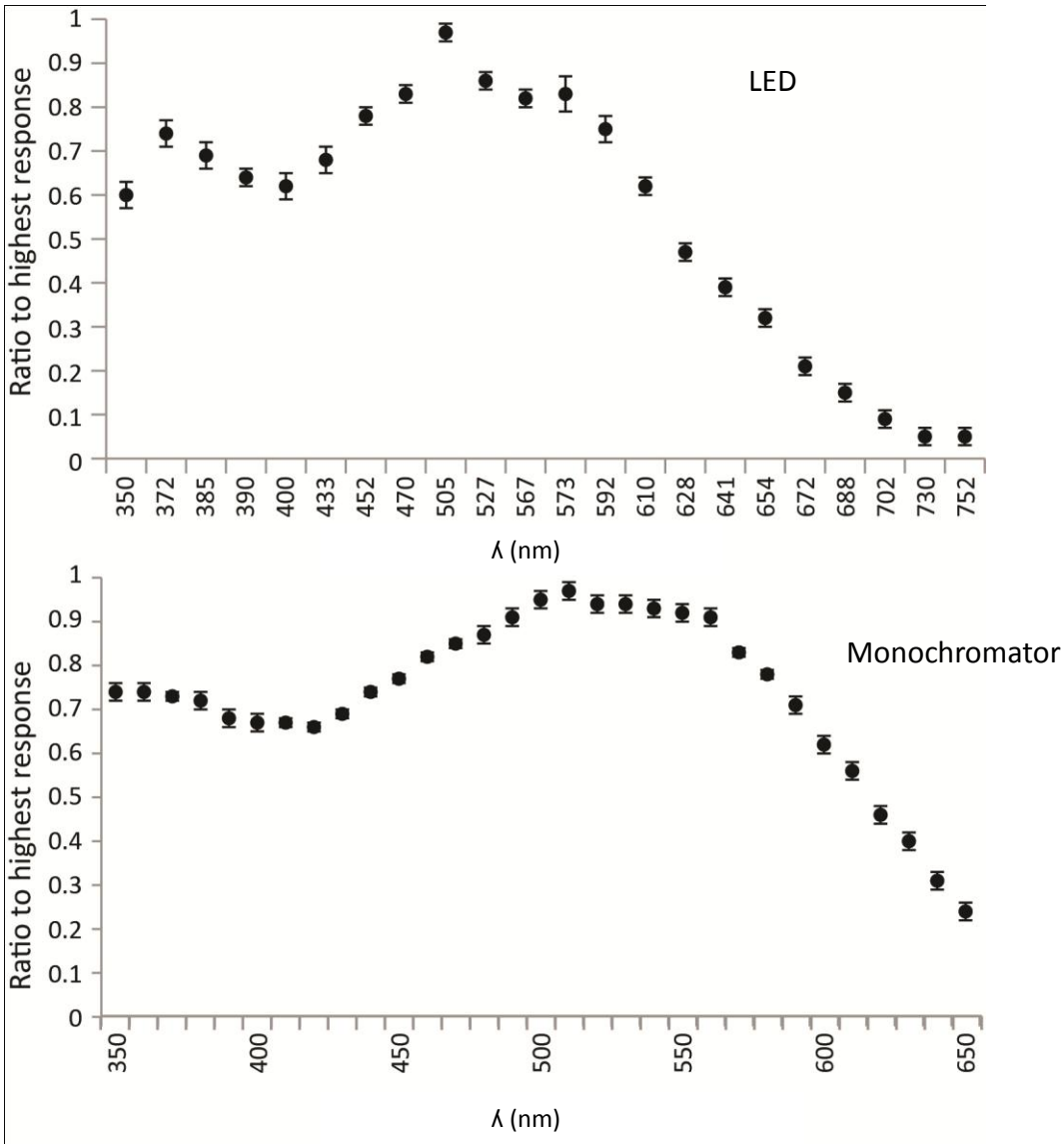

LED
Ratio to highest response
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
350
372
385
390
400
433
452
470
505
527
567
573
592
610
628
641
654
672
688
702
730
752
λ (nm)
Monochromator
Ratio to highest response
350
400
450
500
550
600
650
λ (nm)

Figure 4.3

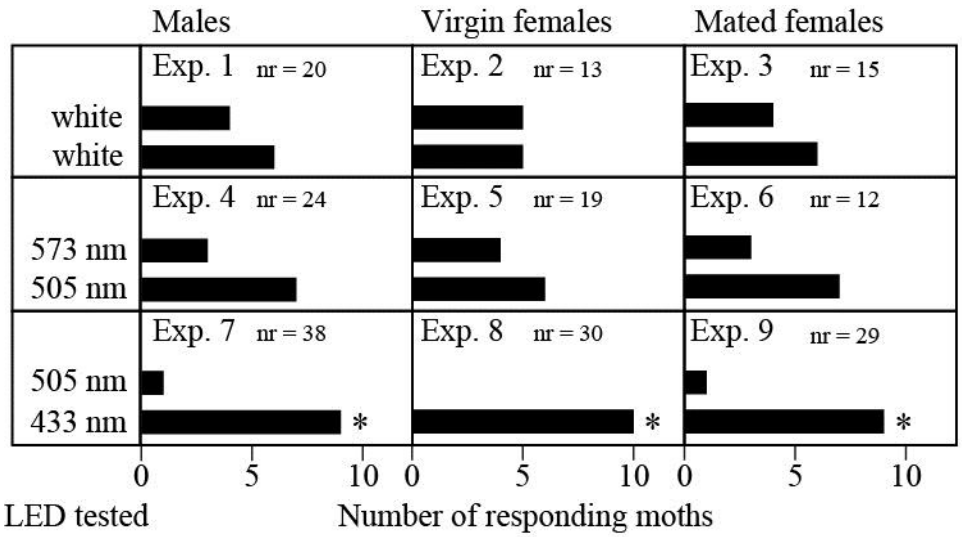
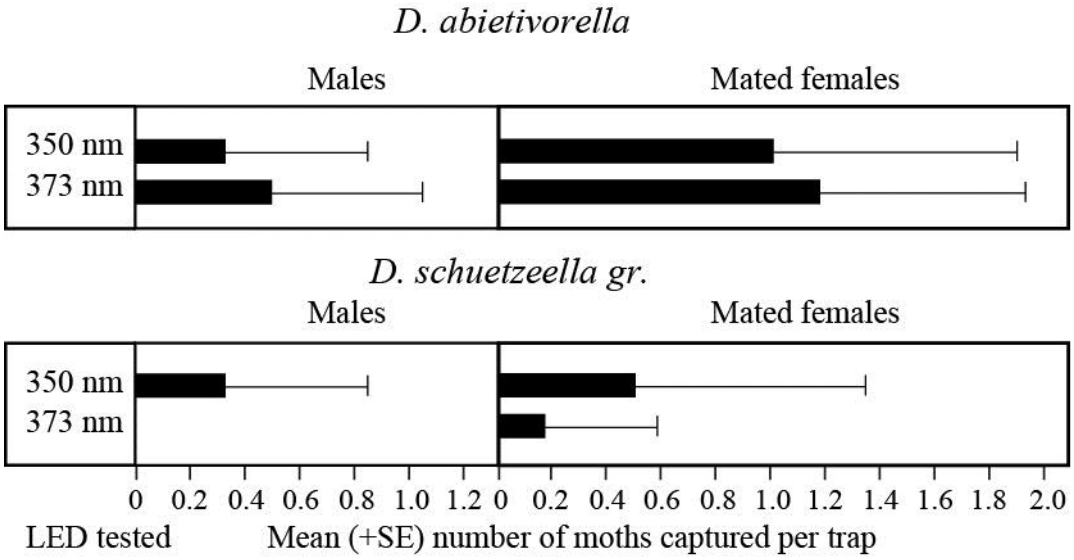

Males
Virgin females
Mated females
Exp. 1 nr = 20
Exp. 2 nr = 13
Exp. 3 nr = 15
white
white
Exp. 4 nr = 24
Exp. 5 nr = 19
Exp. 6 nr = 12
573 nm
505 nm
Exp. 7 nr = 38
Exp. 8 nr = 30
Exp. 9 nr = 29
505 nm
433 nm
*
*
*
0 5 10 0 5 10 0 5 10
LED tested
Number of responding moths

Figure 4.4


D. abietivorella
Males
Mated females
350 nm
373 nm
D. schuetzeella gr.
Males
Mated females
350 nm
373 nm
0 0.2 0.4 0.6 0.8 1.0 1.2 0 0.2 0.4 0.6 0.8 1.0 1.2 1.4 1.6 1.8 2.0
LED tested
Mean (+SE) number of moths captured per trap

5. Can conophagous Western conifer seed bugs use radiant IR to discern between objects with differing temperature and surface texture?[4]

5.1. Abstract

It has recently been shown that the conophagous Western conifer seed bug, *Leptoglossus occidentalis*, is attracted to radiant infrared (IR) from conifer cones. However, locating these cones might be challenging because cone temperature and resulting radiant IR may vary according to cone and diurnal characteristics, and because other structures in coniferous forests may resemble the IR signature (thermal contrast) of cones. In thermographic field surveys and in laboratory experiments, we tested the hypotheses (1) that cone temperatures vary with time of day, age (first year, second year), size and colour, and (2) that *L. occidentalis* prefers radiant IR from objects with

[4]Authors: Tracy Zahradnik, Audrey Labrie, Laurie Lin, Ward Strong, Gerhard Gries. This Chapter has been formatted to the requirements of the Bulletin of Entomological Research.

cone-like temperature and surface texture. Mean and hot-spot temperatures of western white pine cones, *Pinus monticola,* ranged between 15 °C and 35 °C from 09:00 to 18:30 h. Colour, length or width of within-year cones did not affect cone temperature but second-year cones were warmer than the smaller first-year cones. When given a choice between radiant IR from heat sources well within (40 °C) and just outside (60 °C) the natural cone temperature range, males and females of *L. occidentalis* were attracted to the former. However, they failed to discern between radiant IR emanating from cones, Brussel sprouts and copper pipes all heated to identical temperature (35 °C), indicating that the contrasting surface texture of these objects did not modulate their IR signature to an extent that affected the behavioural response of *L. occidentalis*. Our data support the conclusion that the IR receptors of *L. occidentalis* can discern between temperature differentials of at least 20 °C, and support the concept that *L. occidentalis* exploits the IR signature of conifer cones as foraging cues.

Keywords: Conophagous insects; surface properties; conifer cones; electromagnetic spectrum; infrared radiation detection

5.2. Introduction

Both vertebrates and invertebrates exploit infrared (IR) radiation as foraging cues. IR receptors of crotaline snakes (Moiseenkóvà *et al*., 2003), *Python* snakes (Grace *et al*., 1999), and vampire bats (Kürten & Schmidt, 1982) aid in locating warm-bodied prey or hosts. IR receptors of the blood-sucking bugs *Rhodnius prolixus* and *Triatoma infestants* detect radiant IR from warm-bodied hosts (Lazzari & Núñez 1989; Schmitz *et al.,* 2000a). The pyrophilic jewel beetle, *Merimna acuminata*, Australian fire beetle, *M. atrata*, little ash beetle, *Acanthocnemus nigricans,* and the Australian flat bug, *Aradus albicornis,* respond to radiant IR from forest fires to locate smoldering wood as oviposition sites (Schmitz & Bleckmann, 1997, 1998; Schmitz *et al*., 2000b, 2002, 2008; Schmitz & Trenner, 2003). *Merimna acuminata* may be able to detect a 10-ha fire from 10 km away (Schmitz & Bleckmann, 1998).

Only recently have insect herbivores been shown to exploit radiant IR as a foraging cue from live plants. Both the conophagous Western conifer seed bug, *Leptoglossus occidentalis* Heidermann (Hemiptera: Coreidae) (Takács *et al*., 2009), and the fir coneworm, *Dioryctria abietivorella* Groté (Lepidoptera: Pyralidae) (Chapter 3), have IR receptors and may orient toward high-frequency (intense) radiant IR from cones and tree branches. The Douglas-fir cone gall midge, *Contarinia oregonensis* Foote (Diptera: Cecidomyiidae), also responds to intense radiant IR (Chapter 2) but IR receptors have not been located (see Chapter 2).

A resource is likely best detected if it emanates radiant IR of different wavelength or intensity than that of the surroundings. The resulting contrast (or IR signature) depends on such aspects as the angle of the sun, cloud cover, time of day, and the property and size of the resource (Thánh *et al*., 2009). A stark thermal contrast is required when IR receptors are small (Bakkan & Krochmal, 2007), such as those of *L. occidentalis* (< 100 µm; Takács *et al.*, 2009). A stark thermal contrast between the target and background still facilitates foraging decisions by animals which have large IR receptors, such as snakes. They responded more strongly to freshly killed (warm) prey placed on cold background than to warm prey placed on warm background (Theodoratus *et al*., 1997). It is unknown whether animals respond to properties of IR of a resource or certain aspects of the resource, such as hot spots.

Resource colour, size, texture or life traits may mediate resource temperature. For example, the interior of purple cones of white fir, *Abies concolor,* is warmer than that of green cones (Sturgeon & Mitton, 1980) and thus may affect the cones' IR signature. Similarly, differences in size texture of male and female catkins of the artic willow *Salix artica* cause temperature differentials between catkins (Kevan 1990). Male catkins are smaller and have less insulating hair than do their female counterparts (Kevan 1990). The high artic midge *Smittia valutina* seeks the warmest part of these catkins (Kevan 2007). Moreover, some gymnosperm and angiosperm plants are capable of generating heat within reproductive parts (Thien *et al.* 2000) and are not entirely reliant on solar radiation.

IR receptors seem most sensitive to IR wavelengths which radiate from essential resources. IR receptors of *M. acuminata*, for example, have a peak sensitivity at 3 µm (Evans, 1966) which match the 3-5 µm wavelengths radiating from forest fires that burn

at heat intensities of 600-1000 °C (Schmitz & Bleckmann, 1997). Similarly, IR receptor of *Python* snakes absorb radiant IR in the 3- to 5- and 8- to 12-µm range, the latter corresponding with the peak wavelength of 10 µm emitted by prey (Grace *et al.*, 1999).

Heating of IR receptor membranes increases their neural activity in *M. acuminata* (Schmitz & Trenner, 2003) and in crotaline snakes (de Cock Bunings *et al*., 1981; Moiseenkóvà *et al*., 2003). In snakes, temperature differentials as little as 0.003 °C and 0.2 °C elicit neurological and behavioural responses, respectively (Bullock & Diecke, 1956; Noble & Schmidt, 1937). Foraging boid and viperid snakes integrate radiant IR and visual information to form a spatial (thermal) image of their environment (Hartline *et al*., 1978).

Analogous to snakes which integrate visual and radiant IR information (Grace et al. 2001), IR detection technology is designed to create visual thermographic images of target objects in relation to their surroundings (Thánh *et al*., 2009). Even though this technology becomes increasingly more sophisticated, some inaccuracies exist when using IR technology for biological research (Katsburger & Stachl, 2003) resulting in more subtle differences not being observed. As natural IR detectors are much more sensitive than currently available IR detection technology (Grace *et al.* 1999), it is conceivable, and prompted us to test experimentally, that natural IR receptors detect even subtle differences in surface texture, and help discern between target and non-target objects. Honeybees are capable of recognizing microsurface textures of flower petals (Kevan & Lake 1985).

Adults and nymphs of *L. occidentalis* are attracted to radiant IR from cones (Takács *et al*., 2009), where they feed on the content of seeds (Krugman & Koerber,

1969; Blatt & Borden 1999; de Groot *et al*., 1994; Strong *et al*., 2007). Cones may be as warm as 50 °C, contrasting well against the much cooler foliage (Takács *et al.*, 2009). To the best of our knowledge, plant parts greater than 50 °C have not yet been recorded within the geographic distribution of *L. occidentalis.*

If *L. occidentalis* indeed relies, in part, upon radiant IR from conifer cones as a foraging cue (Takács et al. 2009), then one would expect a match between cue properties and peak receptor sensitivities. However, to obtain electrophysiological recordings from IR receptors, and to demonstrate differential responses to radiant IR from target or non-target resource temperatures is exceedingly difficult. Moreover, the amplitude of electrophysiological responses does not imply preference or avoidance behaviour. Thus, we decided to run behavioural instead of electrophysiological experiments to investigate any preference by *L. occidentalis* for specific radiant IR.

In surveys and experiments, we tested the hypotheses (1) that cone temperature varies with time of day, age (first year *vs*. second year), size and colour, and (2) that *L. occidentalis* prefers radiant IR from objects which are cone-like in temperatures and surface texture.

5.3. Methods and materials

5.3.1. Thermographic surveys to determine whether cone temperature is affected by time of day, age (first year, second year), size or colour

Thermographs were taken with a mid-range (3-5 µm) Agema Thermovision 550 camera (FLIR Systems Ltd., Burlington, ON, Canada) and/or a long-range (8-20 µm) Fluke TI-20 camera (Fluke Corp., Everett, WA, USA). Corresponding colour photographs were taken with a Kodak EasyShare C613 camera (Kodak, Rochester, NY, USA). The recoding distance was set to ~ 90-150 cm from the cone, and the emissivity (the ratio of the radiation emitted by a surface to the radiation emitted by a black body at the same temperature) was set to 0.95 [the approximate value of the emissivity of vegetated areas (Jiang *et al.* 2006)]. Absolute reflectance was measured using a disk of aluminium hung from the tree. Ambient temperature and relative humidity were determined from an Environment Canada Weather archive. Using ThermaCam Reporter 2000 Pro software (FLIR Systems Ltd.), thermographic images were analyzed to determine the mean temperature of cones and that of hot spots (the hottest area of the cone). If there was more than one cone per image, cone temperatures were averaged.

Survey 1 was designed to record the difference in ambient mean temperature of cones of a western white pine for a 24-h period. Images of three side-by-side *in-situ* cones were recorded hourly or every two hours at four, 24-h recording events. Events were on 07 August 2008, 03 September 2008, and 29 June 2009 at the Sechelt Seed Orchard (Canadian Forestry Products, Vancouver, BC, Canada), Sechelt, BC, Canada

(123°43′W, 49°27′N), and on 09 July 2009 at the Kalamalka Forestry Centre (British Columbia Ministry of Forests and Range) in Vernon, BC, Canada (119°16′W, 50°14′N).

For the results of survey 2, we compared mean temperature between brown and green cones. At the Sechelt Orchard on 29 July 2009 between 13:00 and 13:30 h, 10 images each of green and brown western white pine cones were recorded. Mean and hot spot temperatures of brown and green cones were compared by the two-tailed Wilcoxon signed-rank test (Zar 1999) using JMP software (SAS®, Cary, NC, USA).

For the results of survey 3, we compared potential differences in mean temperature between first- and second-year cones on the same western white pine trees. At the Sechelt Orchard on 06 August 2009 between 17:00-17:30, 15 images each of first- and second-year cones on each of 15 trees were recorded. Mean and hot spot temperatures of first and second year cones were compared by two-tailed Wilcoxon signed-rank (Zar 1999) in JMP.

In surveys 4 and 5, we assessed the relationship between cone dimension and mean cone temperature. Thermographs were taken, and cone length and width measured, at the Kalamalka Forestry Centre on 17 July 2009 between 11:00-11:30 h for western white pine (*Pinus monticola)* (Survey 4), and between 12:00-12:30 for Douglas-fir (*Pseudotsuga menziesii)* (Survey 5). Length and width of cones were regressed against mean temperature.

5.3.2. Experimental insects

Adults of *L. occidentalis* were collected from cones at the Kalamalka Forestry Centre and Sechelt Seed Orchard. They were provided with water and fresh cones of

western white pine, Douglas-fir or spruce (*Picea engelmanni* x *glauca*), and kept in wooden and mesh cages (47 × 47 × 92 cm) outdoors at Simon Fraser University. Cage interiors were misted daily with water. Because *L. occidentalis* forages during the photophase, experiments were run between 10:00 and 17:00 h in August and September 2010. Prior to bioassays, specimens were dark-adapted for 30-60 min.

5.3.3. Response of L. occidentalis *to IR sources in laboratory two-choice experiments 1-10*

All experiments were run in a cooled chamber designed to eliminate external thermal cues or radiant IR (Takács et al. 2009). The chamber consisted of a glass aquarium (outside dimensions: 50.5 × 26.7 × 33.0 cm) which was covered with a glass lid (50.5 × 26.7 cm) and rested on 5-cm tall rubber stoppers inside a larger glass aquarium (outside dimensions: 61.0 × 33.0 × 41.0 cm), with cooled water (16 ± 2 °C) between them. A black PVC pipe (7 cm inside diameter; IPEX, Toronto, ON, Canada) was sealed with aquarium grade black silica (All Glass Aquarium, Franklin, WI, USA) between the two aquaria in each of the two end sections, allowing external stimuli to enter the inner aquarium without passing through water.

A glass T-tube (2.5 cm diam, stem: 10 cm, side arms: 16 cm each) was mounted inside the inner aquarium on a metal stub (Fig. 5.1). An IR source was mounted outside the aquarium apparatus. Convective or conductive heat was separated from radiant IRby reflecting the radiation with face-first mirrors (10.2 × 10.2 cm bare gold-coated BK7 mirrors, reflecting ca. 96% of wavelengths between 0.7 - 20 µm (Tempotec Optics, Fuzhou, Fujian, China) (Fig. 5.1). IR treatments (see below) were randomly assigned (with a coin flip) to position (left/right) and temporal order. Surface temperature of IR

sources was measured with a thermocouple in order to maintain the target temperature. To ensure that insects inside the T-tube within the inner aquarium could receive the radiant IR, a horizontal laser was used to properly position the mirrors so that the beam of radiant IR entered the T-tube in the inner aquarium.

All experiments were run in darkness. In each test, an insect was placed into the stem of the T-tube. Insects that approached within 2.5 cm of the distal end of one of the side arms within 2-30 min were considered responders. After use, T-tubes were baked overnight at 120 °C, and the inside of the inner aquarium was wiped with 70% ethanol to remove potential chemical residues (Cowan & Gries 2009). Experiments were terminated when 10 responders, or 12 responders in experiments 3 and 4, were obtained. Number of responders for each stimuli in Experiments 1-10 (see below) were analyzed by a Fisher's exact two-sided test (Zar 1999) using JMP software.

Experiments 1-6 were designed to explore the effect of temperature and its resulting radiant IR on the response of *L. occidentalis.* Each experiment tested the choice of *L. occidentalis* between two objects of identical surface texture [capped copper pipe (2.5 cm inside diameter × 7 cm)] but contrasting radiant IR due to different object temperature. Target temperatures were produced by resistors coupled to a custom-built control device (R. Holland, Science Technical Centre, Simon Fraser University, Burnaby, BC, Canada) and placed inside the pipe.

Experiments 1 and 2 tested the choice of males (Exp. 1) and females (Exp. 2) between radiant IR from a 40 ± 5 °C warm pipe (within cone temperature range) and a 60 ± 5 °C warm pipe (outside cone temperature range). Experiments 3 and 4 tested the choice of males (Exp. 3) and females (Exp. 4) between a 40 ± 5 °C warm pipe and a 100

± 5 °C hot pipe, a temperature typically not encountered by *L. occidentalis* in its habitat. Experiments 5 and 6 tested the choice of males (Exp. 5) and females (Exp. 6) between a 60 ± 5 °C warm pipe and a 100 ± 5 °C hot pipe.

Experiments 7-10 were designed to explore the effect of object surface texture on the response of *L. occidentalis.* Each experiment tested the choice of *L. occidentalis* between objects of identical temperature (35 ± 5 °C) but contrasting surface texture: copper pipes (see above), freshly collected Douglas-fir cones (~ 3 × 5 cm) and fresh Brussels sprouts (*Brassica olercea* cultivar group Gemmifera) peeled to cone size (~ 4 × 4 cm). Copper pipe was selected for tests to determine whether objects with a highly homogeneous surface texture would be less attractive than cones. Brussel sprouts were selected to determine whether objects with a surface texture as smooth as that of Douglas-fir cones but lacking their prominent tridentine bracts would be as attractive as cones. A single cone or brussel sprout was cored out to accommodate the resistor which heated the surface of the object to 35 °C. Experiments 7 and 8 tested the choice of males (Exp. 7) and females (Exp. 8) between a cone and a pipe. Experiments 9 and 10 tested the choice of males (Exp. 9) and females (Exp. 10) between a cone and a Brussel sprout.

5.4. Results

In thermographic survey 1, the temperature of Western white pine cones ranged from 15 °C to 31 °C between 09:00 and 18:30 h (see results of the 9 June 2009 recording in Fig. 5.2). Ambient temperature ranged from 6.2 ˚C to 8.7 ˚C.

In survey 2, there was no significant difference in mean or hot-spot temperature between brown and green Western white pine cones (two-tailed Wilcoxon signed-rank test; mean: $Z = 0.19$, $p = 0.85$; hottest spot: $Z = 0.68$, $p = 0.50$; Fig. 5.3). In survey 3, second-year White pine cones weresignificantly warmer than first-year cones (two-tailed Wilcoxon signed-rank test; mean: $Z = 3.90$, $p < 0.0001$; hottest spot: $Z = 4.65$, $p < 0.0001$; Fig. 5.3). In surveys 4-6, there was nosignificant correlation between (*i*) cone temperature and cone length (western white pine: mean: $r^2 = 0.006$; hottest spot: $r^2 = 0.007$; Douglas-fir mean: $r^2 = 0.05$; hottest spot: $r^2 = 0.12$) or (*ii*) cone temperature and cone width (Western white pine: mean: $r^2 = 0.05$; hottest spot: $r^2 = 0.02$; Douglas-fir: mean: $r^2 = 0.02$; hottest point: $r^2 = 0.01$) (Fig 5.4).

In experiments 1 and 2, males and females significantly preferred radiant IR from a 40-°C pipe to radiant IR from a 60-°C pipe (Fig. 5.5) (males: $\chi^2 = 3.81$, $p = 0.05$; females: $\chi^2 = 6.67$, $p = 0.01$). In experiments 3 and 4, neither males ($\chi^2 = 0.17$, $p = 0.41$) nor females ($\chi^2 = 0.17$, $p = 0.681$) exhibited a preference for radiant IR from a 40-°C or a 100-°C pipe. In experiments 5 and 6, males ($\chi^2 = 3.81$, $p = 0.05$), but not females ($\chi^2 = 0.00$, $p = 1.00$), preferred radiant IR from a 100-°C pipe to radiant IR from a 60-°C pipe. In experiments 7 and 8, neither males ($\chi^2 = 0.00$, $p = 1.00$) nor females ($\chi^2 = 0.2$, $p = 0.65$) discriminated between radiant IR from a cone or pipe. Similarly, in experiments 9 and 10, neither males ($\chi^2 = 0.2$, $p = 0.65$) nor females ($\chi^2 = 0.00$, $p = 1.00$) discriminated between radiant IR from a cone or a brussel sprout.

5.5. Discussion

Our data support the hypotheses that the temperature of Western white pine cones varies with time of day and with some but not all cone attributes, and that *L. occidentalis* prefers radiant IR from objects with cone-like temperature.

Diel temperatures of White pine cones fluctuated from 5 ºC to 31 ºC (Fig. 5.2). They started to increase sharply at around 07:00 h, peaked around noon, and then steadily decreased until about 18:00 h. Similar data were obtained with cones of Douglas-fir and spruce trees (Chapter 4), which are also fed on by *L. occidentalis*. The IR signatures of cones indicate that extrinsic factors such as solar radiation and ambient temperature have greater bearing on cone temperature than intrinsic factors such as metabolic activity. The radiant IR from warm cones could then become a reliable foraging cue for *L. occidentalis*. This concept is supported by observations that *L. occidentalis* takes flight, forages and mates during times (10:00 - 18:30 h) when cones are warmest. That foraging periods of *L. occidentalis* are much shorter on cloudy days (W.S., unpublished data) also supports this concept.

Thermographic Survey 3 showed (Fig. 5.3), that the larger, second-year cones are warmer than the smaller first-year cones. By preferentially responding to IR cues from such cones, *L. occidentalis* would be able to exploit resources that are relatively more profitable than first-year cones. Although the length and width of within-year cones varied, size differences were too subtle to detect any effect on cone temperature and radiant IR.

In our study, cone colour had no effect on cone temperature (Fig. 5.3). This is consistent with findings that oviposition decisions by female *C. oregonensis* were not affected by cone colour (Chapter 2), and that green and purple Douglas-fir cones had similar IR signatures (Chapter 2).

Leptoglossus occidentalis responded preferentially to 40 °C over °60 C IR sources (Fig 5.5), indicating that the IR receptors can discern between heat differentials of at least 20 °C and possibly below. It is not very likely, though, that *L. occidentalis* can discern between heat differentials as low as 0.003 ºC, as the pit organs of some snakes can (Noble & Schmidt, 1937; Bullock & Diecke, 1956). The IR receptors of *L. occidentalis* are simply too small (< 100 µm) (Takács et al. 2009), and the sensitivity of IR receptors decreases with size (Bakken & Krochmal, 2007). The response to IR is unlikely to be an avoidance of high temperatures: there was no differential response between 40 °C and 100 °C. We can find no ready explanation for the preferential response of males to 100 °C. The relatively high number of non-responding insectsin the laboaratory may be due to reluctance to move in an artificial setting or may be due to ambient temperature cooler than in the field.

Copper pipes, Douglas-fir cones and Brussels sprouts differed in surface texture, but *L. occidentalis* was unable to discern between host and non-host objects (Fig. 5.5; Exps. 7-10). It appears that the shape and size of objects are more important foraging cues than surface texture. This concept is supported by findings that small cone-like traps were more effective than large traps in attracting *L. occidentalis* (Takács, person. com.), and that branch-like traps were more effective than can-like traps in attracting *C. oregonensis* (Chapter 2).

Host-finding by *L. occidentalis* using electromagnetic radiation appears to include a complex of object shape, size, colour, surface texture and IR signature (see Chapter 7). This complexity provides fertile grounds for further laboratory and field research.

5.6. Literature cited

Bakken, G.S. & Krochmal, A.R. (2007) The imaging properties and sensitivity of the facial pits of pitvipers as determined by optical and heat-transfer analysis. *The Journal of Experimental Biology*, **210:** 2801–2810.

Blatt, S.E. & Borden, J.H. (1999) Physical characteristics as potential host selection cues for *Leptoglossus occidentalis* (Heteroptera: Coreidae). *Environmental Entomology*, **28:** 246–254.

Bullock, T.H. & Diecke, P.J. (1956) Properties of an infrared receptor. *Journal of Physiology*, **134:** 47–87.

de Groot, P., Turgeon, J.T. & Miller G.E. (1994) Status of cone and seed insect pest management in Canadian seed orchards. *The Forestry Chronicle*, 7**0:** 745–761.

Evans, W.G. (1966) Perception of infrared radiation from forest fires by *Melanophila acuminata* De Greer (Buprestidae: Coleoptera). *Ecology*, **47:** 1061–1065.

Grace, M.S., Church, D.R., Kelly, C.T., Lynn, W.F. & Cooper, T.M. (1999) The *Python* pit organ: imaging and immunocytochemical analysis of an extremely sensitive natural infrared detector. *Biosensors and Bioelectronics*, **14:** 53–59.

Grace, M.S., Woodward, O.M., Church, D.R. & Calisch, G. (2001) Prey targeting by the infrared-imaging snake *Python*: effects of experimental and congenial visual deprivation. *Behavioural Brain Ressearch*, **119:** 23–31.

Hartline, P.H., Kass, L. & Loop, M.S. (1978) Merging modalities in the optic tectum: infrared and visual integration in rattlesnakes. *Science*, **199:** 1225–1229.

Jiang, G., Li. Z., & Nerry, F. (2006) Land surface emissivity retrieval from combined mid-infrared and thermal infrared data of MSG-SEVIRI. *Remote Sensing of Environment*, **105:** 326–355.

Katsburger, G. & Stachl, R. (2003) Infrared imaging technology and biological applications. *Behavior Research Methods, Instruments & Computers,* **35:** 429–439.

Kevan, P.G. (1990) Sexual differences in the temperature of blossoms on a dioecious plant Salix arctica: Significance for life in the Arctic. *Arctic and Alpine Research,* **22:** 283-289.

Kevan, P.G. (2007) Diaheliotaxis and ombrophobia in a anthophilous high artic midge *Smittia velutina* (Lundbeck, 1898) (Chironomiidae). *Chironomus*, **20:** 29-31

Kevan, P.G. & Lane M.A. (1985) Flower petal microtexture is a tactile cue for bees. *Proceedings of the National Academy of Science USA,* **82:** 4750-4752.

Krugman, S.L. & Koerber, T.W. (1969) Effects of cone feeding by *Leptoglossus occidentalis* on ponderosa pine seed development. *Forest Science*, **16:** 104–111.

Kürten L, & Schmidt U. (1982) Thermoperception in the common vampire bat (*Desmondus rotundus*). Journal of Comparative Physiology **146**:223-228.

Lazzari, C.R. & Núñez, J.A. (1989) The response to radiant heat and the estimation of the temperature of distant sources in *Triatoma infestans*. Journal of *Insect Physiology*, **35:** 525–**529.**

Moiseenkóvà, V., Bell, B., Motamedi, M., Wozniak, E. & Christensen, B. (2003) Wide-band spectral tuning of heat receptors in the pit organ of the copperhead snake (*Crotalinae*). *American Journal of Physiology - Regulatory, Integrative and Comparative Physiology*, **284:** 598–606.

Noble, G.K. & Schmidt, A. (1937) The structure and function of the facial and labial pits of snakes. *Proceedings of the American Philosophical Society*, **77:** 263–288.

Schmitz, H. & Bleckmann, H. (1997) Fine structure and physiology of the infrared receptor of beetles of the genus *Melanophila* (Coleoptera: Burprestidae). *International Journal of Insect Morphology and Embryology*, **26:** 205–215.

Schmitz, H. & Bleckmann, H. (1998) The photomechanic infrared receptor for the detection of forest fires in the beetle *Melanophila acuminata* (Coleoptera: Buprestidae). *Journal of Comparative Physiology A*, **182:** 647–657.

Schmitz, H., Trenner, S., Hofmann, M.H. & Bleckmann, H. (2000a) The ability of *Rodnius prolixus* (Hemiptera: Reduviidae) to approach a thermal source solely by its infrared radiation. *Journal of Insect Physiology*, **46:** 745–751.

Schmitz, H., Schmitz, A. & Bleckmann H. (2000b) A new type of infrared organ in the Australian “fire-beetle” *Merimna atrata* (Coleoptera: Buprestidae). *Naturwissenschaften*, **87:** 542–545.

Schmitz, H, Schmitz, A, Trenner, S. & Bleckmann H. (2002) A new type of insect infrared organ of low thermal mass. *Naturwissenschaften*, **89:** 226–229.

Schmitz, H. & Trenner, S. (2003) Electrophysiological characterization of the multipolar thermoreceptors in the “fire-beetle” *Merimna atrata* and comparison with the infrared sensilla of *Melanophia acuminata* (both Coleoptera, Buprestidae). *Journal of Comparative Physiology A*, **189:** 715–722.

Schmitz, A., Gebhardt, M. & Schmitz, H. (2008) Microfluidic photomechanic infrared receptors in a pyrophilous flat bug. *Naturwissenschaften*, **95:** 455–460.

Sturgeon, K.B. & Mitton, J.B. (1980) Cone color polymorphism associated with elevation in white fir, *Abies concolor,* in Southern Colorado*. American Journal of Botany*, **67:** 1040–1045.

Strong, W.B., Bates, S.L. & Stoehr, M.U. (2001) Feeding by *Leptoglossus occidentalis* Heidermann (Hemiptera: Coreidae) reduces seed set in lodgepole pine. *The Canadian Entomologist*, **133:** 857–865.

Takács, S., Bottomley, H., Andreller, I., Zahradnik, T., Schwarz, J., Bennett, R., Strong, W. & Gries, G. (2009) Infrared radiation from hot cones on cool conifers attracts seed-feeding insects. *Proceedings of the Royal Society B*, **276:** 649–655.

Thánh, N.T., Háo, D.N. & Sahli H. (2009) Infrared thermography for land mine detection. In: Hammond RI (ed) Augmented vision perception in infrared: Algorithms and applied systems, advances in pattern recognition. Springer-Verlag London Limited, London, pp 3–36.

Theodoratus, D. H., Chiszar, D. & Smith, H. M. (1997). Rattlesnake orientation to prey as a function of thermal background and edges. *Psychological Records,* **47:** 461–472.

Thien, L.B., Azuma, H. & Kawano, S. (2000). New perspectives on the pollination biology of basal angiosperms. International Journal of Plant Science, 161: S225-S235.**Zar, J.H.** (1999) Biostatistical analysis. 4th edition. Prentice Hall, Upper Saddle River.

5.7. Figure captions

Figure 5.1. Experimental design to test behavioural responses of *Leptoglossus occidentalis* to radiant IR from objects of different temperature or surface texture in a double aquarium design with dark adapted insects and radiant IR emitted from a heated copper pipe, Douglas-fir cone or brussel sprout.

Figure 5.2. Representative example of mean and hottest-spot temperatures of cones recorded in survey 1 in mid-range (3-5 µm; Agema Thermovision) and hottest-spot temperatures of cones recorded in survey 1 in long-range (8-15 µm; Fluke) thermographic images of *in situ* Western white pine cones during a 24-h period on 29 June 2009.

Figure 5.3. Mean and hottest-spot temperatures recorded in mid-range (3-5 µm) thermographs of *in situ* Western white pine cones which differed in colour [survey 2 (n = 10): green *vs.* brown cones; Sechelt, BC, 29 July 2009, 01:00-01:30] or age [survey 3 (n = 15): 1^{st}-year- vs 2^{nd}-year-cones; Sechelt, BC, 06 August 2009, 17:00-17:30]. In each survey, an asterisk (*) indicates a significant temperature difference (two-tailed Wilcoxon signed-rank test; $p < 0.05$).

Figure 5.4. Correlations between cone attributes (length, width) and mean and hot-spot temperatures of *in situ* Western white pine and Douglas-fir cones, as determined in thermographic surveys 4 and 5.

Figure 5.5. Numbers of male and female *Leptoglossus occidentalis* responding in two-choice laboratory experiments (n = 10 each) to radiant IR from objects of (*i*) similar shape but at different surface temperature (Exps. 1-6), or (*ii*) identical temperature but different surface texture (Exps. 7-10). In each experiment, an asterisk (*) indicates a significant preference for a test stimulus (Pearson's chi square test; $p < 0.05$). nr = non-responding insects.

Figure 5.1

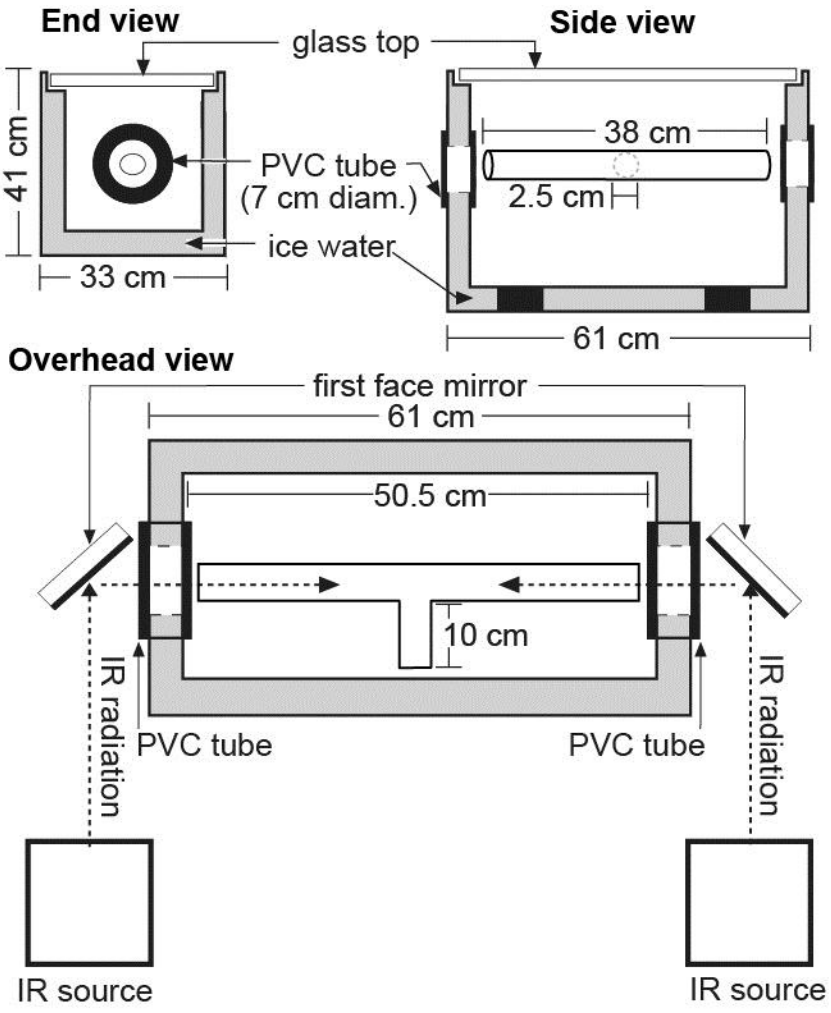

End view
glass top
Side view
41 cm
PVC tube
(7 cm diam.)
38 cm
2.5 cm
ice water
33 cm
61 cm
Overhead view
first face mirror
61 cm
50.5 cm
10 cm
IR radiation
PVC tube
PVC tube
IR radiation
IR source
IR source

Figure 5.2

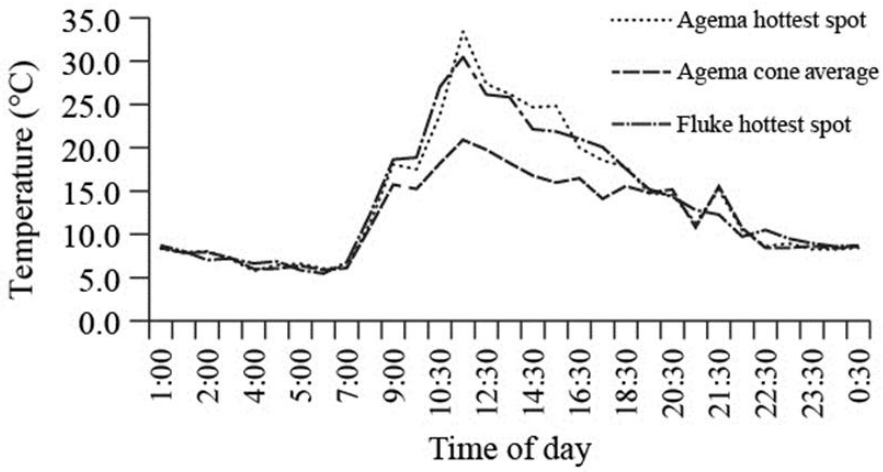

Agema hottest spot
Agema cone average
Fluke hottest spot
Temperature (°C)
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
1:00
2:00
4:00
5:00
7:00
9:00
10:30
12:30
14:30
16:30
18:30
20:30
21:30
22:30
23:30
0:30
Time of day

Figure 5.3

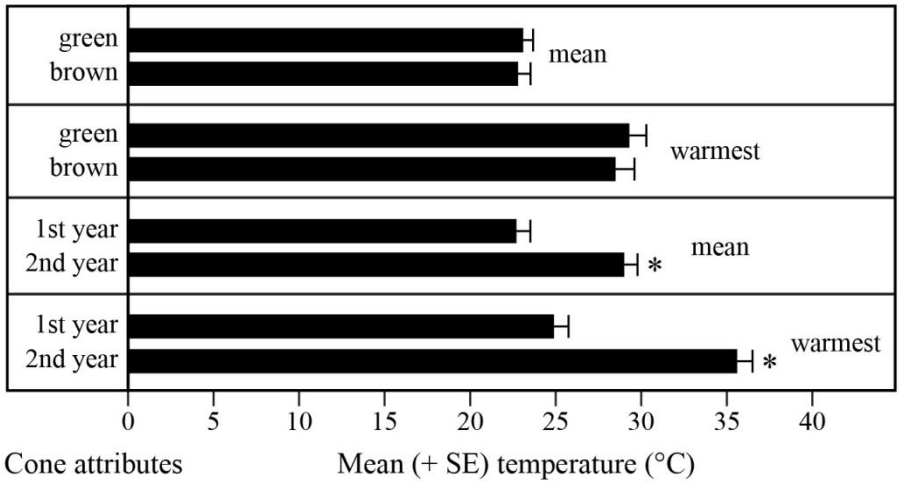

green
brown
mean
green
brown
warmest
1st year
2nd year
mean
*
1st year
2nd year
warmest
*
0
5
10
15
20
25
30
35
40
Cone attributes
Mean (+ SE) temperature (°C)

Figure 5.4

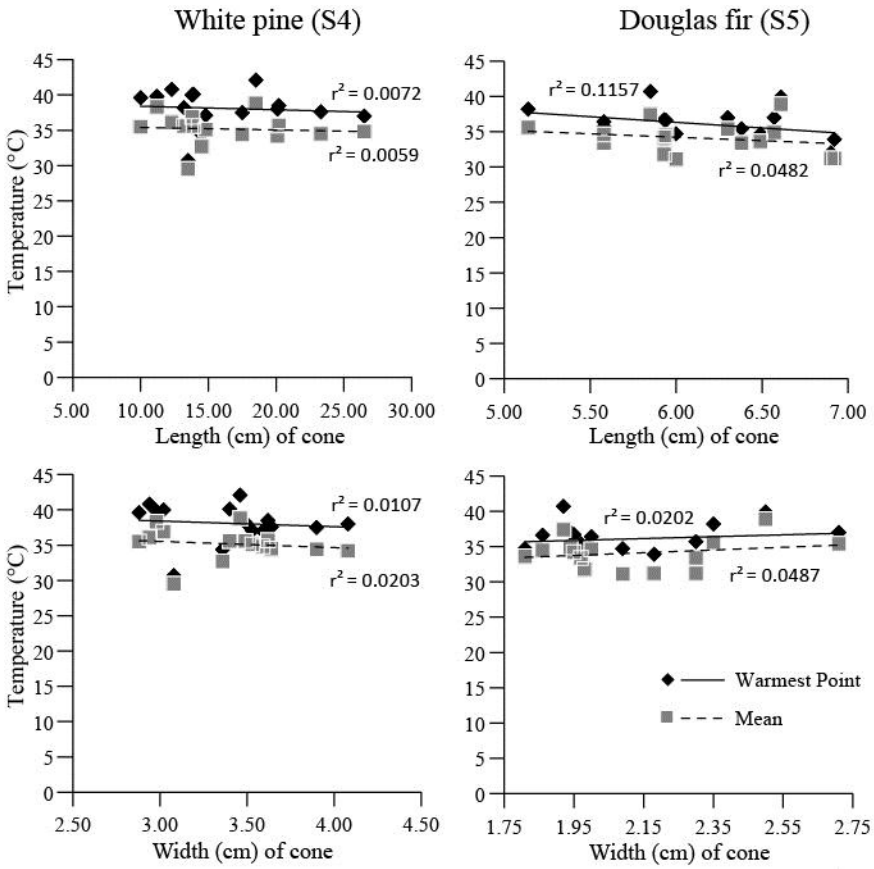
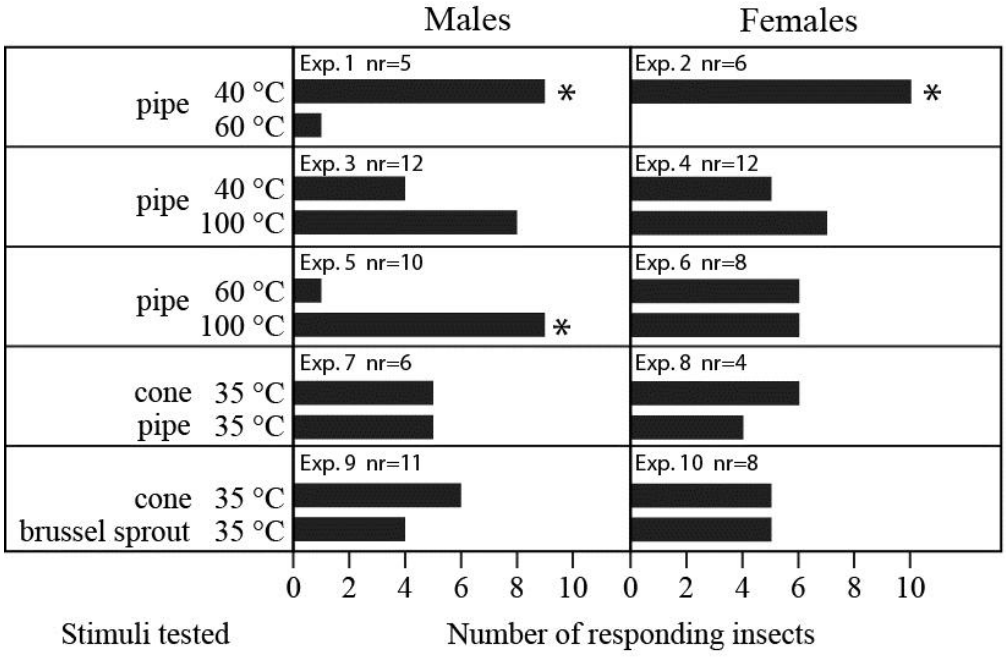

White pine (S4)
Douglas fir (S5)
Temperature (°C)
Length (cm) of cone
Width (cm) of cone
r² = 0.0072
r² = 0.0059
r² = 0.1157
r² = 0.0482
r² = 0.0107
r² = 0.0203
r² = 0.0202
r² = 0.0487
Warmest Point
Mean

Figure 5.5


Males
Females
Exp. 1 nr=5
Exp. 2 nr=6
pipe 40 °C
60 °C
Exp. 3 nr=12
Exp. 4 nr=12
pipe 40 °C
100 °C
Exp. 5 nr=10
Exp. 6 nr=8
pipe 60 °C
100 °C
Exp. 7 nr=6
Exp. 8 nr=4
cone 35 °C
pipe 35 °C
Exp. 9 nr=11
Exp. 10 nr=8
cone 35 °C
brussel sprout 35 °C
0 2 4 6 8 10 0 2 4 6 8 10
Stimuli tested
Number of responding insects

6. Retinal and behavioural responses by the Western conifer seed bug, *Leptoglossus occidentalis*[5]

6.1. Abstract

Insects exhibit wavelength-specific or -dependent behaviour provided they have at least two photoreceptors with overlapping spectral sensitivities in their eyes. Visual systems with multiple sensitivities to different wavelengths may also have regional specialization, with some regions more sensitive than others to particular wavelengths. Herein we investigated potential wavelength-specific retinal and/or behavioural responses of the conophagous Western conifer seed bug, *Leptoglossus occidentalis*, to light emitting diodes (LEDs), monochromatic light and OneLight spectra. Method-dependent, electroretinograms revealed spectral sensitivities in the blue (430-460 nm) region, green (480-530 nm) region, and possibly ultraviolet region. In two-choice behavioural bioassays, males preferred blue (433 nm) to red (621 nm) and to the

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absence of light. They avoided red light. Females preferred blue and green (505 nm) light to red light. In combination, these data support the conclusion that *L. occidentalis* receives different wavelengths and engages in wavelength-specific behaviour.

Keywords: electroretinogram; two choice-bioassay; conophagous; monochromatic light; light emitting diodes (LED); OneLight

6.2. Introduction

True colour vision requires discrimination and choice between two colours independent of intensity (Menzel 1979, Cutler et al. 1995, Kelber 1999, Nation 2002). In true colour vision, the brain integrates information of colour or hue to allow an animal to make choices based on this information (Nation 2002). Even when no choice is displayed, behavioural responses to specific wavelengths of light (wavelength-specific or -dependent behaviour) do not require a choice between colours but simply differential responses dependent on the wavelength of one stimulus (Menzel 1979, Cutler et al. 1995, Kelber 1999). The two criteria (neurophysiological output such as electroretinograms and behavioural response such as movement toward or away from a stimulus) that must be met to demonstrate wavelength-specific behaviour are maximal spectral sensitivities to one of several wavelengths, and behavioural responses to different wavelengths (Skorupski & Chittka 2011).

The methodologies developed for exploring spectral sensitivities comprise electroretinograms, intracellular optical recordings, microspectrophotometery (Briscoe & Chittka 2001), and inference of spectral sensitivities by DNA sequences (Osorio & Vorobyev 2008) or from behavioural responses. Examples of liht sources that can be used in electroretinograms include light emitting diodes (LEDs) (Cowan & Gries 2009, Chapter 4), monochromatic light (Groberman & Borden 1980, Meyer-Rochow 1981, Bennett et al. 1997, Townson et al. 1998, Chapter 3) and light with bandpass filters (Gilbert & Anderson 1996).

In some insects, spectral sensitivities may be shaped by important resources in their environment (Wallace 1878, Kevan and Backhaus 1998). For example, species-specific spectral arrangements of visual pigments in the eyes of sympatric species of *Lycanea* butterflies are thought to help in mate distinction (Bernard & Remington 1991). The swallowtail butterfly *Papilio aegeus* has spectral sensitivities in the red wavelength region, aiding discrimination of green foliage (Kelber 1999). In honeybees, sensitivity to green wavelengths of light mediates the detection of motion, shape and size recognition as well as depth perception (Giurfa *et al.* 1999, Kevan *et al.* 2001)

The butterfly *Pieris rapae* displays wavelength-specific behaviour in regards to feeding, egg-laying, drumming and open space locomotion. However, electrophysiological responses of eyes (Scherer & Kolb 1987) and maximal spectral sensitivities of visual pigments (Shimohigashi & Tominaga 1991) do not overlap with the bandwidth of visible light that elicits a behavioural response (Skorupski & Chittka 2011). Thus, a behavioural response to a certain bandwidth of visible light is not mediated by a specific photoreceptor spectrally sensitive to that wavelength (Skorupski & Chittka 2011) but by multiple receptors being stimulated at specific ratios. Wavelength discrimination requires two photoreceptors with overlapping spectral sensitivities so that different wavelengths of stimuli excite the two photoreceptors at different ratios (Skorupski & Chittka 2011). This ability to distinguish between two wavelengths at the same intensity is refered to as the wavelength discrimination function (Goldsmith et al. 1981).

The spectral contrast between conifer cones and foliage (Blatt & Borden 1999, Takács et al. 2009, Chapter 8), and the diverse and often contrasting cone colours (Copes 1972, Sturgeon & Mitton 1980), prompted us to predict that conophagous insects might exploit cone colour as a foraging cue. Male and female adults and 2nd to 5th instar

nymphs of the Western conifer seed bug, *Leptoglossus occidentalis* Heiderman, consume the contents of cone seeds, whereas 1st instars feed on needles (Krugman & Koerbezr 1969). Females lay eggs on the needles of conifer trees (Bates and Borden 2005). Cone-derived infrared (IR) radiation has recently been described to attract *L. occidentalis* (Takács et al. 2009), suggesting that other cone characteristics, such as colour, could complement radiant IR as a foraging cue.

Our objectives were to investigate retinal and behavioural responses by *L. occidentalis* to specific wavelengths of light.

6.3. Materials and methods

6.3.1. Experimental insects

Adult *L. occidentalis* were collected from conifer cones at the Kalamalka Forestry Centre (Ministry of Forests and Range), Vernon, BC, Canada (119°16′W, 50°14′N), and at the Sechelt Seed Orchard (Canadian Forestry Products), Sechelt, BC, Canada (123°43′W, 49°27′N). Specimens were provided with water and fresh cones of Western white pine (*Pinus monticola*), Douglas-fir (*Pseudotsuga menziesii*) or spruce (*Picea engelmanni* x *glauca*), and kept in mesh cages (47 × 47 × 92 cm) outdoors at Simon Fraser University. The insides of cages were misted with tap water daily. As *L. occidentalis* forages during the photophase, experiments were run between 10:00 and 17:00, from June to October in 2009 and 2010.

6.3.2. General design of electroretinogram experiments

Leptoglossus occidentalis, with their legs and antennae amputated, were immobilized laterally on glass slides with plasticine. An electrically sharpened (Cools et al. 1970) bare tungsten wire recording electrode (0.2 mm diameter; A-M Systems Inc., Carlsborg, WA, USA) was inserted with a micromanipulator (Leitz, Leica, Vienna, Austria) into the central region of the equatorial section of eyes facing upwards. An indifferent tungsten electrode was inserted between the third and fourth abdominal sterna. Eyes were dark-adapted by keeping insects in total darkness for 45 min before recordings. All recordings were made in a Faraday cage completely covered in black cloth to exclude both electrical noise and any light (Cowan & Gries 2009) other than that of test stimuli (see below). Electrical responses from eyes were pre-amplified (Syntech Auto Spike, Syntech, Hilversum, The Netherlands), processed (IDAC signal interface box, Syntech), and analyzed for amplitude with oscilloscope software (EAG, Version 2.4, Syntech).

Light emitting diodes (LEDs) served as one of three light sources for test stimuli. They were calibrated to an intensity of 5.0×10^{13} photons/cm^2/s at 2.5 cm from point source, using an HR4000 spectrometer with cosine collector (Ocean Optics, Dunedin, FL, USA) and SpectraSuite (Ocean Optics) software. They were powered by custom-built LED drivers [P. Kowalski, Science Technical Centre, Simon Fraser University (SFU)], each of which could adjust the intensity of up to eight LEDs. Calibrated LEDs were placed in front of the sub multi assembly (SMA) terminus of a 1000-µm single fiber optic cable (fused silica solarized UV resistant patch, Multimode Fiber Optics, Hackettstown, NJ, USA). The other end of the fiber optic was fitted with a collimator assembly (LC-4U-THD, Multimode Fiber Optics) that shone through a programmable

shutter (R. Holland, Science Technical Centre, SFU). The shutter was programmed to open for 500 ms every 9.5 s. When closed, it prevented light transmission, allowed LEDs to be changed and the eye to recover between stimuli. The insect's dark adapted eye can recover in < 2 s. Light transmitted through the open shutter was collected in a collimator assembly attached to a 1000-µm single fiber optic cable. The far terminus, which had only an SMA terminus (Multimode Fiber Optics), was held ca. 5 mm above the insect's right eye, in which the recording electrode was inserted.

A 35-watt Xenon light (Mikropak GmbH, Ostfildern, Germany) and a fiber optic scanning monochrometer (Monoscan2000, Ocean Optics, Dunedin, FL, USA) served as the second light source for test stimuli. Stimuli consisted of 31, 10-nm bandwidths tested consecutively at 10-nm increments from 345 nm to 655 nm. The technical set-up did not allow for randomly presented wavelengths. A 600-µm optical fibre (premium-grade solarized-resistant assembly, Ocean Optics) attached to the monochrometer transmitted light to a 0-2 stop circular variable neutral density wheel [Fused Silica (200 nm to 2500 nm), Reynard, Calle Sombra, San Clemente, California, USA] directly in front of a 70:30 beam splitter ("polka dot" 4-7001, Optometrics, Ayer, MA, USA). Thirty percent of a light beam was transmitted to the spectrometer, and calibrated such that each increment had an intensity of 3.0×10^{12} photons/cm^2/s at a distance of 0.5 cm from point source, using a spectrometer with SpectraSuite software. The remainder (70% or 7.0×10^{12} photons/cm^2/s) of the test stimulus was delivered to an eye as described above through a secondary fiber optic cable directly above the insect eye. For each bandwidth tested, data were standardized using the ratio to the highest response (set to 1.0) per eye, and then averaged between eyes (Kirchner et al. 2005).

The third light source for test stimuli was a OneLight instrument (from here on "OneLight") (Onelight Corp., Vancouver, BC, Canada), a light engine capable of replicating and modifying spectral profiles of target objects. Output intensity of the OneLight was set to 80%. Programmed spectra (see below) were projected onto a set of neutral density filters (see below) situated in front of a programmable shutter (R. Holland, Science Technical Centre, SFU) which when closed prevented light transmission. The shutter was programmed to open for 500 ms and to close for 9.5 s, during which the eye could recover and the next test spectrum be turned on. Directly beyond the programmable shutter was a 1000-µm single fiber optic cable (fused silica solarized UV resistant patch, Multimode Fiber Optics, Hackettstown, NJ, USA) fitted with a collimator assembly (LC-4U-THD, Multimode Fiber Optics). Its sub multi assembly (SMA) terminus was positioned ca. 5 mm above the insect's right eye.

To reduce traces of white light noise continuously emitted from the OneLight, ≤ 9 stops of neutral density filters were used during recordings. The Onelight continuously emits a low level of non-programmed light when turned on. This light cannot be eliminated from the spectral profiles emitted from the device. Eyes were first exposed for 500 ms to OneLight-emitted but neutral density-filtered (9 stops) light noise. If they did not respond, thus indicating no light noise, they were exposed for 500 ms to the first test spectrum. If they failed to respond to it because the light intensity was too low, the procedure was repeated with 8.5 stops of filters. This process was continued until eyes responded to the first test stimulus but not to the white light noise.

6.3.3. Electroretinogram experiments 1-3: spectral sensitivity of eyes

Experiments 1-3 were designed to determine the spectral sensitivity curve of eyes of *L. occidentalis.* In experiment 1, the eyes of five males and five females from an interior BC (Kalamalka Forestry Centre) and a coastal BC (Saanichton Seed Orchard) population were exposed to LEDs with peak wavelengths (nm) 370, 372, 376, 390, 400, 408, 425, 433, 470, 474, 480, 505, 527, 541, 567, 571, 572, 573, 590, 592, 595, 604, 610, 621, 636, 642, and 648 (Roithner LaserTechnic, Vienna, Austria) in a random order (assigned by a random number generator). Commercially available LEDs were selected for wavelengths representative of various sections of the electromagnetic spectrum. In experiment 2, the eyes of 10 males and 10 females from the Saanichton Seed Orchard were exposed to monochromatic light stimuli (see above). They consisted of 31 10-nm bandwidths consecutively tested at 10-nm increments from 345 nm to 655 nm. In experiment 3, the eyes of 10 males and 10 females from the Saanichton Seed Orchard were exposed to OneLight (see above) spectra. They consisted of 25 10-nm bandwidths consecutively tested at 10-nm increments from 400 nm to 650 nm. For each bandwidth tested, data were standardized using the ratio to the highest response per eye, and then averaged between eyes (Kirchner et al. 2005). Standardized responses for each wavelength were compared with a one-way ANOVA and post-hoc Tukey-Kramer test blocked by insect (Zar 1999) using JMP software (SAS®, Cary, NC, USA).

6.3.4. General design of two-choice behavioural experiments

All experiments were run in a cooled chamber designed to eliminate external thermal cues or radiant IR (Takács et al. 2009). The chamber consisted of a glass

aquarium (outside dimensions: 50.5 × 26.7 × 33.0 cm) which was covered with a glass lid (50.5 × 26.7 cm) and rested on 5-cm tall rubber stoppers inside a larger glass aquarium (outside dimensions: 61.0 × 33.0 × 41.0 cm), with cooled water (16 ± 2 °C) between them. A black PVC pipe (7 cm inside diameter; IPEX, Toronto, ON, Canada) was sealed with aquarium grade black silica (All Glass Aquarium, Franklin, WI, USA) between the two aquaria in each of the two end sections, allowing external stimuli to enter the inner aquarium without passing through water. A glass T-tube with a 2.5 cm diameter (Fig. 6.1) placed in the center of the inner aquarium was mounted on a stub which was held in place with black plasticine. After each replicate, the T-tube was baked over-night at 120°C to remove any potential pheromones adhering to it (Takács et al. 2009), and the inside of the inner aquarium and the lid were wiped with 70 % ethanol (Cowan & Gries 2009).

Experiments were run in the dark except for the light stimuli being tested. Single LEDs run by custom-built LED drivers (Pavel Kowalski, Science Technical Centre, SFU) were placed 5 cm apart from each of the two end sections of the outer aquarium behind a piece of glass which excluded LED-derived radiant IR (Hsieh & Su 1979) (Fig. 6.1A). LEDs were calibrated to an intensity of 1.0×10^{15} photons/cm^2/s at 1 cm from the source, using a spectrometer with cosine collector and SpectaSuite software.

For each replicate, the bug was acclimated for 30-60 min in darkness before being placed in the stem of the T-tube (Fig. 6.1B) which was then mounted on a stub inside the inner aquarium. Bugs that approached within 2.5 cm of the orifice of one of the T-tube's side arms within 2-30 min were considered responders. Experiments were terminated when 10 responders (18 in experiments 6 and 7) had been obtained. The

proportion of responders was compared using a Pearson's chi-square test (Zar 1999) using JMP software.

6.3.5. Two-choice behavioural experiments 4-19

To determine any asymmetry in T-tubes or the experimental set up, possibly inducing a biased response in bioassay insects, experiments 6 and 7 tested a white light sun LED on either distal end (Table 6.1).

Experiments 6-11 tested the attractiveness of a single light stimulus, consisting of an LED with peak-intensity wavelength of 505 nm, 433 nm or 621 nm (Table 6.1), of which the latter was outside the peak spectral sensitivity range of the insect (see Fig.6. 2), as determined in experiment 1-3.

Experiments 12-17 compared the relative attractiveness of LED light stimuli with a peak intensity wavelength within or outside the peak spectral sensitivity range of the insect eye (see above) (Table 6.1).

Experiments 18 and 19 compared two equal-intensity light stimuli, one stimulus being a 433-nm LED and the other monochromatic light with the same peak intensity wavelength of light (Table 6.1). Monochromatic, 428-nm to 438-nm light was produced by a Monoscan2000 fiber optic scanning monochrometer with a Xenon light source and transmitted through a 600-µm optical fibre (premium-grade solarized-resistant assembly, Ocean Optics) to one distal end of the aquarium.

6.4. Results

6.4.1. Electroretinogram experiments 1-3: Spectral sensitivity of eyes

The eyes of *L. occidentalis* are sensitive to wavelengths from 350 nm to 660 nm (Figure 6.2 A-C). The spectral sensitivity curve obtained in experiment 2, using the monochrometer, showed a predominant peak in the green (480-530 nm) region (Figure 6.2B). The spectral sensitivity curve obtained in experiment 3, using the OneLight, showed a predominant peak in the blue (430-460 nm) region (Figure 6.2C).

6.4.2. Two-choice behavioural experiments 4-19

In experiments 4 and 5, males and females revealed no side bias when test stimuli were identical (Figure 6.4; Pearson's chi-square test, males: $\chi^2 = 0.11$, $p = 0.74$, females: $\chi^2 = 0.45$, $p = 0.50$). In experiments 6 and 7, males and females responded equally to the 505-nm LED or to no light (males: $\chi^2 = 0.83$, $p = 0.36$; females: $\chi^2 = 0.00$, $p = 1.00$). In experiments 8 and 9, males ($\chi^2 = 3.81$, $p = 0.05$), but not females ($\chi^2 = 0.20$, $p = 0.65$), preferred the 433-nm LED to no light. In experiments 10 and 11, males ($\chi^2 = 3.81$, $p = 0.05$), but not females ($\chi^2 = 0.83$, $p = 0.36$), preferred no light to the 621-nm LED.

In experiments 12 and 13, females ($\chi^2 = 6.67$, $p = 0.01$), but not males ($\chi^2 = 0.83$, $p = 0.36$), preferred a 505-nm LED to a 621-nm LED. In experiments 14 and 15, both males ($\chi^2 = 6.67$, $p = 0.01$) and females ($\chi^2 = 6.67$, $p = 0.01$) preferred a 433-nm LED to

a 621-nm LED. In experiments 16 and 17, neither males (χ^2 = 0.00, p = 1.00) nor females (χ^2 = 0.20, p = 0.65) showed a preference for a 475-nm LED or a 505-nm LED.

In experiments 18 and 19, neither males (χ^2 = 0.20, p = 0.65) nor females (χ^2 = 0.00, p = 1.00) showed a preference for a 433-nm LED or monochromatic light with identical peak wavelength.

6.5. Discussion

In electroretinograms and two-choice behavioural bioassays, we investigated spectral sensitivities of the eyes of *L. occidentalis,* and behaviour associated with peak spectral sensitivities.

The various methods used to determine spectral sensitivities yielded different results. Method-dependent contrasting results were also obtained in studies with the green peach aphid, *Myzus periscae* (Kirchener et al., 2005). Dark-adapted eyes of *M. periscae* showed a sensitivity peak in the green region, white-adapted eyes exhibited another peak in the UV region, and yellow- adapted eyes revealed a peak in the blue region due to bleaching of the green receptor (Kirchener et al., 2005). Exposure of dark-adapted *L. occidentlis* eyes to monochrometer-derived light revealed a sensitivity peak in the green region, and possibly the UV region (Figure 6.2B). Eye-exposure to OneLight-derived stimuli, which contained some light contamination, may have sufficiently bleached the eyes' green-light sensitivity for blue spectral sensitivity to be revealed (Figure 6.2C). Alternatively, the results may be an artifact of the Bezold-Brücke effect, and insects perceived the same hue differently due to differences in intensity of the light sources (Backhaus 1992).

Other conophagous insects appear to share spectral sensitivity characteristics with *L. occidentalis*. The peak sensitivity in the green region in *L. occidentalis* (480-530 nm; Figure 6.2) may also be present in the conophagous nocturnal moth *D. abietivorella* (Chapter 4). Moreover, like male and female *L. occidentalis* (Figure 6.3, Exps. 13, 14), male and female *D. abietivorella* also orient toward a blue 433-nm wavelength (Chapter 4), implying recognition of the same foraging cue. It is uncertain, though, why these two species, and also the hairy rose beetle, *Tropinota sqaulida* (Ali 1993), are more strongly attracted to blue wavelengths of light than to other colours.

Leptoglossus occidentalis is sensitive to light in the red region (Figure 6.2). The swallowtail butterfly *Papilio aegeus* has spectral sensitivity in the red range with a visual receptor sensitive to red light. This is thought to aid in the discrimination of green foliage, allowing *P. aegeus* to find young shoots (Kelber, 1999). Similarly, the nymphalid butterfly *Heliconius erato* can see light in the red region, although it has only one long-wavelength-sensitive visual pigment. This is believed to be possible due to filtering pigments in the eye close to the rhadome (Zaccardi et al. 2006). Spectral sensitivity of *L. occidentalis* in the red range (Figure 6.2), and avoidance of 621-nm red light (Figure 6.4, Exp. 10), may help *L. occidentalis* discriminate against over-ripe cones which turn reddish brown when they have dispersed their seeds and are no longer a profitable food source. The relatively high number of non-responding insectsin the laboaratory may be due to reluctance to move in an artificial settings or may be due to ambient temperature cooler than in the field.

In summary, we present evidence (1) that the eyes of *L. occidentalis* are sensitive to wavelengths from 350 nm to 660 nm with peak spectral sensitivities in the green and UV region, and (2) that males and females show different behavioural

responses to different wavelengths of light. Combined these data support the hypothesis that *L.occidentalis* receives multiple wavelengths, some of which elicit behavioural responses. Thus visible light may be used during foraging to locate resources.

6.6. Literature cited

Ali MA (1993) Influence of trap colors and placement on captures of the hairy rose beetle, *Tropinota squalida* Scop. (Coleoptera, Scarabaeidae). Insect Science and its Application 14: 215-219.

Backhaus W (1992) The Bezold-Brücke effect in the color vision system of honeybees. Vision Research 32: 1425-1431.

Bates SL & Borden JH (2005) Life table for *Leptoglossus occidentalis* Heidermann (Heteroptera: Coreidae) and prediction of damage in lodgepole pine seed orchards. Agricultural and Forest Entomology 7: 145–151.

Bennett RR, White RH & Meadows J (1997) Regional specialization in the eye of the sphingid moth *Manduca sexta*: Blue sensitivity of the ventral retina. Visual Neuroscience 14: 523–526.

Bernard GD & Remington CL (1991) Colour vision in *Lycanena* butterflies: Spectral tuning of receptor arrays in relation to behavioural ecology. Proceedings of the National Academy of Sciences of the United States of America 88: 2783–2787.

Blatt SE & Borden JH (1999) Physical characteristics as potential host selection cues for *Leptoglossus occidentalis* (Heteroptera: Coreidae). Environmental Entomology 28: 246–254.

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

Cowan T & Gries G (2009) Ultraviolet and violet light: attractive orientation cues for the Indian meal moth, *Plodia interpunctella*. Entomologia Experimentalis et Applicata 131: 148–158.

Cutler DE, Bennett RR, Stevenson RB & White RH (1995) Feeding behaviour 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.

Gilbert HL & Anderson M (1996) The spectral efficiency of the eye of *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae). Journal of Stored Products Review 32: 285–291.

Giurfa M Zaccardi G & Vorobyev M (1999) How bees detect coloured targets using different regions of the compound eye. Journal of Comparative physiology A 185: 591-600.

Goldsmith TH, Collins JS & Perlman JL (1981) A wavelength discrimination function for the hummingbird *Archilochus alexandri*. Journal of Comparative Physiology A 143: 103-110.

Groberman LJ & Borden JH (1980) Electrophysiological response of *Dendroctonus pseudotsgugae* and *Ips paraconfusus* (Coleoptera: Scolytidae) to selected wavelength regions of the visible spectrum. Canadian Journal of Zoology 60: 2180–2189.

Hsieh CK & Su KC (1979) Thermal radiative properties of glass from 0.32 to 106 microns. Solar Energy 22: 37–43.

Kelber A (1999) Ovipositing butterflies use a red receptor to see green. The Journal of Experimental Biology 202: 2619–1630.

Kevan PG & Backhaus WGK (1998) Color vision: Ecology and evolution in making the best of photic environment. In WGK Backhaus R Kliegl & JS Werner (Eds.) Color Vision: Perspectives from different disciplines. Berlin, Germany, Walter de Gruyter.

Kevan, PG, Chittka L, and Dyer G. (2001) Limits to the salience in ultraviolet: Lessons from colour vision in bees and birds. Journal of Experimental Biology 204: 2571-2580.

Kirchner SM, Döring TF & Saucke H (2005) Evidence for trichromacy in green peach aphid, *Myzus periscae* (Sulz.) (Hempitera: Aphidae). Journal of Insect Physiology 51: 1255-1260.

Krugman SL & Koerber TW (1969) Effects of cone feeding by *Leptoglossus occidentalis* on ponderosa pine seed development. Forest Science 15: 104–111.

Menzel R (1979) Spectral sensitivity and colour vision in invertebrates. Handbook of Sensory Physiology, vol. VII/6A (ed. H. Autrum). Berlin, Heidelberg, New York: Springer.

Meyer-Rochow VB (1980) Electrophysiologically determined spectral efficiencies of the compound eye and median ocellus in the bumblebee *Bombus hortorum tarhakimalainen* (Hymenoptera, Insecta). Journal of Comparative Physiology A 139: 261–266.

Nation JL (2002) Insect physiology and biochemistry. CRC Press, Boca Raton, Florida, USA.

Osorio D & Vorobyev M (2008) A review of the evolution of colour vision and visual communication signals. Vision Research 48: 2042–2051.

Scherer C & Kolb G (1987) Behavioural experiments on the visual processing of colour stimuli in *Pieris brassicae* (Lepidoptera). Journal of Comparative Physiology A Sensory and Behavioural Physiology 160: 645-656.

Shimohigashi M & Tominaga Y (1991) Identification of UV, green and red receptors, and their projection to lamina in the cabbage butterfly, *Pieris rapae*. Cell and Tissue Research 263: 49-59.

Skorupski P & Chittka L (2011) Is colour cognitive? Optics and Laser Technology 43: 251-260.

Strong WB, Bates SL & Stoehr MU (2001) Feeding by *Leptoglossus occidentalis* (Hemiptera: Coreidae) reduces seed set in lodgepole pine (Pinaceae). The Canadian Entomologist 133: 857–865.

Sturgeon KB & Mitton JB (1980) Cone color polymorphism associated with elevation in white fir, *Abies concolor,* in Southern Colorado. American Journal of Botany, 67: 1040–1045.

Townson TM, Chang BSW, Salcedo E, Chadwell LV, Pierce NE & Britt SG (1998) Honeybee blue- and ultraviolet-sensitive opsins: Cloning heterologous expression in *Drosophila* and physiological characterization. The Journal of Neuroscience 18: 2412–2422.

Wallace AR (1878) Tropical nature and other essays. London, UK: Macmillan and Company.

White RH, Xu HH, Münch TA, Bennett RR & 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.

Zaccardi G, Kelber A, Sison-Mangus MP & Briscoe AD (2006) Colour discrimination in the red range with only one long-wavelength sensitive opsin. The Journal of Experimental Biology 209: 1944–1955.

Zar JH (1999) Biostatistical Analysis, 4th ed. Prentice Hall, Upper Saddle River, NJ.

Table 6.1: Stimuli and sex of *Leptoglossus occidentalis* tested in experiments 4-19.

Exp.	Sex	Stimulus A	Stimulus B
4	male	white light LED	white light LED
5	female	white light LED	white light LED
6	male	505-nm LED	no LED
7	female	505-nm LED	no LED
8	male	433-nm LED	no LED
9	female	433-nm LED	no LED
10	male	621-nm LED	no LED
11	female	621-nm LED	no LED
12	male	505-nm LED	621-nm LED
13	female	505-nm LED	621-nm LED
14	male	433-nm LED	621-nm LED
15	female	433-nm LED	621-nm LED
16	male	505-nm LED	473-nm LED
17	female	505-nm LED	473-nm LED
18	male	433-nm LED	433-nm monochrometer
19	female	433-nm LED	433-nm monochrometer

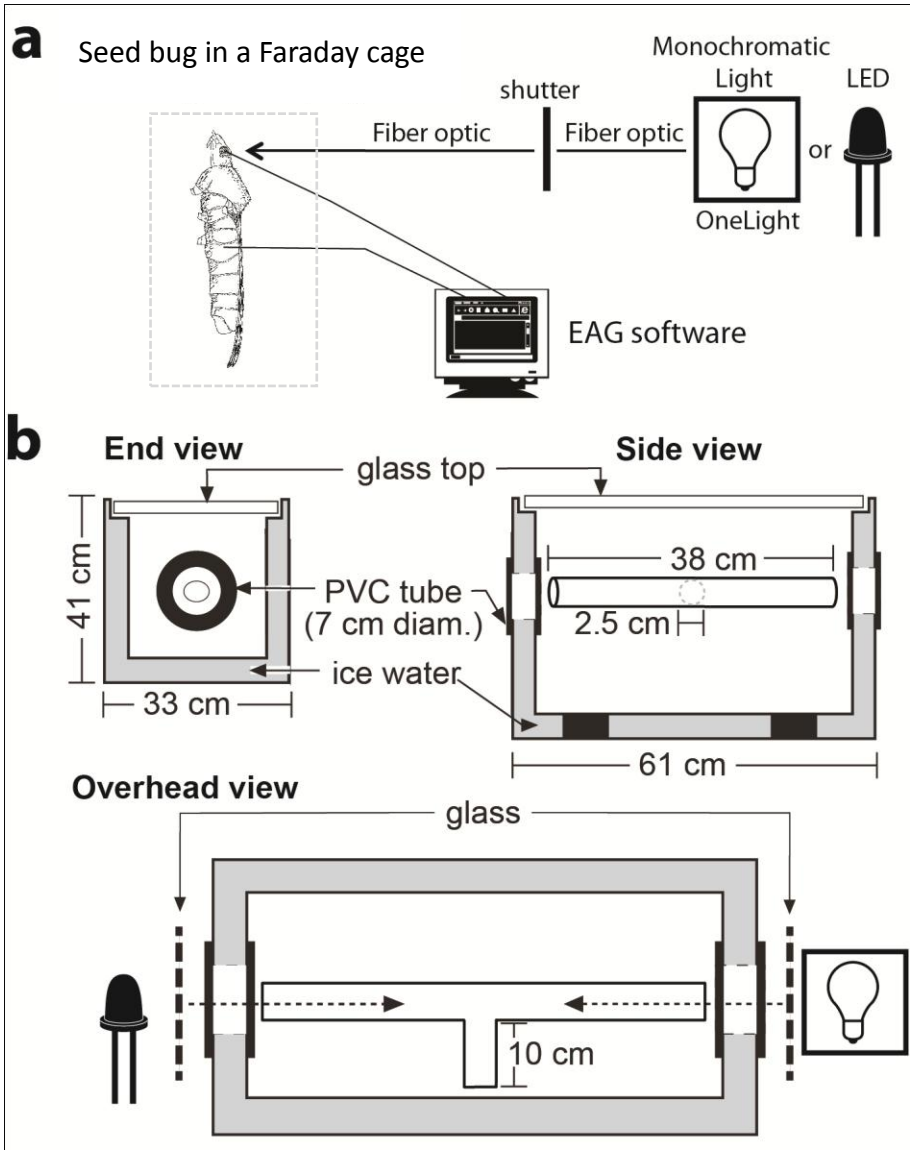
6.7. Figure captions

Figure 6.1. Drawings of the experimental design in (a) electrophysiological recordings and (b) laboratory experiments 4-19; see methods for details.

Figure 6.2. Mean (+ SE) retinal responses (standardized to 1.0 of the highest response for each eye) obtained in electroretinograms from eyes of 10 male and 10 female *Leptoglossus occidentalis*, exposed to (A) light-emitting diodes (LEDs), (B) monochromatic light (at 10-nm bandwidth) or (C) OneLight spectra (at 15-nm bandwidth) as test stimuli.

Figure 6.3. Number of male and female *Leptoglossus occidentalis* responding in two-choice laboratory experiments 4-19 to various light stimuli. In each experiment, bars with an asterisk indicate a significant preference for the test stimulus (Pearson's chi-square test, $p < 0.05$). nr denotes the number of non-responding insects; nr = non-responding insects.

Figure 6.1


a
Seed bug in a Faraday cage
Monochromatic Light
LED
shutter
Fiber optic
Fiber optic
or
OneLight
EAG software
b
End view
glass top
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41 cm
PVC tube
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38 cm
2.5 cm
ice water
33 cm
61 cm
Overhead view
glass
10 cm

Figure 6.2

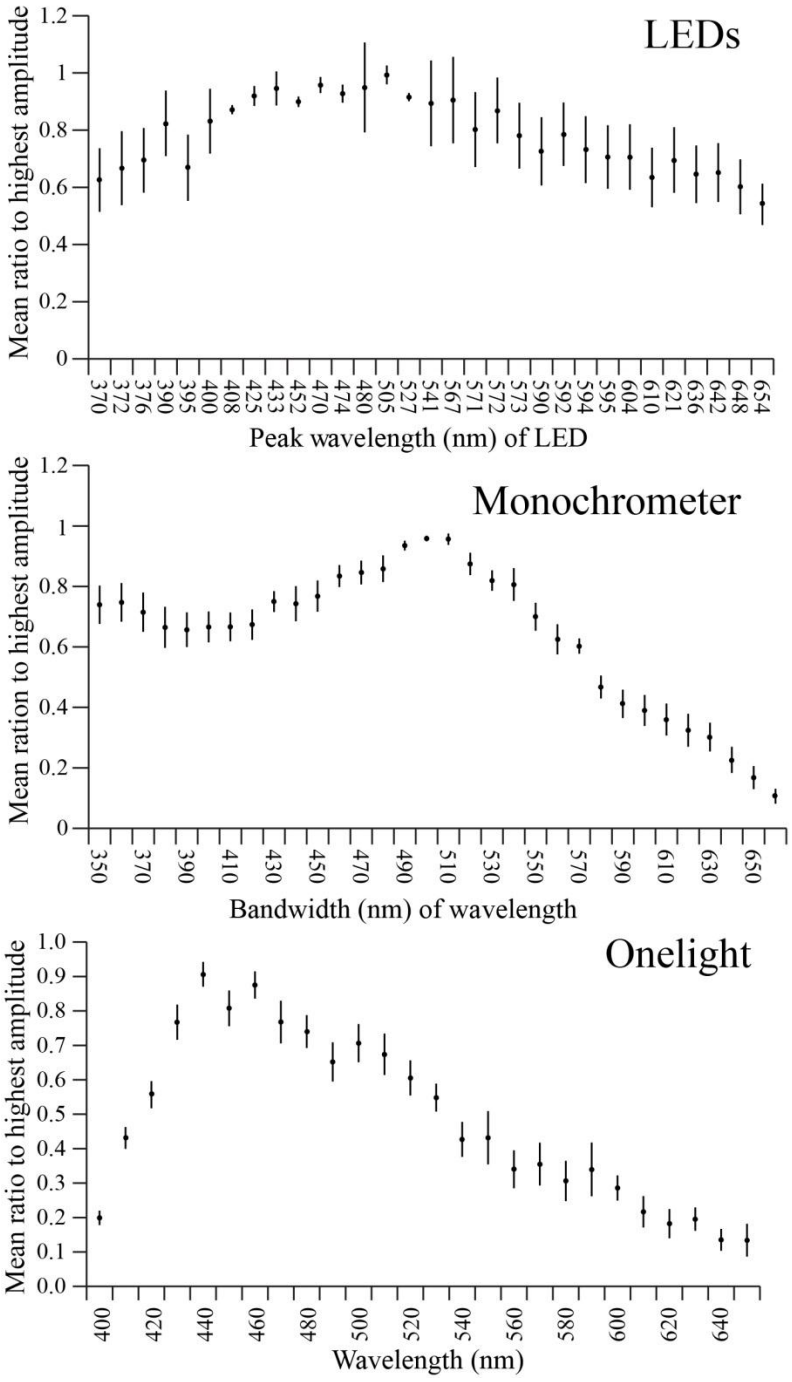
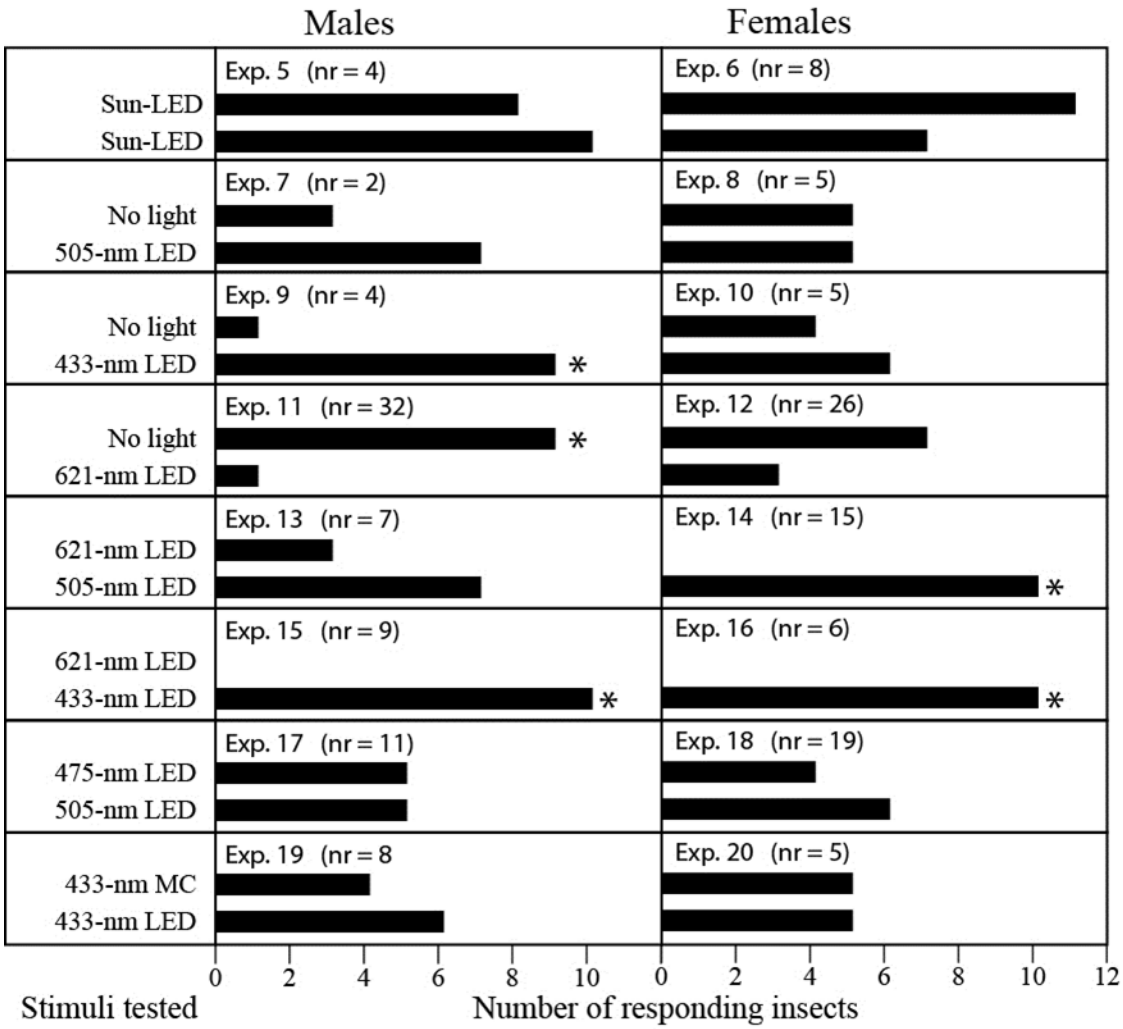

LEDs
Mean ratio to highest amplitude
1.2
1
0.8
0.6
0.4
0.2
0
370 372 376 390 395 400 408 425 433 452 470 474 480 505 527 541 567 571 572 573 590 592 594 595 604 610 621 636 642 648 654
Peak wavelength (nm) of LED
Monochrometer
Mean ration to highest amplitude
1.2
1
0.8
0.6
0.4
0.2
0
350 370 390 410 430 450 470 490 510 530 550 570 590 610 630 650
Bandwidth (nm) of wavelength
Onelight
Mean ratio to highest amplitude
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
400 420 440 460 480 500 520 540 560 580 600 620 640
Wavelength (nm)

Figure 6.3


Males
Females
Exp. 5 (nr = 4)
Sun-LED
Sun-LED
Exp. 6 (nr = 8)
Exp. 7 (nr = 2)
No light
505-nm LED
Exp. 8 (nr = 5)
Exp. 9 (nr = 4)
No light
433-nm LED
*
Exp. 10 (nr = 5)
Exp. 11 (nr = 32)
No light
621-nm LED
*
Exp. 12 (nr = 26)
Exp. 13 (nr = 7)
621-nm LED
505-nm LED
Exp. 14 (nr = 15)
*
Exp. 15 (nr = 9)
621-nm LED
433-nm LED
*
Exp. 16 (nr = 6)
*
Exp. 17 (nr = 11)
475-nm LED
505-nm LED
Exp. 18 (nr = 19)
Exp. 19 (nr = 8
433-nm MC
433-nm LED
Exp. 20 (nr = 5)
0 2 4 6 8 10
0 2 4 6 8 10 12
Stimuli tested
Number of responding insects

7. Females of the Western conifer seed bug, *Leptoglossus occidentalis* (Hemiptera: Coreidae), are attracted to visible light spectra (400-640 nm) of Western white pine needles[6]

7.1. Abstract

We investigated electrophysiological and behavioural responses of an insect herbivore, the Western conifer seed bug (*Leptoglossus occidentalis*), to visible light spectra of various plant parts, in the absence of plant shape, size or odour to determine whether colour alone is an attractive trait of plant parts. This was made possible with the advent of Onelight Spectra (OneLight) technology, a light engine capable of reproducing and modifying electromagnetic spectra of objects. Female *L. occidentalis* seek conifer

[6]Authors: Tracy Zahradnik, Ward Strong, Gerhard Gries. This Chapter has been formatted for the requirements of Entomologia Experimentalis et Applicata.

needles as oviposition sites, but they feed on the contents of cone seeds as do adult males and 2nd to 5th instar nymphs. We hypothesized that the complete visible light spectrum of conifer [White pine (*Pinus monticola*)] needles guides females to oviposition sites. In electrophysiological recordings, the spectrum of White pine needles induced stronger retinal responses from eyes of females than did spectra of White pine or Douglas-fir (*Pseudotsuga menziesii*) cones. In two-choice bioassays, white pine needles attracted females but not males, suggesting a functional role in the context of oviposition rather than of food-foraging. The complete spectrum of White pine needles was effective in attracting females, but the yellow/green 562- to 582-nm section of the spectrum seemed particularly important, because a spectrum lacking this section was not attractive to females. As a novel technology, the OneLight comes with challenges, such as an inability to produce ultraviolet wavelengths and low-level white light noise, but it offers new opportunities to investigate colour vision in vertebrates and invertebrates.

Keywords: spectral profiles; foraging cue; oviposition; light emitting diode; monochrometer; OneLight Spectra

7.2. Introduction

Insects are capable of true colour vision and behavioural responses to specific wavelengths of light (wavelength-specific behaviour) (Kevan & Backhaus 1998, reviewed by Briscoe & Chittka 2001, Kelber et al. 2003a, Osorio & Vorobyev 2008). Their behavioural response to colour has been tested with light sources or paints, which are wide-band and limited to broad categories of colour such as blue or green, regardless of hue(s) emitted or reflected (Vernon & Bartel 1985, Kamm et al. 1991, Kelber et al. 2003b, Chu et al. 2005, Blackmer et al. 2008, Cowan & Gries 2009). Testing such stimuli may discount the complication that some surfaces may appear different to human and to insect eyes, and may reflect wavelengths outside the intended test range (Vukusic 2010). Also, complex spectra appear more attractive than simple spectra (Blatt & Borden 1999), further adding to the complexity of light research.

Honey bees more easily learn to be attracted to visual cues that are more natural and meaningful to their evolutionary history (Clarke & Lotto 2009). Realizing the importance of full spectrum reflectance for pollinators (Chittka & Menzel 1992), the Floral Reflectance Spectral Database was established which provides complete electromagnetic spectra of various flower types and parts (Arnold et al. 2008), and allows correlation of this information to the guild of respective pollinators (Chittka & Menzel 1992). For example, peak spectral sensitivities of Hymenoptera are linked to the evolutionary fine-tuning of flower colour (Chittka & Menzel, 1992). Butterflies also prefer certain flower colours (Yurtserver et al. 2010), and have diverse, almost species-specific

arrangements of visual pigments in their eyes (Awata et al. 2010), perhaps with spectral sensitivities evolutionarily tuned to colour patterns, similar to the Hymenoptera.

The resource-tuned spectral sensitivities of insect eyes could be a widespread phenomenon (Wallace 1878, Kevan and Backhaus 1998) and may occur in members of the conophagous insect guild, such as the Western conifer seed bug, *Leptoglossus occidentalis* Heiderman. This bug has a broad spectral sensitivity in the visible light range (Chapter 6), and may exploit the spectral contrast between conifer cones and foliage as foraging cues (Blatt & Borden 1999). Adults and 2nd to 5th instar nymphs consume the contents of seeds (Krugman & Koerber 1973, de Groot et al. 1994), whereas 1st instars feed on conifer needles (Krugman & Koerbezr 1969). Females seek needles as oviposition sites (Bates & Borden 2005).

The recent advent of the OneLight Spectra (from here on “OneLight”) (Onelight Corp., Vancouver, BC, Canada), a light engine capable of replicating and modifying spectral refelectance profiles of target objects, has made it possible to investigate behavioural responses of insect herbivores to human visible (henceforth “visible”) spectral profiles of plant or plant parts, excluding their shape, size or odor. The OneLight represents the first technology capable of recreating the visible spectra using reflectance measures from a spectrometer. The visible spectromatic profile of any object can be programed into the OneLight to generate output. However, the OneLight comes with challenges including a narrow wavelength range, flickering light at < 80% output, considerable heat, and low but persistent white light noise.

Herein we tested the hypothesis that the complete visible light spectrometric profile of conifer cones or needles induces stronger retinal and behavioural responses of

L. occidentalis than do narrow-bandwidth sections of such profiles or complete visible light spectra of non-host plants.

7.3. Materials and methods

7.3.1. Leptoglossus occidentalis

Adult *L. occidentalis* were collected from cones at the Kalamalka Forestry Centre (Ministry of Forests and Range), Vernon, BC, Canada (119°16′W, 50°14′N) and the Sechelt Seed Orchard (Canadian Forestry Products), Sechelt, BC, Canada (123°43′W, 49°27′N). Specimens were provisided with water and fresh cones of Western white pine (*Pinus monticola*), Douglas-fir (*Pseudotsuga menziesii*) or spruce (*Picea engelmanni* x *glauca*) and kept outdoors at Simon Fraser University in wooden cages with mesh-covered ventilation holes (47 × 47 × 92 cm). The insides of the cages were misted daily. Because *L. occidentalis* forages during the photophase, behavioural experiments were run between 10:00 and 17:00 h, from August to October 2010.

7.3.2. Electroretinograms: experiments 1-3

For electrophysiological recordings, legs and antennae of seed bugs were amputated and bugs were restrained laterally left-side down on glass slides by using plasticine. An electrically sharpened (Cools et al. 1970) bare tungsten wire electrode (0.2 mm inside diameter; A-M Systems Inc., Carlsborg, WA, USA) was inserted with a micromanipulator (Leitz, Leica, Vienna, Austria) into the central region of the equatorial section of the right eye. Another tungsten electrode was inserted into the lateral side

between the third and fourth abdominal sterna (Fig. 7.1a). Eyes were dark-adapted by keeping insects in total darkness for 45 min before recordings. All recordings were made in a Faraday cage completely covered in black cloth to exclude both electrical noise and any light (Cowan & Gries 2009) other than that of test stimuli (see below). Electrical responses from eyes were pre-amplified (Syntech Auto Spike, Syntech, Hilversum, The Netherlands), processed (IDAC signal interface box, Syntech), and analyzed for amplitude with oscilloscope software (EAG, Version 2.4, Syntech).

For experiments 1 and 2, spectra in the 400- to 650-nm range were recorded from natural items found in seed orchards, including Western white pine needles, branches, and cones, Douglas-fir cones, and dandelion (*Taraxacum officinale*) flowers (Fig. 7.2). Spectra were obtained with an HR4000 spectrometer (Ocean Optics, Dunedin, FL, USA) and SpectraSuite (Ocean Optics) software, and programmed into the OneLight. Eyes of 10 males (Exp. 1) and 10 females (Exp. 2) were exposed to synthesized OneLight spectra in random order.

Output spectra from the OneLight were calibrated to an intensity of 1.0×10^{14} photons/cm^2/s 2.5 cm from the fiber optic output cable of the OneLight, using the HR4000 spectrometer, cosine collector and SpectraSuite. Standardized responses to each stimulus were compared with a one-way ANOVA and post-hoc Tukey-Kramer test blocked by insect (Zar 1999) using JMP software (SAS®, Cary, NC, USA).

7.3.3. Two-choice behavioural experiments 3-12

All experiments were run in a cooled chamber designed to eliminate external thermal cues or radiant IR (Takács et al. 2009). The chamber consisted of a glass aquarium (outside dimensions: 50.5 × 26.7 × 33.0 cm) which was covered with a glass lid (50.5 × 26.7 cm) and rested on 5-cm tall rubber stoppers inside a larger glass aquarium (outside dimensions: 61.0 × 33.0 × 41.0 cm), with cooled (16 ± 2 °C) water between them. A black PVC pipe (7 cm inside diameter; IPEX, Toronto, ON, Canada) was sealed with aquarium grade black silica (All Glass Aquarium, Franklin, WI, USA) between the two aquaria in each of the two end sections, allowing external stimuli to enter the inner aquarium without passing through water. A glass T-tube with a 2.5 cm diameter (Fig. 7.1b) placed in the center of the inner aquarium was mounted on a stub which was held in place with black plasticine. After each replicate, the T-tube was baked overnight at 120°C to remove any potential pheromones adhering to it (Takács et al. 2009), and the inside of the inner aquarium and the lid were wiped with 70 % ethanol (Cowan and Gries 2009).

Experiments were run in darkness except for light stimuli that were being tested. Stimuli consisted of either a single LED with peak wavelength 505 nm or 573 nm (Roithner LaserTechnik, Vienna, Austria) controlled by custom-built LED drivers (P. Kowalski, Science Technical Centre, SFU), or a programmed OneLight-emitted light spectrum. Stimuli were randomly assigned (with a coin flip) to, and placed 5 cm from, one of the two end sections of the outer aquarium, behind a piece of glass to exclude radiant IR (Hsieh & Shu 1979) from the test stimulus (Fig. 7.1a). Light sources were calibrated to an intensity of 1.0×10^{15} photons/cm^2/s at 1 cm from the source, using a spectrometer with cosine collector and SpectaSuite software.

For each replicatetest, a bug was acclimated for 30-60 min in darkness before being placed in the opening of the 10-cm portion of the T-tube (Fig. 7.1b). Bugs that approached within 2.5 cm of the distal end of one of the T-tube's side arms within 2-30 min were considered responders. Each experiment was terminated when 10 responders had been obtained. The proportion of responders was compared using a Pearson's chi-square test (Zar 1999) using JMP software.

Experiments 3 and 4 (Table 7.1) tested the choice of male and female *L. occidentalis* between an LED with peak wavelength 505 nm and the equivalent spectrum emitted from the OneLight. Experiments 5-12 (Table 7.1) tested the choice of male and female *L. occidentalis* between a 572-nm LED and profiles of Douglas-fir cones and Western white pine needles, with or without wavelengths 562-582 nm.

7.3.4. Relative engery calcuations

Relative energy calculations were taken for each of the five spectral profiles. Relative energy was calculated using the formula $E=(h*c/\lambda)*$relative intensity count, wherein E is engery, h is plank's constant, c is the speed of light and λ is wavelength.

7.4. Results

7.4.1. Electroretinogram experiments 1-2

Complete visible light spectra of White pine bark, cones and needles, Douglas-fir cones, and dandelion flowers (Fig. 7.2) elicited different response amplitudes by eyes of both males (Exp. 2) and females (Exp. 3) (one-way ANOVA; males: $F_{(4,49)} = 6.95$, p

=0.0003; females: $F_{(4,49)} = 13.77$, $p < 0,0001$) (Fig. 7.3). Spectra of Western white pine needles and bark elicited stronger responses from eyes of females than did spectra of Douglas-fir cones (Tukey test: needles, $p = 0.0003$; bark, $p = 0.015$), White pine cones (Tukey test: needles, $p = 0.0004$; bark, $p = 0.016$), and dandelion flowers (Tukey test: needles, $p < 0.0001$ 0.001; bark, $p = 0.0007$) (Fig. 7.3). Spectra of White pine needles and bark elicited stronger responses from eyes of males than did spectra of dandelion flowers (Tukey test: needles, $p = 0.00123$; bark, $p = 0.017$), and Douglas-fir cones (Tukey test: needles, $p = 0.003$; bark, $p = 0.033$) (Fig. 7.3)

7.4.2. Two-choice behavioural experiments 3-12

Males (Exp. 3) and females (Exp. 4) were equally attracted to a 505-nm LED and an equivalent spectrum emitted by the OneLight (Pearson's chi-square test: males, $\chi^2 = 0.20$, $p = 0.65$; females, $\chi^2 = 0.20$, $p = 0.65$) (Fig. 7.4). Males (Exp. 5) and females (Exp. 6) were equally attracted to a 572-nm LED and the spectrum of a Douglas-fir cone (Pearson's chi-square test: males, $\chi^2 = 0.20$, $p = 0.65$; females, $\chi^2 = 0.83$, $p = 0.36$) (Fig. 7.4). Males (Exp. 6) and females (Exp. 7) were equally attracted to a 572-nm LED and the spectrum of a Douglas-fir cone lacking wavelengths 562-582 nm (Pearson's chi-square test: males, $\chi^2 = 0.83$, $p = 0.36$; females, $\chi^2 = 0.20$, $p = 0.65$) (Fig. 7.4). When given a choice between a 572-nm LED and the spectrum of Western white pine needles, females (Exp. 10), but not males (Exp. 9), preferred the latter (Pearson's chi-square test: males: $\chi^2 = 0.20$, $p = 0.65$; females: $\chi^2 = 3.81$, $p = 0.05$) (Fig. 7.4). However, females (Exp. 12) and males (Exp. 11) were equally attracted to a 572-nm LED and the spectrum of Western white pine needles lacking wavelengths 562-582 nm (Pearson's chi-square test: males, $\chi^2 = 0.20$, $p = 0.65$; females, $\chi^2 = 0.83$, $p = 0.36$) (Fig. 7.4)

7.4.3. Relative energy calcuations

The spectrum of White pine bark had the least relative energy followed by the the spectra of the dandelion flower and White pine needles. The spectra of White pine cones and Douglas-fir cones had the most relative engery (Table 7.2).

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7.5. Discussion

The advent of OneLight technology made it possible to reproduce and modify spectral profiles of target objects, and to investigate responses of *L. occidentalis* to complete or partial electromagnetic spectra of natural resources including conifer cones and needles.

Our data support the hypothesis that the complete visible light spectrum of White pine needles (Fig. 7.2) induces stronger retinal or behavioural responses of female *L. occidentalis* than does a narrow-bandwidth section of the needle spectrum or the complete spectrum of a non-host flower (Fig. 7.4). Unexpectedly, though, complete spectra of White pine or Douglas-fir cones elicited relatively weak retinal responses (Fig. 7.3), and the complete spectrum of Douglas-fir cones failed to attract *L. occidentalis* in two-choice experiments (Exps. 5, 6; Fig. 7.4). These results suggest that the eyes of *L. occidentalis* are not evolutionarily tuned to cone colour, and that cone colour as a singular foraging cue is ineffective, even though it contrasts well against foliage (Blatt &

Borden 1999, Takács et al. 2009, Fig. 7.2). The same conclusion applies to the Douglas-fir cone gall midge, *Contarinia oregonensis*, which is not attracted to any type of cone colour (Chapter 2).

The spectra of White pine needles and White pine bark induced a stronger electrophysiological response than the spectra of White pine cones or Douglas fir cones (7.4) even though the spectra of the former had less relative energy than the latter (Table 7.2). This supports the conclusion that eyes of *L. occidentalis* may be spectrally tuned to locate White pine needles or bark.

The relatively high number of non-responding insects in the laboaratory may be due to reluctance to move in an artificial setting or may be due to ambient temperature cooler than in the field.

The complete visible light spectrum of White pine needles may guide female *L. occidentalis* to oviposition sites. In electrophysiological recordings, it induced stronger retinal responses from females than did spectra of White pine or Douglas-fir cones (Fig. 7.3), and it attracted females in two-choice bioassays (Fig. 7.4, Exp. 10). Its functional role in the context of oviposition, rather than food-foraging, is also supported in that males failed to orient toward the needle spectrum (Fig. 7.4, Exp. 9). Our conclusion that females are truly attracted to the White pine needle spectrum is further supported by observations that this stimulus had the lowest number of non-responders.

Although the complete spectrum of White pine needles is effective in attracting females, some sections of the spectrum seem important for the recognition of needles. This inference was supported by an experimental modification of the spectrum. When

we removed the yellow-green, 562- to 582-nm section from it (Fig. 7.2), we rendered it unattractive to females (Fig. 7.4, Exp. 12).

OneLight spectra cover a narrower wavelength range (400-640 nm) than do LEDs or monochrometers. Our instrument was custom-shifted towards UV light (400-640 nm) but could still not emit UV light, which is part of light spectra of many natural sources (Kevan et al. 2001), and which on its own or in combination with visible light attracts many insects (Roe et al. 2006, Whitehouse et al. 2011). Foraging bees, *Apis mellifera*, in particular respond to UV and visible light from flowers (Kevan et al. 2001; Dyer & Chittka 2004). Weed flowers, such as the dandelion tested in this experiment, have an UV component in their spectrum which is attractive to pollinators (Mulligan & Kevan 1973). This missing UV component may have affected our results.

Programmed OneLight spectra set to < 80% light output exhibited distinct flickering, a phenomenon which was less pronounced or only intermittent at > 80% light intensities. Nonetheless, if not noticed or addressed, such a phenomenon could complicate the interpretation of results. Houseflies, *Musca domestica*, e.g., respond more strongly to flickering than to non-flickering UV light, even if the latter has stronger intensity (Syms & Goodman 1987). Moreover, the OneLight generates heat which, if not properly excluded (Takács et al. 2009), could modulate responses of insects that orient toward infrared radiation from warm-bodied resources (Takács et al. 2009). Both the flickering and heat challenge were addressed in our experiments 3 and 4 (Fig. 7.1b) and did not alter responses of *L. occidentalis.* In experiment 4 and 5, *L. occidentalis* showed no preference for either the 505-nm LED or the Onelight emitting a very similar light spectrum. If flickering were attractive or repulsive, a different result would have been obtained.

Low levels of white light noise in programmed OneLight spectra posed a particular challenge. In retinograms, this light noise elicited retinal responses which could have altered responses to test stimuli by bleaching the eyes' photoreceptors and thus reducing their response (Bernard 1982) or it could have altered results due to the Bezold-Brücke effect (Backhaus 1992). To address this challenge, we used neutral density filters which excluded the light noise, such that the insect's eye could not detect and respond to it, but did respond to the programmed test stimulus. Unfortunately, this method could not be standardized. Each insect and preparation required a different number of neutral density filters.

Although neutral density filters worked relatively well with the diurnal *L. occidentalis* they were not applicable for retinograms with the nocturnal moth *D. abietivorella.* Nocturnal moths are highly sensitive to light and capable of colour vision even at starlight intensities (Kelber et al. 2003b).

All the above-mentioned challenges associated with the OneLight are offset by one superior benefit, the ability to reproduce, modify, and bioassay spectra of natural resources. While next-generation OneLight instruments are projected to include the UV light range, current-generation OneLight instruments already offer new opportunities to investigate color vision in vertebrates and invertebrates.

7.6. References

Arnold SEJ, Savolainen V & Chittka L (2008) FReD: The floral reflectance spectral database. Nature Proceedings doi:10.1038/npre.2008.1846.1

Bates SL & Borden JH (2005) Life table for *Leptoglossus occidentalis* Heidermann (Heteroptera: Coreidae) and prediction of damage in lodgepole pine seed orchards. Agricultural and Forest Entomology 7: 145–151.

Bernard GD & Remington CL (1991) Colour vision in *Lycaena* butterflies: Spectral tuning of receptor arrays in relation to behavioural ecology. Proceedings of the National Academy of Sciences of the United States of America 88: 2783–2787.

Blackmer JL, Byers JA & Rodreiguez-Saona C (2008) Evaluation of colour traps for monitoring *Lygus* spp.: Design, placement, height, time of day and non-target effects. Crop Protection 27: 171–181.

Blatt SE & Borden JH (1999) Physical characteristics as potential host selection cues for *Leptoglossus occidentalis* (Heteroptera: Coreidiae). Environmental Entomology 28: 246–254.

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

Chittka L & Menzel R (1992) The evolutionary adaptation of flower colours and the insect pollinators' colour vision. Journal of Comparative Physiology A 171: 171–181.

Chu CC, Chen TY, Natwick ET, Fitzgerald, G, Tuck S, Alexander P & Henneberry TJ (2005) Light response by *Frankliniella occidentalis* to white fluorescent light filtered through colour films and ultraviolet- and blue light-emitting diodes. Southwestern Entomologist 30: 149–154.

Clarke R & Lotto BR (2009) Visual processing of the bee innately encodes higher-order image statistics when the information is consistent with natural ecology. Vision Research 49: 1455–1464.

Cowan T & Gries G (2009) Ultraviolet and violet light: attractive orientation cues for the Indian meal moth, *Plodia interpunctella*. Entomologia Experimentalis et Applicata 131: 148–158.

Dyer AG & Chittka L (2004) Bumblebee search time without ultraviolet light. The Journal of Experimental Biology 207: 1683–1688.

Kamm JA, Fairchild CE, Gavin WE & Cooper TM (1991) Influence of celestial light on visual and olfactory behaviour of seed chalcids (Hymenoptera: Eurytomidae). Journal of Insect Behaviour 5: 273–287.

Kelber A (1999) Ovipositing butterflies use a red receptor to see green. The Journal of Experimental Biology 202: 2619–1630.

Kelber A, Vorobyev M & Orsorio D (2003a) Animal colour vision – behavioural tests and physiological concepts. Biological Review 78: 81–118.

Kelber A, Balkenius A & Warrant EJ (2003b) Colour vision in diurnal and nocturnal hawkmoths. Integrative Comparative Biology 43: 571–579.

Kevan PG & Backhaus WGK (1998) Color vision: Ecology and evolution in making the best of photic environment. In WGK Backhaus R Kliegl & JS Werner (Eds.) Color Vision: Perspectives from different disciplines. Berlin, Germany, Walter de Gruyter.

Krugman SL & Koerber TW (1969) Effects of cone feeding by *Leptoglossus occidentalis* on ponderosa pine seed development. Forest Science 15: 104–111.

Menzel R (1979) Spectral sensitivity and colour vision in invertebrates. Handbook of sensory physiology, vol. VII/6A (ed. H. Autrum). Berlin, Heidelberg, New York: Springer.

Mulligan GA & Kevan PG (1973) Color, brightness, and other floral characteristics attracting insects to the blossoms of some Canadian weeds. Canadian Journal of Botony 51: 1939-1952.

Osorio D & Vorobyev M (2008) A review of the evolution of colour vision and visual communication signals. Vision Research 48: 2042–2051.

Roe AD, Stein JD, Gillette NE & Sperling FAH (2006) Identification of *Dioryctria* (Leidoptera: Pyralidae) in a seed orchard at Chico, California. Annals of the Entomological Society of America 99: 433–448.

Syms PR & Goodman LJ (1987) The effect of flickering UV light output on the attractiveness of an insect electrocutor trap to the house-fly, *Musca domestica*. Entomologia Experimentalis et Applicata 43: 81–85.

Takács S, Bottomley H, Andreller I, Zahradnik T, Schwarz J, Bennett R, Strong W & Gries G (2009) Infrared radiation from hot cones on cool conifers attracts seed-feeding insects. Proceedings of the Royal Society B 276: 649–655.

Vernon RS & Bartel DL (1985) Effects of hue, saturation and intensity of colour selection by the onion fly, *Delia antiqua* (Meigen) (Diptera: Anthomyiidae) in the field. Environmental Entomology 14: 210–216.

Vukusic P (2010) Natural designs for manipulating the appearance of surfaces. Opthalmic and Physiological Optics 30: 435–445.

Wallace AR (1878) Tropical nature and other essays. London, UK: Macmillan and Company.Whitehouse CM, Roe AD, Strong WB, Evenden ML & Sperling FAH (2011) Biology and management of North American cone-feeding *Dioryctria* species. The Canadian Entomologist 143: 1–34.

Yurtserver S, Okyar Z & Guler N (2010) What colour of flowers do Lepidoptera prefer for foraging. Biologia 65: 1049–1056.

Zar JH (1999) Biostatistical Analysis, 4th ed. Prentice Hall, Upper Saddle River, NJ.

Table 7.1. Stimuli and sex of *Leptoglossus occidentalis* tested in two-choice experiments 3-12.

		Stimuli tested	
Exp. #	Insect sex	LED[1]	OneLight[2]
3	male	505 nm	505 nm
4	female	505 nm	505 nm
5	male	572 nm	Douglas-fir spectrum
6	female	572 nm	Douglas-fir spectrum
7	male	572 nm	Douglas-fir spectrum without 562-582 nm
8	female	572 nm	Douglas-fir spectrum without 562-582 nm
9	male	572 nm	White pine needle spectrum
10	female	572 nm	White pine needle spectrum
11	male	572 nm	White pine needle spectrum without 562-582 nm
12	female	572 nm	White pine needle spectrum without 562-582 nm

[1]Peak intensity wavelength of Light Emitting Diode (LED)
[2]Light engine capable of replicating and modifying spectral profiles of target objects

Table 7.2. Relative energy of spectra generated from the OneLight instrument.

Stimuli tested	Relative energy
Douglas-fir cone	5.94E-15
White pine cone	3.89E-15
White pine needles	3.62E-15
Dandelion flower	2.57E-15
White pine bark	7.96E-16

7.7. Figure captions

Figure 7.1. Drawings of the experimental design for (a) electrophysiological recordings and (b) laboratory two-choice experiments 4-13; see methods for details.

Figure 7.2. Spectral profiles of (*i*) Western white pine (*Pinus monticola*) needles, bark, and cones, Douglas-fir (*Pseudotsuga menziesii*) cones, and dandelion (*Taraxacum officinale*) flowers as tested in experiments 1-2 and 7-12, and (*ii*) Western white pine needles and Douglas-fir cones lacking wavelengths 562-582 nm, as tested in experiments 7-8 and 11-12.

Figure 7.3. Mean (+ SE) retinal responses (standardized to 1.0 of highest response for each eye) obtained in electroretinograms from eyes of male (n = 10) and female (n = 10) *Leptoglossus occidentalis* exposed to OneLight-emitted 400- to 650-nm spectra of Western white pine bark, needles and cones, Douglas-fir cones, and dandelion flowers (see Fig. 7.2 and Table 7.2) (one-way ANOVA, $p < 0.05$).

Figure 7.4. Number of male and female *Leptoglossus occidentalis* responding in two-choice laboratory experiments 3-12 to various light stimuli. In each experiment, bars with an asterisk indicate a significant preference for the test stimulus (Pearson's chi-square test, $p < 0.05$); nr denotes the number of non-responding insects; OL = OneLight.

Figure 7.1.

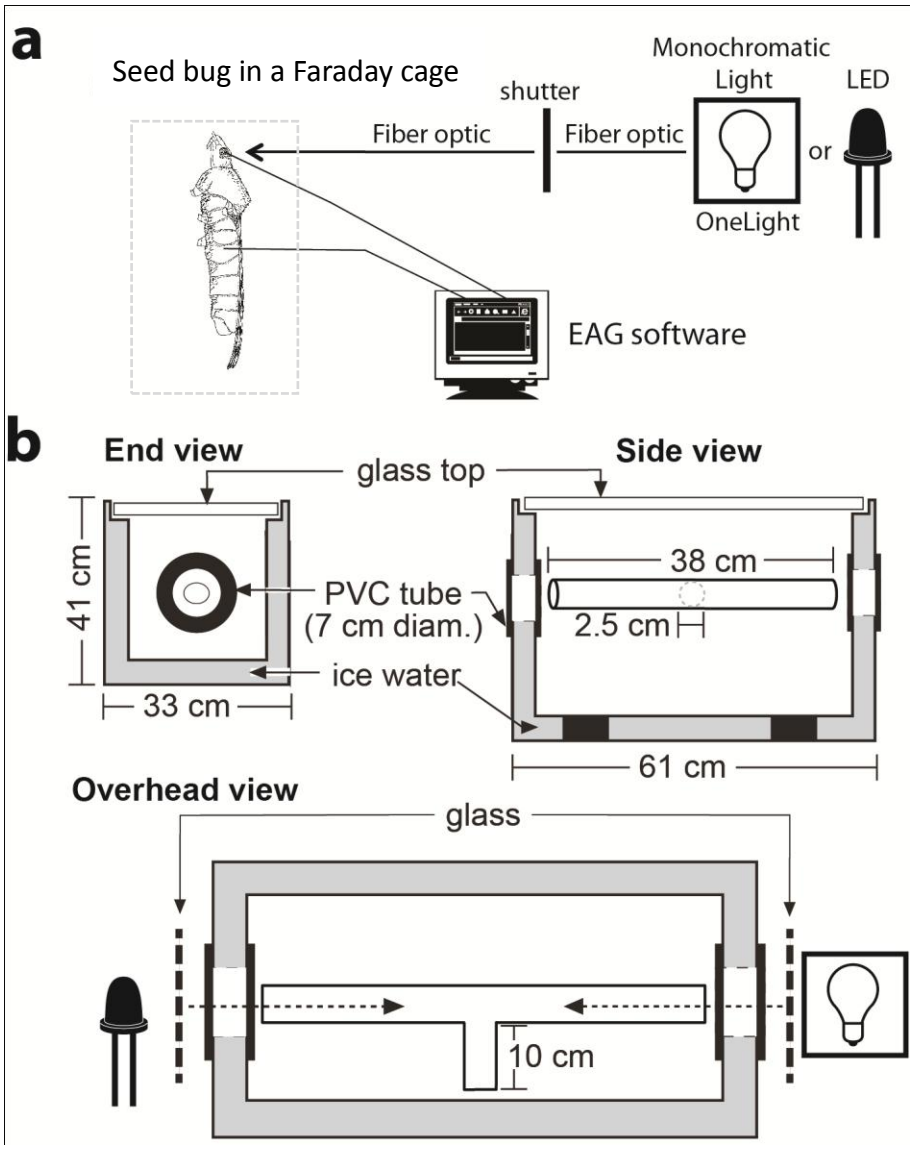

a
Seed bug in a Faraday cage
shutter
Monochromatic Light
LED
Fiber optic
Fiber optic
or
OneLight
EAG software
b
End view
glass top
Side view
41 cm
PVC tube
(7 cm diam.)
38 cm
2.5 cm
ice water
33 cm
61 cm
Overhead view
glass
10 cm

Figure 7.2

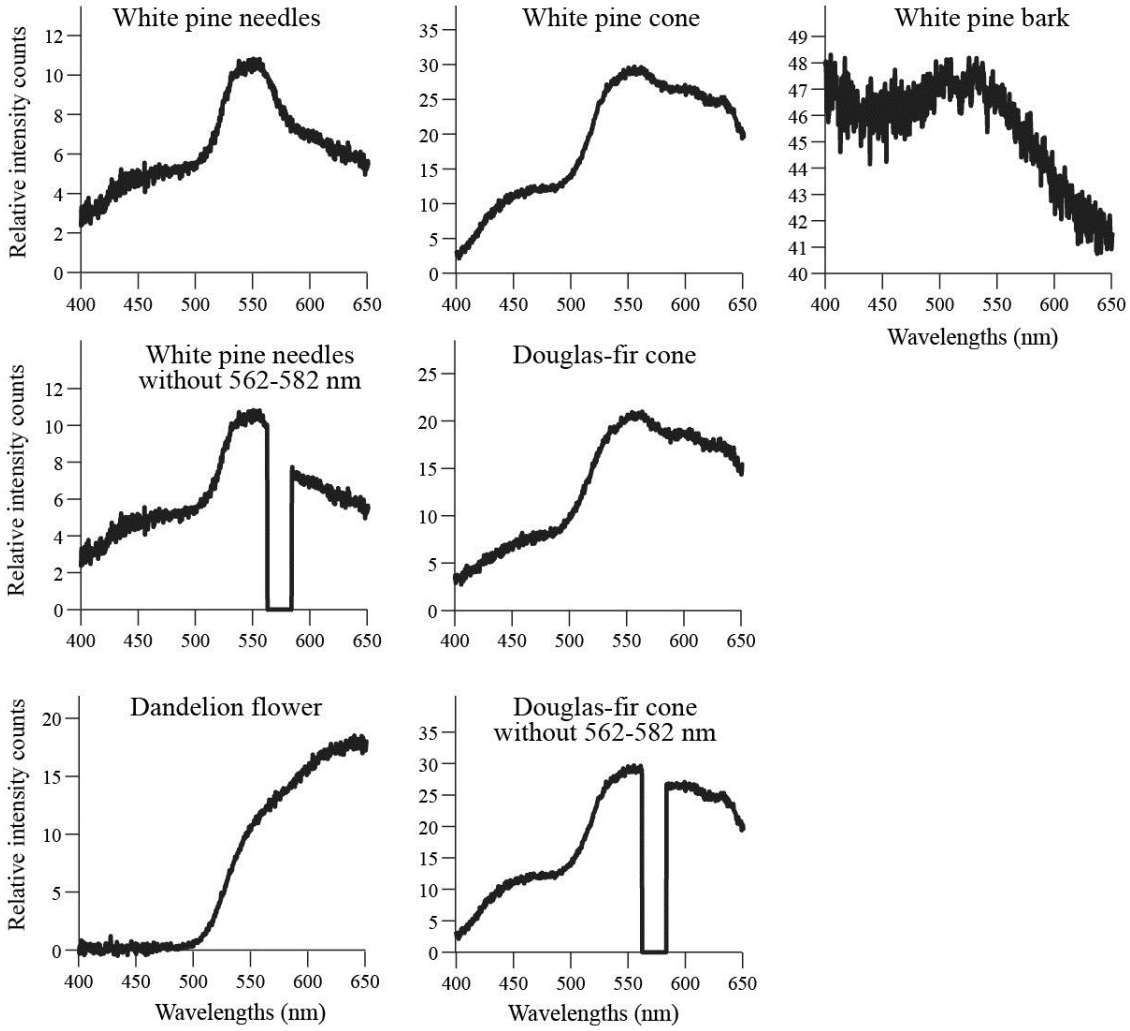

White pine needles
White pine cone
White pine bark
White pine needles without 562-582 nm
Douglas-fir cone
Dandelion flower
Douglas-fir cone without 562-582 nm
Relative intensity counts
Wavelengths (nm)

Figure 7.3

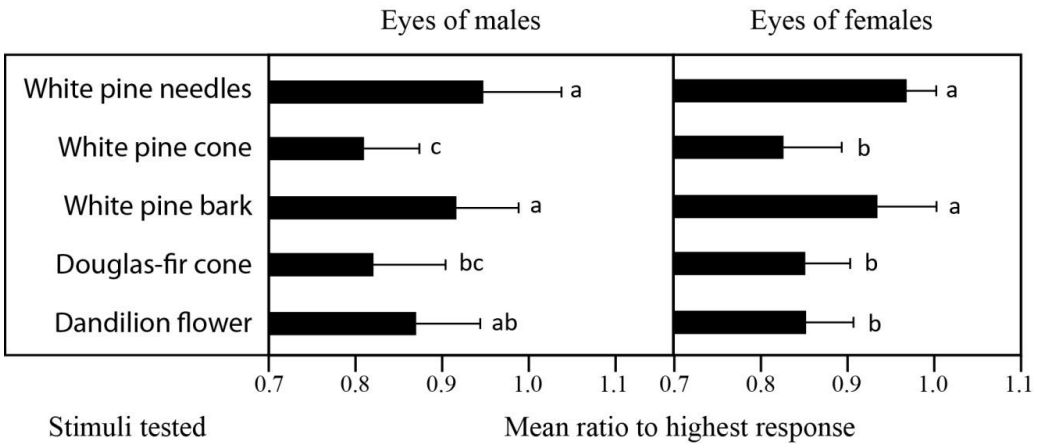
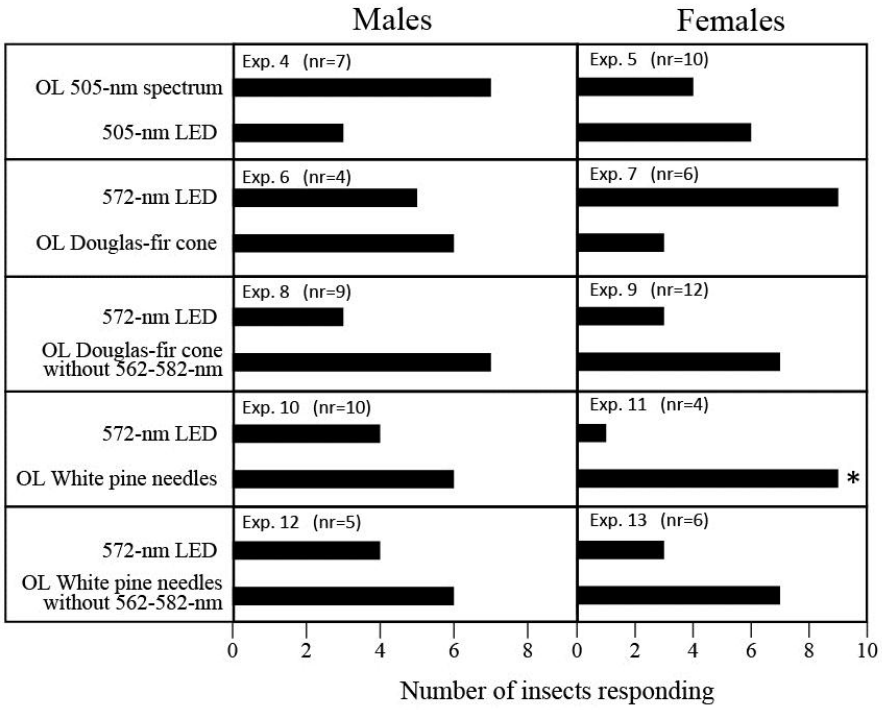

Eyes of males
Eyes of females
White pine needles
White pine cone
White pine bark
Douglas-fir cone
Dandilion flower
a
c
a
bc
ab
a
b
a
b
b
0.7
0.8
0.9
1.0
1.1
0.7
0.8
0.9
1.0
1.1
Stimuli tested
Mean ratio to highest response

Figure 7.4


Males
Females
Exp. 4 (nr=7)
Exp. 5 (nr=10)
OL 505-nm spectrum
505-nm LED
Exp. 6 (nr=4)
Exp. 7 (nr=6)
572-nm LED
OL Douglas-fir cone
Exp. 8 (nr=9)
Exp. 9 (nr=12)
572-nm LED
OL Douglas-fir cone without 562-582-nm
Exp. 10 (nr=10)
Exp. 11 (nr=4)
572-nm LED
OL White pine needles
*
Exp. 12 (nr=5)
Exp. 13 (nr=6)
572-nm LED
OL White pine needles without 562-582-nm
0 2 4 6 8 0 2 4 6 8 10
Number of insects responding

8. Do conophagous insects integrate information from the UV, visual and IR range when they forage for cones?[7]

8.1. Abstract

Most insects exhibit retinal and behavioural responses to electromagnetic wavelengths in the ultraviolet (UV; ~300-400 nm) and visual (400-750 nm) range, but a few species are known to detect and orient towards infrared radiation (IR). Among these few species are the nocturnal fir cone worm, *Dioryctria abietivorella* Groté, and the diurnal Western conifer seed bug, *Leptoglossus occidentalis* Heiderman. As they have also been shown to be spectrally sensitive to specific wavelengths in the UV (360-372 nm), green (505-510 nm), yellow (560-573 nm) and blue (433 nm) range, we tested the hypothesis that combinations of wavelengths from the UV, visual and IR range are more effective in attracting *L. occidentalis* and *D. abietivorella* than is a single, discreet range of these wavelengths. Data obtained in laboratory and field experiments did not support

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this hypothesis for *D. abietivorella.* However, data obtained in laboratory experiments with male and female *L. occidentalis* revealed a synergistic effect between blue light (433-nm LED) and high-frequency radiant IR (from a 40 °C heat source). These data support the conclusion that the central nervous system of *L. occidentalis* is capable of processing and integrating information from two types of sensory receptors, compound eyes on the head which perceive visual light, and IR receptors on the ventral abdomen which perceive radiant IR. Such integration was previously known only in snakes.

Keywords: *Leptoglossus occidentalis*; *Dioryctria abietivorella*; colour vision; ultraviolet (UV) vision; infrared (IR) radiation; IR receptor

8.2. Introduction

Insects exploit at least two ranges of the electromagnetic spectrum as a foraging cue. They detect electromagnetic wavelengths in the ultraviolet (UV; ~300-400 nm) and human visual (henceforth "visible") (400-750 nm) range (e.g., Briscoe and Chittka 2001, Kelber et al. 2003a) using their eyes, and they detect radiant infrared (IR) wavelengths (> 700 nm) using special IR receptors (Schmitz and Bleckmann 1997).

The compound eyes of insects have UV light receptors, resulting in UV-shifted vision (Kevan and Backhaus 1998, Briscoe and Chittka 2001). The eyes contain visual pigments that abosorb specific wavelengths of visible light (Townson et al. 1998, Briscoe and Chittka 2001, Osorio and Vorobyev 2002) and typically are trichromatic, with wavelength sensitivity maxima in the UV, violet/blue and green range (Höglund et al. 1973, Kevan and Backaus 1998, Townson et al. 1998, Briscoe and Chittka 2001, Kelber et al. 2003b). This spectral tuning may aid in the detection of essential resources, such as mates (Bernard and Remington 1991, Osorio and Vorobyev 2008), food (White et al. 1994, Gilburt and Anderson 1996, Townson et al. 1998) or oviposition sites (Cutler et al. 1995). For example, diurnal honey bees, *Apis mellifera* L., exploit visual and UV light cues from flowers to find nectar (Chittka and Menzel 1992). Foraging nocturnal moths respond to UV light (Roe et al. 2006, Whitehouse et al. 2011), but can also see colour even at very low (star light) illumination levels (Kelber et al. 2002, 2003b).

Although many insects can sense visual and UV light cues associated with essential resources (Wallace 1878, Kevan and Backhaus 1998), few insects are known to sense radiant IR (0.7-15 µm). For example, the blood-sucking bug *Rhodnius prolixus*

orients toward radiant IR from warm-bodied prey (Schmitz et al. 2000), and buprestid fire beetles orient towards radiant IR (2.2-4 µm) from forest fires (Evans 1964; Schmitz and Bleckmann 1998) to locate smoldering wood in which they oviposit.

Neural integration of electromagnetic wavelengths in the visual and IR range has not yet been studied in insects, but it is known to occur in snakes. Eyes and pit organs of *Python* snakes detect concurrently visible and IR electromagnetic wavelengths (Grace et al. 2001). In rattlesnakes, different neurons in the tectum of the brain respond to visual and IR cues, or combinations thereof (Hartline et al. 1978). Such neural systems allow neural integration of visual and IR signals, improving prey detection and capture.

Here we studied behavioral responses by the Western conifer seed bug, *Leptoglossus occidentalis* Heiderman, and the fir cone worm, *Dioryctria abietivorella* Groté, to electromagnetic wavelengths in the UV, visual and IR range. Both species are part of the conophagous insect guild, are capable of sensing UV, visual, and radiant IR, and respond to cues which thermally contrast well against background (Takács et al. 2009, Chapter 3). *Diorctria abietivorella* can see wavelengths of light in the UV (360-372 nm), and green (505-510 nm and 560-573 nm) range, and behaviourally responds to blue light (433-nm LED) (Chapter 4). *Leptoglossus occidentalis* eyes can see wavelengths of visible light in the UV (372 nm), blue (433 nm), and green (505 nm) range (Chapter 6).

Both species exploit similar resources but their life cycle varies. *Leptoglossus occidentalis* forages during the photophase (Krugman and Koerber 1969). Unlike first instar nymphs which feed on conifer needles, second to fifth instars and adults feed on the seeds of cones, primarily in the genera *Pinus*, *Abies*, *Pseudotguga* and *Tsuga*

(Krugman and Koerber 1973, de Groot et al. 1994). *Dioryctria abietivorella* forages at night. Gravid females lay eggs on cones (Shu et al. 1997), needles (Shu et al. 1997, Whitehouse et al. 2011) or twigs (Shu et al. 1996) of conifers in the genera *Abies*, *Larix*, *Picea*, *Pinus*, *Pseudotsuga*, and *Tsuga* (de Groot et al. 1994). Neonate larvae bore into cones and feed on the content of seeds (Grant et al. 2009).

We tested the hypothesis that combinations of electromagnetic wavelengths (UV, visual and IR) are more attractive to *L. occidentalis* and *D. abietivorella* than is any single type of electromagnetic radiation.

8.3. Methods and materials

8.3.1. Insects

Nymphs and adults of *L. occidentalis* were collected from the Sechelt Seed Orchard (123°43′W, 49°27′N; Canadian Forestry Products, Ltd., Vancouver, BC, Canada) in Sechelt, BC, and the Kalamalka Forestry Centre (119°16′W, 50°14′N; British Columbia Ministry of Forests and Range) in Vernon, BC, Canada. They were provisided with water and fresh cones of western white pine (*Pinus monticola*), Douglas-fir (*Pseudotsuga menziesii*) and spruce (*Picea engelmanni* x *glauca*), and they housed outdoors in wooden, mesh-covered cages (47 × 47 × 92 cm), the inside of which was misted daily.

Pupae of *D. abietivorella* were provided by Insect Production Services (Canadian Forest Service, Sault Ste. Marie, ON, Canada). They were kept in growth chambers [25 °C, 60 % relative humidity, 16:8 (L:D) photoperiod], and provided with a 10-% sugar

solution (Trudel et al. 1995). At least 4-day old male and virgin female moths were bioassayed during their natural flight period, 2-6 hours into the scotophase (Chapter 4). To obtain mated females, > 2-day old virgin females were retained together with males for at least two days.

8.3.2. Laboratory two-choice bioassays

All experiments used a cooled chamber designed to eliminate external thermal cues (Fig. 8.1a) (Takács et al. 2009). The chamber consisted of a glass aquarium (outside dimensions: 50.5 × 26.7 × 33.0 cm) covered with a glass lid (50.5 × 26.7 cm) and rested on 5-cm tall rubber stoppers inside a larger aquarium (outside dimensions: 61.0 × 33.0 × 41.0 cm), with cooled water (16 ± 2 °C) between them. A black PVC tube (7 cm inside diameter; IPEX, Toronto, ON, Canada) was sealed with aquarium grade black silica (All Glass Aquarium, Franklin, WI, USA) between the two aquaria in each of the two end sections to exclude water, allowing external radiant IR to enter the inner aquarium.

Light emitting diodes (LED) that elicited strong electrophysiological or behavioural responses from *L. occidentalis* and *D. abietivorella* as described in Chapters 3, 6 and 7 were tested in laboratory and field experiments. These LEDs had peak wavelengths in the UV (373 nm; NS370L-5RLO 370 nm), blue (433 nm; LED430-05U 430nm), green (505 nm; B5-433-B505 505-nm) and yellow-green (573 nm; B5-433-20 572nm) range (all LEDs: Roithner LaserTechnic GmbH, Vienna, Austria). LEDs were calibrated to the desired intensity at a distance of 2.5 cm from the source, using a spectrometer with cosine collector (HR4000, Ocean Optics, Dunedin, FL, USA) run on SpectraSuite software (Ocean Optics) for intensity of light emittance (Table 8.1). LEDs

were placed at either side of the aquarium behind glass plates (Fig. 8.1a) to ensure that radiant IR emitted from the LEDs did not enter the chamber (Hsieh and Su 1979).

To test combinations of visible light and radiant IR, IR stimuli were added to the experimental design (Fig. 8.1a). Higher- or lower-frequency radiant IR was generated from Pyrex glass flasks (1000 ml) containing heated (40 ± 2 °C) or ice-cooled (2 ± 2 °C) water. Flasks were randomly assigned to either end section of the chamber, and placed horizontal and diagonal to the tube openings (Fig. 8.1a). First-face mirrors [10.2 cm × 10.2 cm bare gold-coated first-face BK7 mirrors reflecting ca. 96% of wavelengths between 700 nm - 20 µm (Tempotec Optics Co. Ltd., Fuzhou, Fujian, China)] (Fig. 8.1a)] reflected a beam of radiant IR (but not conductive or convective heat) from the IR sources at 90 ° angles into the inner aquarium. To ensure that insects inside a bioassay glass T-tube (see below) within the inner aquarium could perceive the radiant IR, a horizontal laser was used to properly position the mirrors so that the beam of radiant IR entered the T-tube (see below) in the inner chamber. An AGEMA Thermovision 550 camera (FLIR Systems Ltd., Burlington, ON, Canada) confirmed the apparent temperature of heated (40 ± 2 °C) or cooled (2 ± 2 °C) water which generated the IR raddition that was reflected by the mirrors.

A glass T-tube with a 2.5 cm diameter (Fig. 8.1a) placed in the center of the inner aquarium was mounted on a stub which was held in place with black plasticine. For experiments with *D. abietivorella,* each tube was fitted with small sheets of cut paper towel to provide traction for the walking insects during locomotion. After each replicate, the T-tube was baked over-night at 120 °C to remove any potential pheromones adhering to it (Takács et al. 2009), and the inside of the inner aquarium and the lid were wiped with 70 % ethanol (Cowan and Gries 2009).

Bioassay insects to be tested were dark-adapated for 30-60 min (*L. occidentlis*), or acclimated for 30-60 min to a glass holding tube (2.5 × 6 cm) in the dark (*D. abietivorella*), before a single specimen was inserted into the stem of the T-tube which was then mounted on the stub inside the inner aquarium (see above). Insects which approached within 2.5 cm of the terminal orifice of one of the side arms within 2-30 min (*L. occidentalis*) or 2-60 min (*D. abietivorella*) were considered responders. All others were not included in statistical analyses (Pearson's chi-square, Zar 1999).

8.3.3. Effect of visible light (433 nm, 505 nm, 573 nm) and visible light or UV light (373 nm) on attraction of D. abietivorella *and* L. occidentalis *in two-choice laboratory experiments 1-12*

Experiments 1-3 (Table 8.1) explored a potential synergistic effect between wavelengths of visible light and UV light on attraction of *D. abietivorella,* by testing the response of males (Exp. 1), virgin females (Exp. 2), and mated females (Exp. 3) to two 505-nm LEDs *versus* one 505-nm LED and one 373-nm LED.

Experiments 4-13 (Table 8.1) explored potential synergistic effects between (*i*) wavelengths of visible light and (*ii*) wavelengths of visible light and UV light on attraction of *L. occidentalis.* Experiments 4 and 5 were designed to ascertain for subsequent experiments that the number of LEDs in a test stimulus had no effect on the response of bioassay insects, as long as the intensity of each of the two test stimuli was kept identical. Experiments 4 and 5 thus tested the response of males (Exp. 4) and females (Exp. 5) to one *versus* two 433-nm LEDs (Table 8.1). As the two stimuli were equally attractive (see Results), experiments 6 and 7 then tested the response of males (Exp. 6) and females (Exp. 7) to one 433-nm LED alone or in combination with one 373- nm LED.

Experiments 8 and 9 tested the response of males (Exp. 8) and females (Exp. 9) to one 505-nm LED alone or in combination with one 573-nm LED. Experiments 10 and 11 tested the response of males (Exp. 10) and females (Exp. 11) to one 505-nm LED alone or in combination with one 433-nm LED. Experiments 12 and 13 tested the response of males (Exp. 12) and females (Exp. 13) to one 433-nm LED *versus* one 505-nm LED, the combination of which had attracted males (see Results, Exp. 10).

8.3.4. Effect of visible light and radiant IR on attraction of L. occidentalis *in two-choice laboratory experiments 14-17*

Experiments 14 to 17 were designed to explore a potential synergistic effect between two stimuli, each of which was previously shown to be attractive to *L. occidentalis*. The design of these experiments took into account (*i*) that malessignificantly preferred the 433-nm LED (attractive light) over the 505-nm LED (non-attractive light) (see Results, Exp. 10), and (*ii*) that higher-frequency radiant IR (from a 40 ± 2 °C water source), but not lower-frequency radiant IR (from a 2 ± 2 °C water source), attract *L. occidentalis* (Takács *et al.* 2009). Experiments 14-15 tested the response of males (Exp. 14) and females (Exp. 15) to non-attractive light combined with attractive IR *versus* attractive light combined with non-attractive IR. Conversely, experiments 16-17 tested the response of males (Exp. 16) and females (Exp. 17) to non-attractive light with non-attractive IR *versus* attractive light combined with attractive radiant IR.

8.3.5. Effect ofvisible and UV light on attraction of D. abietivorella *and* D. schuetzeella *group in field experiments 18-20*

The experiments were run at the Kalamalka Forestry Centre from June to July 2010. Each trap (Fig. 8.1b) consisted of an inverted, clear plastic, 473-mL drinking glass, thinly coated with an adhesive (TangleFoot, Contech Enterprises Inc.) on the outside, and suspended by galvanized wire (20 GA, Corfil Products, Montreal, QC, Canada) ~ 2 m above ground between 12 randomly selected (assigned by random number generator) pairs of spruce trees, each tree bearing at least 50 cones. Each trap was baited with a light stimulus comprising four LEDs (1.0×10^{15} photons/cm^2/s per LED for a total intensity of 4.0×10^{15} photons/cm^2/s per trap) which were taped to the inside of the drinking glass (Fig. 8.1b), controlled by a custom-built 4- or 8-channel LED driver (P. Kowalski, SFU Science Technical Centre), and powered by a 9 v battery. The intensity of each LED was calibrated to an intensity of 1.0×10^{15} photons/cm^2/s at a distance of 2.5 cm from point source using a spectrometer (HR4000) with cosine collector run on SpectraSuite software. LEDs were turned on in the evening at 20:00 h. In the morning at 06:00 h, captured moths were collected, preserved in 70-% ethanol, and later dissected to determine their taxonomic identity, sex (Sopow et al. 1996) and mating status.

Experiment 18 (Table 8.1) was designed to compare the relative attractiveness of two visible-light stimuli consisting of four 433-nm LEDs or four 505-nm LEDs. Experiments 19 and 20 (Table 8.1) explored whether combinations of UV and visible light are more attractive than is UV orvisible light alone, by testing one 373-nm LED plus three 505-nm LEDs *versus* four 505-nm LEDs (Exp. 19), or by testing three 373-nm LEDs plus one 505-nm LED *versus* four 373-nm LEDs (Exp. 20). The mean numbers of

male or mated female moths captured were tallied and compared between test stimuli by a by two-tailed Wilcoxon signed-rank test (Zar 1999) using JMP software (SAS®, Cary, NC, USA).

8.3.6. Effect of radiant IR and UV and visible light on attraction of D. abietivorella *and* D. schuetzeella *group in field experiment 21*

Traps used in experiment 21 (Table 8.1) consisted of a tapered plastic bowl (~ 13 cm inside diameter × ~ 6 cm; Fig. 8.1c) which was spray painted with green paint (meadow green Rust-oleum Painter's Touch, RPM International Inc., Medina, OH, USA). Bowls were filled with warm or cold water when used. A clear plastic vial (~ 2.5 cm inside diameter × 7 cm), resting on strips of flat plastic and housing an LED holder, was secured across the open diameter of the bowl (Fig. 8.1c). This ensured that the LEDs were close to the surface of the water without getting wet. Each of 12 traps (four for each of the three treatments) was suspended ~2 m above ground between two spruce trees, each bearing at least 50 cones.

Traps with high or low frequency IR cues were monitored with the AGEMA Thermovision camera, making sure that their IR cue contrasted with the background throughout the night. The thermal contrast of warm traps was maintained by replacing the water with boiling water every 20-30 min. Traps with low frequency IR cue were filled with ice water, 75 mL of which were withdrawn by syringe and replaced by ice cubes every 120-180 min. Responding insects found floating in the water were retrieved, preserved in 70 % ethanol, and identified to species (Sopow et al. 1996). Their sex and mating status (of females) were also determined.

In experiment 21 (Table 8.2), three treatments were tested: (1) four 505-nm LEDs plus higher-frequency IR; (2) three 505-nm LEDs and one 373-nm LED plus higher-frequency IR, and (3) three 505-nm LEDs and one 373-nm LED plus lower-frequency IR. The mean number of males and mated females of *D. abietivorella* and *D. schuetzeella* group captured per trap type were compared with a Kruskal-Wallis one way analysis of variance with Tukey-Kramer post hoc analysis (Zar 1999) using JMP software.

8.4. Results

8.4.1. Effect ofvisible and UV light on attraction of D. abietivorella *and* L. occidentalis *in two-choice laboratory experiments 1-13*

Males (Exp. 1), virgin females (Exp. 2), and mated females (Exp. 3) of *D. abietivorella* all were more strongly attracted to a combination of green and UV light (505-nm LED + 373-nm LED) than to green light (505-nm LED) (Pearson's chi square test: Exp. 1: $\chi^2 = 4.07$, $p = 0.044$; Exp. 2: $\chi^2 = 8.63$, $p = 0.0033$; Exp. 3: $\chi^2 = 4.07$, $p = 0.44$; Fig. 8.2).

Experiments 4 to 13 (Fig. 8.3) were run with males and females of *L. occidentalis.* Males (Exp. 4) and females (Exp. 5) were equally attracted to one or two 433-nm LEDs tested at equal light intensity (Pearson's chi square test; Exp. 4: $\chi^2 = 0.20$, $p = 0.65$; Exp. 5: $\chi^2 = 0.00$, $p = 1.00$). Males (Exp. 6) and females (Exp. 7) were as strongly attracted to a combination of blue and UV light (433-nm LED + 373-nm LED) as

they were to blue light (two 433-nm LEDs) (Exp. 6: $\chi^2 = 0.00$, $p = 1.00$; Exp. 7: $\chi^2 = 0.02$, $p = 0.65$). Males (Exp. 8) and females (Exp. 9) were as strongly attracted to a combination of two different green lights (505-nm LED + 573-nm LED) as they were to two identical green lights (two 505-nm LEDs) (Exp. 8: $\chi^2 = 0.00$, $p = 1.00$; Exp. 9: $\chi^2 = 0.00$, $p = 1.00$). Males (Exp. 10), but not females (Exp. 11), were more strongly attracted to a combination of blue and green lights (433-nm LED + 505-nm LED) than they were to green lights (two 505-nm LEDs) (Exp. 10: $\chi^2 = 8.63$, $p = 0.003$; Exp. 11: $\chi^2 = 0.00$, $p = 1.00$). Finally, males (Exp. 12), but not females (Exp. 13), were more strongly attracted to blue light (433-nm LED) than they were to green light (505-nm LEDs) (Exp. 12: $\chi^2 = 8.14$, $p = 0.004$; Exp. 13: $\chi^2 = 0.10$, $p = 0.74$).

8.4.2. Effect ofvisible light and radiant IR on attraction of L. occidentalis *in two-choice laboratory experiments 14-17*

Males (Exp. 14) and females (Exp. 15) were equally attracted to blue light (433-nm LED) combined with lower-frequency radiant IR and to green light (505-nm LED) combined with higher-frequency IR (Exp. 14: $\chi^2 = 1.68$, $p = 0.20$; Exp. 15: $\chi^2 = 0.93$, $p = 0.34$; Fig. 8.4). However, males (Exp. 16) and females (Exp. 17) were more strongly attracted to blue light (433-nm LED) combined with higher-frequency IR than to green light (505nm LED) combined with lower-frequency IR (Exp. 16: $\chi^2 = 17.26$, $p < 0.0001$; Exp. 17: $\chi^2 = 5.81$, $p = 0.016$; Fig 8.4).

8.4.3. Effect ofvisible and UV light on attraction of D. abietivorella *and* D. schuetzeella *group in field experiments 18-20*

In experiment 18, males and mated females of *D. abietivorella* were attracted equally to blue (433-nm LED) and to green (505-nm LED) lights (two-tailed Wilcoxon signed-rank test: males: z = -0.53, p = 0.59; mated females: z = -0.17, p = 0.87; Fig. 8.5). Virgin females were not captured in any trap.

In experiment 19, males and mated females of *D. abietivorella* were equally attracted to the combination of UV and green (373 nm + 505 nm) lights and to UV (373 nm) lights (two-tailed Wilcoxon signed-rank test: males: Z = 0.92, p = 0.36; mated females: Z = 0.73, p = 0.47; Fig. 8.5). Similarly, males and mated females of *D. schuetzeella* group were equally attracted to the combination of UV and green lights and to UV lights (two-tailed Wilcoxon signed-rank test: males: Z = 1.18, p = 0.24; mated females: Z = 0.16, p = 0.87; Fig. 8.6). Only one virgin female *D. schuetzeella* group was captured.

In experiment 20, males, but not females, of *D. abietivorella* were more strongly attracted to the combination of UV and green (373 nm + 505 nm) lights than to green (505 nm) lights (two-tailed Wilcoxon signed-rank test: males: Z = 2.32, p = 0.02; females: Z = 1.01, p = 0.31; Fig. 8.6). Males and females of *D. schuetzeella* group were equally attracted to the combination of green and UV lights and to green lights (two-tailed Wilcoxon signed-rank test: males: Z = 0.00, p = 1.00; mated females: Z = -1.69, p = 0.09; Fig. 8.6). No virgin females were captured.

8.4.4. Effect of radiant IR, UV, and visible light on attraction of D. abietivorella *and* D. schuetzeella *group in field experiment 21*

Mated females of *D. abietivorella* discriminated between the three test stimuli (S) in experiment 21 (Kruskal-wallis test; $\chi^2 = 7.18$, $p = 0.03$); whereas, other moths did not (male *D. abietivorella*: $\chi^2 = 2.72$, $p = 0.26$; mated female *D. pseudotsgella* group: $\chi^2 = 2.72$, $p = 0.26$; male *D. pseudotsugella* group: $\chi^2 = 2.10$, $p = 0.35$) (Fig. 8.7). The two 3-component test stimuli which included higher-frequency IR [S1 with green light; S2 with green and UV light (Table 8.2)] were equally attractive to mated female *D. abietivorella* (Tukey: S1 *vs.* S2, $p = 0.53$), as were S1 and the lower-frequency IR with green and UV light (S3) (S1 *vs.* S3, $p = 0.11$) (Fig. 8.7), whereas S2 was significantly more attractive than S3 (Tukey: S2 *vs.* S3, $p = 0.04$). There were no significant differences in captures of male *D. abietivorella* (Tukey: S1 *vs*. S2: $p = 0.60$; S1 *vs*. S3: $p = 0.30$; S2 *vs*. S3: $p = 0.13$), mated female *D. pseudotsgella* group (Tukey: S1 *vs*. S2: $p = 0.72$; S1 *vs*. S3: $p = 0.17$; S2 *vs*. S3: $p = 0.29$), or male *D. pseudotsgella* group (Tukey: S1 *vs*. S2: $p = 0.58$; S1 *vs*. S3: $p = 0.12$; S2 *vs*. S3: $p = 0.29$) (Fig. 8.7). Too few virgin female *D. abietivorella* were captured to warrant statistical analysis of data.

8.5. Discussion

Our data support the conclusion that a specific wavelength of visible light (433-nm LED) and higher-frequency radiant IR (from a 40 ºC heat source) interact in attracting male and female *L. occidentalis*. There is no evidence yet for synergistic or additive effects between electromagnetic wavelengths in the UV, visual and IR ranges

for attraction of *D. abietivorella,* but additional experiments are warranted to explore this possibility.

To study potential synergism between visual and UV lights on attraction of *D. abietivorella*, we tested combinations of green (505 nm) and UV (373 nm) lights *versus* either green lights or UV lights alone. Significantly stronger attraction of males, virgin and mated females in the laboratory to green- and UV-light combinations than to green light (Fig. 8.2, Exp. 1-3), and significantly stronger attraction of males and mated females in the field to green- and UV-light combinations than to green light (Fig. 8.6, Exp. 19), all indicated that UV light is more attractive than green light. However, when we tested the green- and UV-light combination *versus* UV light, both stimuli were equally attractive to either male and female *D. abietivorella*, or to male and female *D. schuetzella* (Fig. 8.6, Exp. 20), clearly indicating that the moths were attracted to UV light. These results are consistent with previous findings that *Dioryctria* spp. (Roe et al. 2006, Whitehouse et al., 2011) and other insects (e.g., Sambaraju and Phillips 2008) are attracted exclusively by UV light. Although violet light (405-nm LED) enhanced the attractiveness of UV light (350-nm LED) to mated females of the Indian meal moth, *Plodia interpunctella* (Pyralidae), the effect was due to an increased overall light intensity rather than an interaction between the UV and violet light, as shown by testing violet light *versus* violet/UV light at equal intensities (Cowan and Gries 2009).

That virgin female *D. abietivorella* were attracted to UV light in laboratory experiment 2 (Fig. 8.2), but not in field experiments 19 and 20, is likely due to a contrasting mode of response in these different settings. Although virgin females could walk toward test stimuli in the laboratory, they would have to take flight in the field to respond to, and be captured in, UV light traps. Virgin females, however, tend not to fly

and instead emit sex pheromone that attracts mate-seeking males (Whitehouse et al. 2011). This may also explain why nearly all females of the Eastern hemlock looper, *Lambdina fiscellaria fiscellaria*, captured in light traps are already mated (Delisle et al. 1998). Moreover, many females had already laid at least half of their egg complement, thus reducing their wing loading and increasing their flight capability (Delisle et al. 1998).

Despite the attractiveness of UV light in experiments 1-3 (Fig. 8.2) and 19 (Fig. 8.6), it failed to enhance the attractiveness of a high frequency IR source, and was not attractive when combined with a lower-frequency IR source in field experiment 21 with *D. abietivorella* and *D. schuetzella* (Fig. 8.7). These results imply that higher-frequency radiant IR is an even more attractive foraging cue to mated female *D. abietivorella* than is UV light.

Even though the eyes of *L. occidentalis* can see wavelengths of blue (433 nm) and green (505 nm) lights (Chapter 5), neither blue nor green light alone or in combination attracted female *L. occidentalis* (Fig. 8.3). However, males were more strongly attracted to blue light, either alone (Fig. 8.3, Exp. 12), or combined with green light (Fig. 8.3, Exp. 10), than to green light alone. Furthermore, blue light appeared to have an additive effect with IR. In experiments 10-17 (Figs. 8.3 and 8.4), green light at 505 nm had no behavioural effect, and can be considered benign. In experiments 14-17 (Fig. 8.4), both blue light and higher-frequency IR alone were moderately attractive, but no male *L. occidentalis* responded in their absence. Combining blue light and IR resulted in the greatest attraction, in what appeared to be an additive effect.

The relatively high number of non-responding insects in the laboaratory may be due to reluctance to move in an artificial setting or may be due to ambient temperature cooler than in the field.

The interaction between higher-frequency radiant IR and blue light on attraction of male and female *L. occidentalis* (Fig. 8.4, Exps. 16, 17) supports the hypothesis that the central nervous system of *L. occidentalis* is capable of processing and integrating information from two types of sensory receptors, the compound eyes on the head which perceive visual light, and IR receptors on the ventral abdomen which perceive radiant IR. Such integration has previously been studied only in snakes (Grace et al., 2001). Boid and crotaline snakes possess two distinct types of receptors to image radiant electromagnetic energy: the lateral eye which responds to visible light, and the pit organ which responds to radiant IR. Although binocularly occluded *Python molurus* hunting for mice exhibited strike angles and distances similar to non-occluded snakes, they had a lower strike success, indicating that precise targeting of prey depends to some degree also upon visual information (Grace et al., 2001). The mechanisms by which *L. occidentalis* integrates visible light and radiant IR cues from conifer cones during cone-foraging must be tested in the field by carefully modulating the composition and complexity of test stimuli.

8.6. References cited

Bernard, G.D., and C.L. Remington. 1991. Color vision in *Lycanena* butterflies: spectral tuning of receptor arrays in relation to behavioural ecology. Proc. Natl. Acad. Sci. USA. 88: 2783–2787.

Chittka, L., and R. Menzel. 1992. The evolutionary adaptation of flower colours and insect pollinators' colour vision. J. Comp. Physiol. A. 171: 171–181.

Cowan, T., and G. Gries. 2009. Ultraviolet and violet light: attractive orientation cues for the Indian meal moth, *Plodia interpunctella*. Entomol. Exp. Appl. 131: 148–159.

Cutler, D.E., Bennett, R.R., Stevenson, R.D., and R. White. 1995. Feeding-behaviour in the nocturnal moth *Manduca sexta* is mediated mainly by blue recpetors, but where are they located in the retina. J. Exp. Biol. 198: 1909–1917.

de Groot, P., Turgeon, J.J., and G.E. Miller. 1994. Status of cone and seed insect pest-management in Canadian seed orchards. For. Chron. 70: 745–761.

Delisle, J., West, R.J., and W.W. Bowers. 1998. The relative performance of pheromone and light traps in monitoring the seasonal activity of both sexes of the eastern hemlock looper, *Lambdina fiscellaria fiscellaria*. Entomol. Exp. App. 89: 87–98.

Dyer, A.G. and L. Chittka. 2004. Bumblebee search time without ultraviolet light. J. Exp. Biol. 207: 1683–1688.

Evans, W.G. 1964. Infrared receptors in *Melanophila acuminata* De Geer. Nature 202: 211.

Gilburt, H.L., and M. Anderson. 1996. The spectral efficiency of the ey of *Ephestia cautella* (Walker) (Lepidoptera: Pyralidae). J. Stored Prod. Res.32: 285–291.

Grace, M.S., Woodward, O.M., Church, D.R., and G. Calisch. 2001. Prey targeting by the infrared-imaging snake *Python*: effects of experimental and congenital visual deprivation. Behav. Brain Res. 119: 23–31.

Grant, G.G., Millar, J.G., and R. Trudel. 2009. Pheromone identification of *Dioryctria abietivorella* (Lepidoptera: Pyralidae) from an eastern North American population: geographic variation in pheromone response. Can. Entomol. 141: 129–135.

Hartline, P.H., Kass, L., and M.S. Loop. 1978. Merging of modalities in the optic tectum: Infrared and visual integration in rattlesnakes. Science 4334: 1225–1229.

Höglund, G., Hamdorf, K., and G. Rosner. 1973. Trichromatic visual system in an insect and its sensitivity control by blue light. J. Comp. Physiol. 86: 265–279.

Hsieh, C., and K.C. Su. 1979. Thermal radiative properties glass from 0.32 to 106 microns. Sol. Energy 22: 37–43.

Kelber, A., Balkenius, A., and E.J. Warrant. 2002. Scotopic colour vision in nocturnal moths. Nature 419: 922–925.

Kelber, A., Vorobyev, M., and D. Osorio. 2003a. Animal colour vision – behavioural tests and physiological concepts. Biol. Rev. 78: 81–118.

Kelber, A., Halkenius, A., and E.J. Warrant. 2003b. Colour vision in diurnal and nocturnal hawkmoths. Integr. Comp. Biol. 43: 571–579.

Kevan, P.G. and W.G.K. Backhaus. 1998. Color vision: Ecology and evolution in making the best of photic environment. In WGK Backhaus R Kliegl & JS Werner (Eds.) Color Vision: Perspectives from different disciplines. Berlin, Germany, Walter de Gruyter.

Krugman, S.L., and T.W. Koerber. 1969. Effect of cone feeding by *Leptglossus occidentalis* on ponderosa pine seed development. For. Sci. 15: 104–111.

Osorio, D., and M. Vorobyev. 2008. A review of the evolution of animal colour vision and visual communication signals. Vision Res. 48: 2042–2051.

Roe, A.D., Stein, J.D., Gillette, N.E. and F.A.H. Sperling. 2006. Identification of Dioryctria (Leidoptera: Pyralidae) in a seed orchard at Chico, California. Ann. Entomol. Soc. Am. 99: 433–448.

Sambaraju, K.R., and T.W. Phillips. 2008. Response of adult *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae) to light and combinations of attractants and light. J. Insect. Behav. 21: 422–439.

Schmitz, H. and H. Bleckmann. 1997. Fine structure and physiology of the infrared receptor of beetles of the genus *Melanophila* (Coleoptera: Buprestidae). Int. J. Insect Morphol. Embryol. 26: 205–215.

Schmitz, H., and H. Bleckmann. 1998. The photomechanic infrared receptor for the detection of forest fires in the beetle *Melanophila acuminata* (Coleoptera: Buprestidae). J. Comp. Physiol. A. 182: 647–657.

Schmitz, H., Trenner, S., Hofmann, M.H., and H. Bleckmann. 2000. The ability of *Rhodnius prolixus* (Hemiptera; Reduviidae) to approach a thermal source solely by its infrared radiation. J. Insect. Physiol. 46: 745–751.

Shu, S., Grant, G.G., Langevin, D.A., Lombardo, D.A., and L. MacDonald. 1997. Oviposition and electroantennogram responses of *Dioryctria abietivorella* (Lepidoptera: pyralidae) elicited by monoterpenes and enantiomers from eastern white pine. J. Chem. Ecol. 23: 35–50.

Sopow, S.L., Bennett, R.G., Landry, J., and B. Landry. 1996. Identification of the "grey" *Dioryctria* species of British Columbia (Lepidoptera, Pyralidae). J. Entomol. Soc. B. C. 93: 75–92.

Takács, S., Bottomley, H., Andreller, I., Zahradnik, T., Schwarz, J., Bennett, R., Strong, W., and G. Gries. 2009. Infrared radiation from hot cones on cool conifers attracts seed-feeding insects. Proc. R. Soc. B. 276: 649–655.

Townson, S.M., Chang, B.S.W., Salcedo, E., Chadwell, L.V., Pierce, N.E., and S.G. Britt. 1998. Honeybee blue- and ultraviolet-sensitive opsins: Cloning, heterologous expression in Drosophila, and physiological characterization. J. Neurosci. 18: 2412–2422.

Trudel, R., Bauce, É., Cabana, J., and C. Guertin. 1995. Rearing technique for *Dioryctria abietivorella* (Lepidoptera: Pyralidae). J. Econ. Entomol. 88: 640–643.

Wallace, A.R. 1878. Tropical nature and other essays. London, UK: Macmillan and Company.

White, R.H., Stevenson, R.D., Bennett, R.R., Cutler, D.E., and W.A. Haber. 1994. Wavelength discrimination and the role of ultraviolet vision in the feeding behaviour of hawkmoths. Biotropica 26: 427–435.

Whitehouse, C.M., Roe, A.D., Strong, W.B., Evenden, M.L., and F.A.H. Sperling. 2011. Biology and management of North American cone-feeding *Dioryctria* Species. Can. Entomol. 143: 1–34.

Zar, J.H. 1999. Biostatistical Analysis, 4th ed. Prentice Hall, Upper Saddle River, NJ.

Table 8.1: Stimuli tested in, and location and dates of, experiments 1-21.

Exps.	n[a]	Stimulus 1 (photons/cm^2/s)	Stimulus 2 (photons/cm^2/s)	Location[b]	Dates
1/2/3	10	1 × 505-nm LED (9.0 × 10^{13}) 1 × 505-nm LED (1.0 × 10^{13})	1 × 505-nm LED (9.0 × 10^{13}) 1 × 373-nm LED (1.0 × 10^{13})	SFU	May-Aug 10
4/5	10	1 × 433-nm LED (3.0 × 10^{13})	2 × 433-nm LED (1.5 × 10^{13})	SFU	June-Aug 09
6/7	10	1 × 433-nm LED (3.0 × 10^{13})	1 × 433-nm LED (2.9 × 10^{13}) 1 × 373-nm LED (310 × 10^{12})	SFU	June-Sept 10
8/9	10	1 × 505-nm LED (3.0 × 10^{13})	1 × 505-nm LED (1.5 × 10^{13}) 1 × 573-nm LED (1.5 × 10^{12})	SFU	June-Sept 10
10/11	10	1 × 505-nm LED (3.0 × 10^{13})	1 × 505-nm LED (1.5 × 10^{13}) 1 × 433-nm LED (1.5 × 10^{12})	SFU	June-Sept 10
12/13	20	1 x 505-nm LED (3.0 × 10^{13})	1 x 433-nm LED (3.0 × 10^{13})	SFU	June-Sept 10
14/15	20	1 × 505-nm LED (3.0 × 10^{13}) hot IR stimuli (40 ± 2 °C)	1 × 433-nm LED (3.0 × 10^{13}) cold IR stimuli (2 ± 2 °C)	SFU	June-Sept 10
16/17	20	1 × 505-nm LED (3.0 × 10^{13}) cold IR stimuli (2 ± 2 °C)	1 × 433-nm LED (3.0 × 10^{13}) hot IR stimuli (40 ± 2 °C)	SFU	June-Sept 10
18	6	4 × 505-nm LED (1.0 × 10^{15})	4 × 433-nm LED (1.0 × 10^{15})	Kalamalka	June-July 10
19	6	4 × 505-nm LED (1.0 × 10^{15})	3 × 505-nm LED (1.0 × 10^{15}) 1 × 373-nm LED (1.0 × 10^{15})	Kalamalka	June-July 10
20	6	4 × 373-nm LED (1.0 × 10^{15})	3 × 373-nm LED (1.0 × 10^{15}) 1 × 505-nm LED (1.0 × 10^{15})	Kalamalka	June-July 10

[a]n = number of replicates
[b]SFU = Simon Fraser University; Kalamalka = Kalamalka Forestry Centre.

Table 8.2. Stimuli tested in experiment 21 at the Kalamalka Forestry Centre from June to July 2010.

Stimulus (S) 1 (photons/cm^2/s)	**Stimulus 2 (photons/cm^2/s)**	**Stimulus 3 (photons/cm^2/s)**
4 × 505 nm LED (1.0 × 10^{15})	3 × 505 nm LED (1.0 × 10^{15}) 1 × 373 nm LED (1.0 × 10^{15})	3 × 505 nm LED (1.0 × 10^{15}) 1 × 373 nm LED (1.0 × 10^{15})
”hot IR”[a]	“hot IR”	“cold IR”[b]

[a]Infrared radiation emanating from traps refilled with boiling water every 30 min;
[b]Infrared radiation emanating from traps partially filled with ice water, 50 mL of which were withdrawn by syringe and replaced by ice cubes every 120-180 min

8.7. Figure captions

Figure 8.1. Illustrations of (a) the experimental design used in laboratory experiments 1-14, and (b, c) the light traps used in field experiments 18-20 (b) and 21 (c); see methods for details.

Figure 8.2. Numbers of male (left), virgin female (middle), and mated female (bottom) *Dioryctria abietivorell*a responding in two-choice laboratory experiments 1-3 (n = 10 each) to different types of UV and visual light stimuli. In each experiment, an asterisk (*) indicates a significant preference for a test stimulus (Pearson's chi square test; $p < 0.05$). nr = non-responding insects.

Figure 8.3. Numbers of male and female *Leptoglossus occidentalis* responding in two-choice laboratory experiments 4-13 (n = 10 each) to different types of UV and visual light stimuli. In experiments 10 and 12, an asterisk (*) indicates a significant preference for a test stimulus (Pearson's chi square test; $p < 0.05$). nr = non-responding insects.

Figure 8.4. Number of male and female *Leptoglossus occidentalis* responding in two-choice laboratory experiments 14-17 (n = 20 each) to complex stimuli consisting of visual light from a light-emitting diode (LED) and infrared (IR) radiation from either a hot (40 ± 2 °C; high frequency IR) or cold (2 ± 2 °C; low frequency IR) source. In experiments 16 and 17, an asterisk (*) indicates a significant preference for the test stimulus (Pearson's chi square test; $p < 0.05$).

Figure 8.5. Mean (+ SE) number of male and mated female *Dioryctria abietivorella* captured in field experiment 18 in traps (n = 6) each fitted with four light-emitting diodes (LEDs) with peak wavelength of 433 nm or 505 nm. There was no significant difference between the two stimuli (two-tailed Wilcoxon signed-rank test; $p > 0.05$).

Figure 8.6. Mean (+ SE) number of male and mated female *Dioryctria abietivorella* and *Dioryctria schuetzeella* group captured in field experiments 19 and 20 in traps (n = 6 each) fitted with four light-emitting diodes (LEDs) with peak wavelength in the UV (373 nm) or the visual (505 nm) range. An asterisk (*) indicates a significant preference for a test stimulus (two-tailed Wilcoxon signed-rank test; $p < 0.05$). Only one virgin female moth (*D. schuetzeella* group) was captured in both experiments.

Figure 8.7. Mean (+ SE) number of male, virgin and mated female *Dioryctria abietivorella* and *Dioryctria schuetzeella* group captured in field experiment 21 in traps (n = 4) associated with complex stimuli consisting of green (505 nm) and/or UV (373 nm) light from light-emitting diodes (LEDs) and infrared (IR) radiation from either a hot (40 ± 2°C; high frequency IR) or cold (2 ± 2°C; low frequencr IR) source. The two 3-component test stimulus which included high frequency IR with green and UV light was more attractive to mated female *D. abietivorella* and *D. schuetzeella* group than the one 3-component test stimulus which included low frequency IR. For detailed statistical analyses see the result section.

Figure 8.1

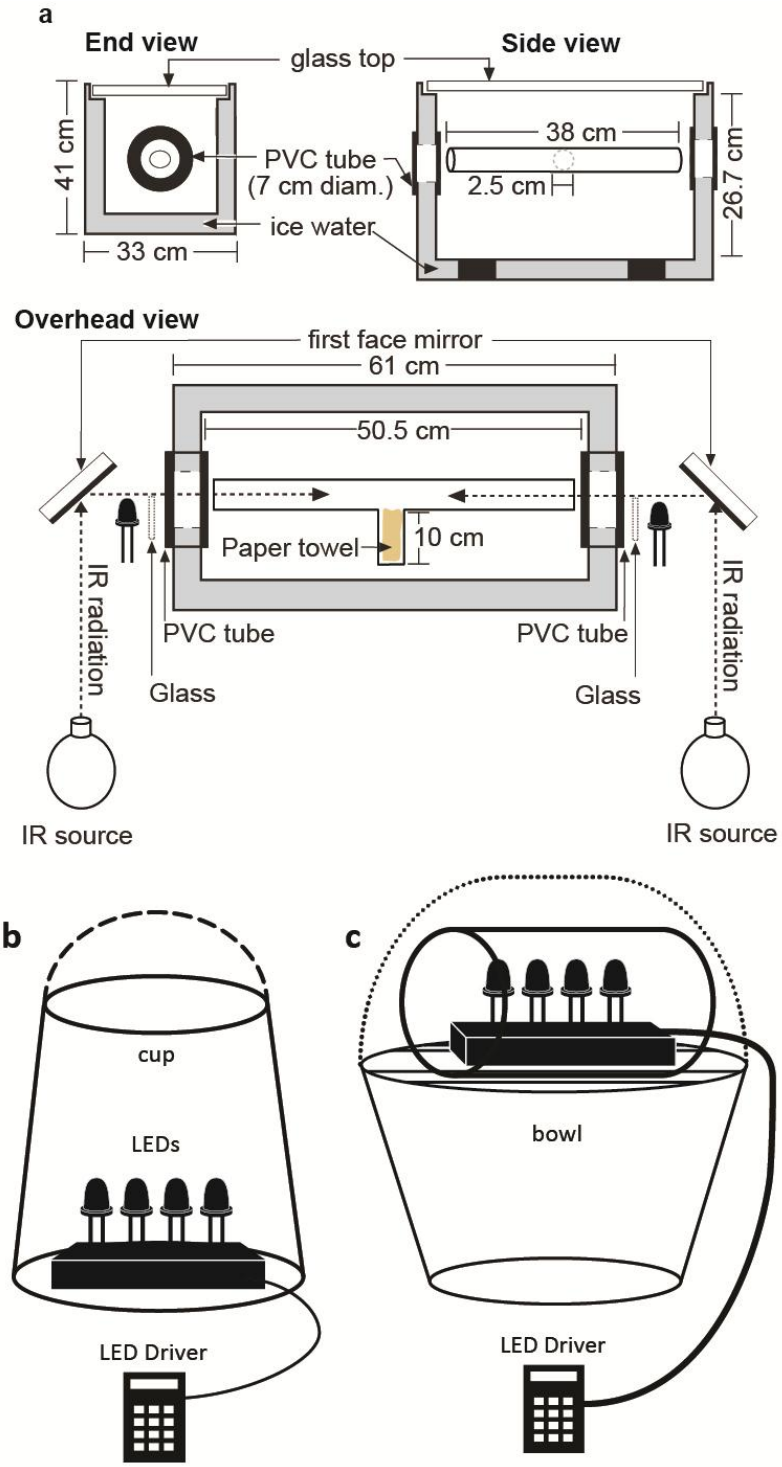

a
End view
glass top
Side view
41 cm
PVC tube
(7 cm diam.)
ice water
33 cm
38 cm
2.5 cm
26.7 cm
Overhead view
first face mirror
61 cm
50.5 cm
Paper towel
10 cm
IR radiation
PVC tube
Glass
PVC tube
Glass
IR radiation
IR source
IR source
b
cup
LEDs
LED Driver
c
bowl
LED Driver

Figure 8.2

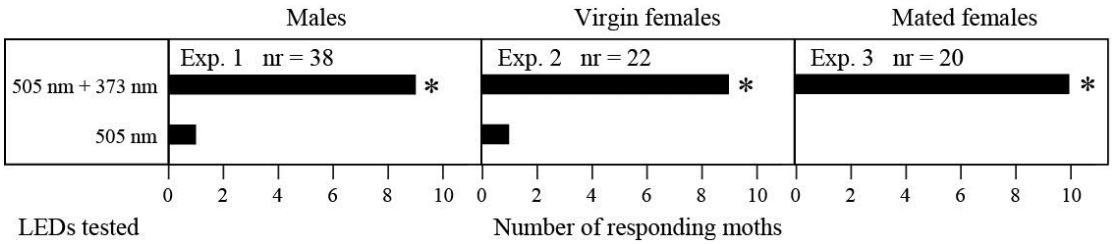

Males
Virgin females
Mated females
Exp. 1 nr = 38
Exp. 2 nr = 22
Exp. 3 nr = 20
505 nm + 373 nm
505 nm
*
*
*
0 2 4 6 8 10 0 2 4 6 8 10 0 2 4 6 8 10
LEDs tested
Number of responding moths

Figure 8.3

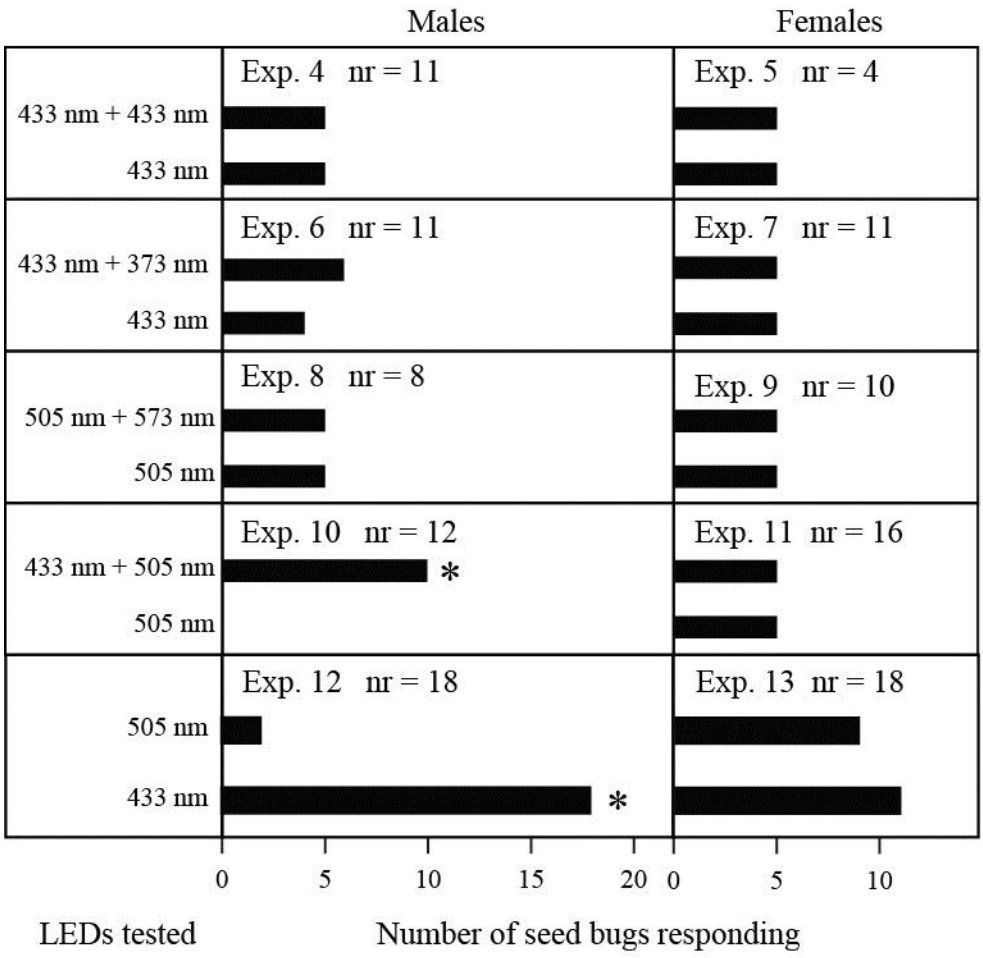

Males
Females
Exp. 4 nr = 11
Exp. 5 nr = 4
433 nm + 433 nm
433 nm
Exp. 6 nr = 11
Exp. 7 nr = 11
433 nm + 373 nm
433 nm
Exp. 8 nr = 8
Exp. 9 nr = 10
505 nm + 573 nm
505 nm
Exp. 10 nr = 12
Exp. 11 nr = 16
433 nm + 505 nm
505 nm
*
Exp. 12 nr = 18
Exp. 13 nr = 18
505 nm
433 nm
*
0 5 10 15 20 0 5 10
LEDs tested
Number of seed bugs responding

Figure 8.4

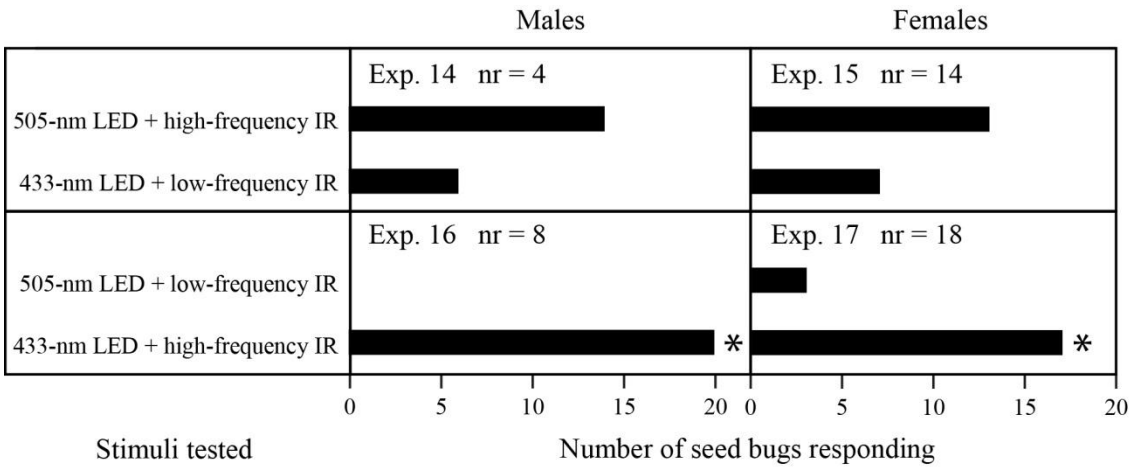

Males
Females
Exp. 14 nr = 4
Exp. 15 nr = 14
505-nm LED + high-frequency IR
433-nm LED + low-frequency IR
Exp. 16 nr = 8
Exp. 17 nr = 18
505-nm LED + low-frequency IR
433-nm LED + high-frequency IR
*
*
0 5 10 15 20 0 5 10 15 20
Stimuli tested
Number of seed bugs responding

Figure 8.5

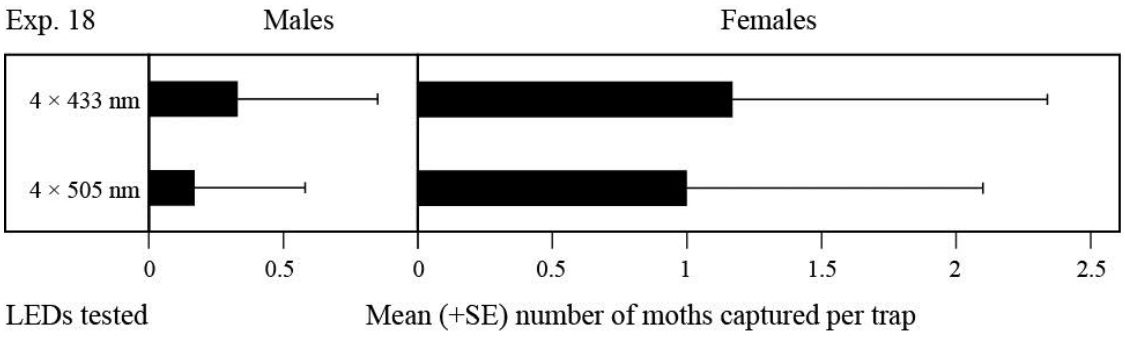

Exp. 18
Males
Females
4 × 433 nm
4 × 505 nm
0
0.5
0
0.5
1
1.5
2
2.5
LEDs tested
Mean (+SE) number of moths captured per trap

Figure 8.6

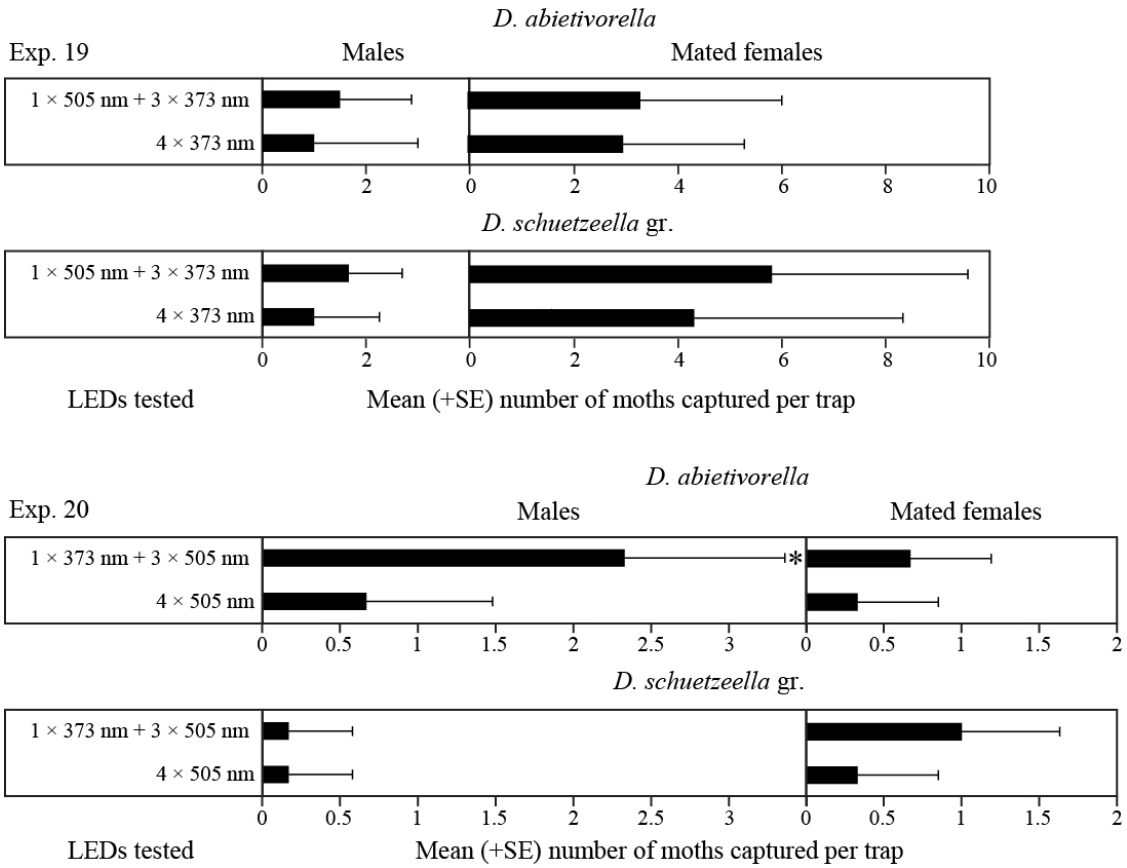
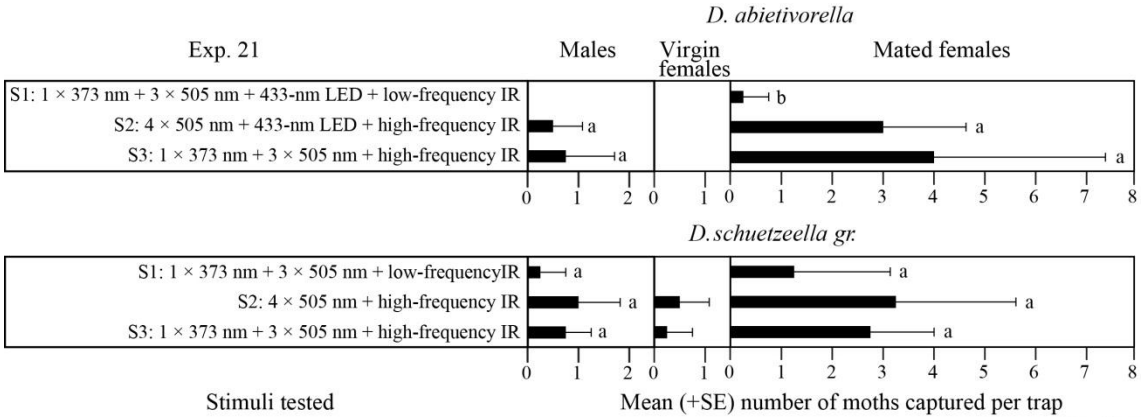

D. abietivorella
Exp. 19
Males
Mated females
1 × 505 nm + 3 × 373 nm
4 × 373 nm
0
2
0
2
4
6
8
10
D. schuetzeella gr.
1 × 505 nm + 3 × 373 nm
4 × 373 nm
0
2
0
2
4
6
8
10
LEDs tested
Mean (+SE) number of moths captured per trap
D. abietivorella
Exp. 20
Males
Mated females
1 × 373 nm + 3 × 505 nm
4 × 505 nm
*
0
0.5
1
1.5
2
2.5
3
0
0.5
1
1.5
2
D. schuetzeella gr.
1 × 373 nm + 3 × 505 nm
4 × 505 nm
0
0.5
1
1.5
2
2.5
3
0
0.5
1
1.5
2
LEDs tested
Mean (+SE) number of moths captured per trap

Figure 8.7


D. abietivorella
Exp. 21
Males
Virgin females
Mated females
S1: 1 × 373 nm + 3 × 505 nm + 433-nm LED + low-frequency IR
S2: 4 × 505 nm + 433-nm LED + high-frequency IR
S3: 1 × 373 nm + 3 × 505 nm + high-frequency IR
b
a
a
a
0 1 2 0 1 0 1 2 3 4 5 6 7 8
D. schuetzeella gr.
S1: 1 × 373 nm + 3 × 505 nm + low-frequencyIR
S2: 4 × 505 nm + high-frequency IR
S3: 1 × 373 nm + 3 × 505 nm + high-frequency IR
a
a
a
a
a
a
0 1 2 0 1 0 1 2 3 4 5 6 7 8
Stimuli tested
Mean (+SE) number of moths captured per trap

9. Concluding summary

I investigated the potential use of electromagnetic foraging or attraction cues in three members of the conophagous insect guild: the diurnal Western conifer seed bug, *Leptoglossus occidentalis* Heidermann (Hemiptera: Coreidae), the nocturnal fir coneworm moth, *Dioryctria abietivorella* Groté (Lepidoptera: Pyralidae), and the diurnal Douglas-fir cone gall midge, *Contarinia oregonensis* Foote (Diptera: Cecidomyiidae). In electrophysiological recordings, two-choice laboratory bioassays, and field trapping experiments, I tested the hypotheses that during (cone) foraging *C. oregonensis*, *D. abietivorella* and *L. occidentalis* (1) rely on IR receptors that receive and respond to cone-derived radiant IR, (2) receive and respond to cone-derived colour cues, and (3) integrate radiant IR, and insect visible light cues.

The following results support hypothesis 1:

Leptoglossus occidentalis: In my study, temperatures of Western white pine cones ranged between 15 °C and 35 °C from 09:00 to 18:30 h, and in previous studies they sometimes exceeded even 40 °C (Takács et al. 2009). Collectively, these data indicate that there is a stark (thermal) contrast between the IR signature of cones and the much cooler foliage throughout the time when *L. occidentalis* forages, and that the IR receptors of *L. occidentalis* (Takács et al., 2009) could exploit this contrast. When given a choice between radiant IR from heat sources well within (40 °C) and just outside (60

°C) the natural cone temperature range, male and female *L. occidentalis* were attracted to the former, supporting the concept that radiant IR from conifer cones serves as a foraging cue for *L. occidentalis.*

Contarinia oregonensis: In early-spring thermographs of Douglas-fir trees, branches were warmer than foliage, and cones were cooler. In choice experiments, *C. oregonensis* preferred warm objects to cool objects, and warm branch-like objects to warm can-like objects, indicating that the shape of, and radiant IR from, Douglas-fir branches could serve as foraging cues for female *C. oregonensis*. Once within the canopy of a host tree, they may then orient towards branch tips to find cones. Assuming that the IR receptors of *C. oregonensis* can be located, and that *C. oregonensis* indeed responded to radiant IR rather than heat at very close range, evidence is emerging that *C. oregonensis* may integrate an object's shape and IR signature when making foraging decisions.

Dioryctria abietivorella: I present the first evidence for IR receptors in a species of the Lepidoptera. Located on the prothorax of male and female *D. abietivorella,* IR receptors responded to radiant IR from heat sources kept at 40-60 °C. In laboratory and field experiments, males and mated females, but not virgin females, were more strongly attracted to higher-frequency than to lower-frequency radiant IR emanating from hot or cold sources, respectively. Natural IR sources are associated with host trees. Thermographs of Douglas-fir and spruce trees showed strong temperature differentials between parts of trees and their surroundings, which could be exploited by moths during their nocturnal flight period (21:00 - 03:00 h).

The following results support hypothesis 2:

I present evidence in electrophysiological recordings with OneLight spectra as test stimuli that the complete human visual spectrum of conifer needles elicits strong responses from the eyes of female *L. occidentalis.* Moreover, in two-choice laboratory bioassays, I show that this complete spectrum attracts females significantly more strongly than simpler spectra in the yellow-green range. However, none of the many other experiments run with *L. occidentalis, D. abietivorella,* and *C. oregonensis* revealed evidence that cone-associated colour serves as a singular foraging cue. Although not yet experimentally tested, it is conceivable that the colour of needles may matter not only to *L. occidentalis,* but also to *D. abietivorella* and *C. oregonensis.*

The following results support hypothesis 3:

Experimental evidence suggested that specific wavelengths of blue light may contribute to a cue complex that triggers foraging or attraction in *L. occidentalis*. This concept was supported when I tested combinations of visible light with radiant IR. High-frequency radiant IR from a ~ 40 ºC heat source coupled with the 433-nm blue light attracted both males and females. That bugs did not respond just to the presence of two types of electromagnetic wavelengths, but to a specific combination of them, became apparent when I tested combinations of either high-frequency radiant IR with non-attractive (505 nm) light, or low-frequency radiant IR (from a 2 ºC source) with attractive (433 nm) light, with either combination failing to significantly attract *L. occidentalis*.

The interaction between high-frequency radiant IR and blue light on attraction of male and female *L. occidentalis* supports the conclusion that the central nervous system of *L. occidentalis* is capable of processing and integrating information from two types of sensory receptors, the compound eyes on the head which detect visual light, and IR

receptors on the ventral abdomen which detect radiant IR. Such interaction or integration has previously been studied only in snakes.

There is no evidence yet for synergistic or additive effects between electromagnetic wavelengths in the UV, visual and IR range for attraction of *D. abietivorella or C. oregonensis* but additional experiments are warranted to explore this further.

Appendix A.

Determining retinal spectral sensitivities of house flies, *Musca domestica*[8]

Introduction

The maximal retinal spectral sensitivities of many insects have been assessed (Briscoe & Chittka 2001). In electrophysiological recordings, a monochrometer has been used to reveal maximal spectral sensitivity (Groberman & Borden 1980, Meyer-Rochow 1981, Bennett et al. 1997, Townson et al. 1998). Hardie (1986) determined the spectral sensitivity of eyes of house flies, *Musca domestica*, by deploying intracellular recordings with dye injections and microspectrophotometry. Spectral sensitivities were shown at 335, 430, 460, 490, 520, and 570 nm (Hardie 1986). These spectral sensitivities do not affect behaviour. Colours predominantly in the blue (490 nm) and green (570 nm) fail to attract more house flies than white (Hanley et al. 2009). Here I show that I obtained similar results in the UV range but fewer less distinct peaks in the blue and green regions of light using a monochromatic light source in electroretinograms. Moreover, my study reveals increased spectral sensitivity in the red (620 nm) region, not previously described.

Materials and Methods

Insects

Insects were obtained from a colony reared at Simon Fraser University (Burnaby, BC, Canada) that was housed indoors and fed a diet of sugar water solution and milk powder (Lam et al. 2007).

Electroretinograms

Ten flies were used in this experiment. Single adult flies were immobilized with their left lateral side on a glass slide using Tanglefoot adhesive (Contec Enterprises Inc., Victoria, BC, Canada). Preperations were placed on a brass stub inside a Faraday cage to help eliminate electrical noise (Cowan and Gries 2009). Using a Leitz micromanipulator (Leica Inc., Vienna, Austria), a recording electrode, made of electrically sharpened 0.2 mm diam bare tungsten wire (A-M Systems Inc., Carlsborg, WA, USA) (Cools et al. 1970), was inserted into the center of the equatorial region of the right eye. Another electrode was inserted in the center of the right side of the abdomen. After an electrode was inserted into the eye, one terminus of a fiber optic cable ending in a sub multi assembly (SMA), originating from the light source (for details see below), was positioned 5 mm above the recording electrode in the insect's eye, and the insect was dark-adapted for 45 min before being exposed to stimuli.

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Electrical responses from the eye were pre-amplified (Syntech Auto Spike, Syntech Inc., Hilversum, The Netherlands) and processed (IDAC signal interface box, Syntech, Inc.) for analysis. Using oscilloscope software, the amplitude of response was determined (EAG, Version 2.4, Syntech Inc.).

A Monoscan2000 fiber optic scanning monochromator (Ocean Optics, Dunedin, FL, USA) powered by an HPX-2000 high-powered, continuous-wave, 35-watt xenon light source (Mikropak GmbHa, Ostfildern, Germany) was programmed to output 10-nm bandwidths of wavelengths between 250-950 nm in 10-nm increments, at 10-s intervals. Test stimuli were projected through a 600-µm premium-grade optical fiber solarized-resistant assembly (Ocean Optics, Dunedin, FL, USA) to a 0-2 stop circular, variable neutral density wheel (Fused Silica (200 nm to 2500 nm), Reynard Corp., Calle Sombra, San Clemente, California, USA). Light passing through the neutral density wheel was directed to a 70:30 beam splitter ("polka dot" 4-7001, Optometrics, Ayer, MA, USA). Thirty percent of the light beam was directed to an HR4000 spectrometer (Ocean Optics) where it was calibrated to an intensity of 3.0×10^{12} photons/cm^2/s for each wavelength at a distance of 0.5 cm from point source using a spectrometer with SpectraSuite software (Ocean Optics). The remaining 70% (7.0×10^{12} photons/cm^2/s) of the light beam was projected toward a programmable shutter (R. Holland, Technical Science Centre, SFU) (Fig. 1). The shutter was continuously closed except for 500 ms every 9.5 s. Beyond the shutter, a LC-4U-THD collimator assembly (Multimode Fiber Optics, Hackettstown, NJ, USA) collected the light into a 1000-µm fused silica solarized UV resistant patch (single fiber) cable (Multimode Fiber Optics) which terminated above the eye. For each wavelength tested, data were standardized using the ratio to the highest response per eye, and then averaged between eyes (Kirchner et al. 2005). Standardized responses of the eye were compared with a one-way ANOVA (with Tukey's post hoc test) blocked by insect (Zar 1999) using JMP software (SAS®, Cary, NC, USA).

Results and Discussion

I recorded four areas of increased spectral sensitivity in the eyes of houseflies (Fig. 2). Hardie (1986) reported spectral sensitivity at 335, 430, 460, 490, 520, and 570 nm. In my study, the regions of increased spectral sensitivity at 340 nm and 430 nm (Fig. 2) are similar to those found by Hardie (1989). Though Hardie (1989) found three distinct spectral sensitivities in the blue-green region, my recordings revealed only a single yet large region of spectral sensitivity ranging between 480 nm to 500 nm (Fig. 2). I found no evidence for increased spectral sensitivity at 570 nm; however, I found increased spectral sensitivity in the red region at 620 nm (Fig. 2). These data indicate that the spectral sensitivities I recorded for *L. occidentalis* using the monochrometer are reliable and form a good basis for comparing the various methods I used, and the results I obtained in my thesis, analyzing spectral sensitivities of three conophagous insect species.

Literature Cited

Bennett RR, White RH & Meadows J (1997) Regional specialization in the eye of the sphingid moth *Manduca sexta*: Blue sensitivity of the ventral retina. Visual Neuroscience 14: 523-526.

Briscoe AD & Chittka L (2001) The evolution of colour vision in insects. Annual Review of Entomology 46: 471-510.

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

Cowan T & Gries G (2009) Ultraviolet and violet light: attractive orientation cues for the Indian meal moth, *Plodia interpunctella*. Entomologia Experimentalis et Applicata 131: 148-158.

Groberman LJ & Borden JH (1980) Electrophysiological response of *Dendroctonus pseudotsgugae* and *Ips paraconfusus* (Coleoptera: Scolytidae) to selected wavelength regions of the visible spectrum. Canadian Journal of Zoology 60: 2180–2189.

Hanley ME Cruickshanks KL Dunn P Stewart-Jones A Goulson D (2009) Luring houseflies (*Musca domestica*) to traps: do cuticular hydrocarbons and visual cues increase catch? Medical and Vetrinary Entomology 23: 26-33.

Hardie RC (1986) The photoreceptor array of the dipteran retina. Trends in Neurosciences 9: 419-423.

Meyer-Rochow VB (1980) Electrophysiologically determined spectral efficiencies of the compound eye and median ocellus in the bumblebee *Bombus hortorum tarhakimalainen* (Hymenoptera, Insecta). Journal of Comparative Physiology A139: 261–266.

Kirchner SM Döring TF & Saucke H (2005) Evidence for trichromacy in green peach aphid, *Myzus periscae* (Sulz.) (Hempitera: Aphidae). Journal of Insect Physiology 51: 1255-1260

Lam K, Babor D, Duthie B, Babor EM, Moore M & Gries G (2007) Proliferating bacterial symbionts on house fly eggs affect oviposition behaviour of adult flies. Animal Behavior 74: 81-92.

Townson SM, Chang BSW, Salcedo,E, Chadwell LV, Pierce NE& Britt.SG (1998) Honeybee blue- and ultraviolet-sensitive opsins: Cloning, heterologous expression in Drosophila, and physiological characterization. Journal of Neuroscience 18: 2412–2422.

Figure Captions

Figure 1. Diagram of the set-up in electroretinogram recordings with an insect prepared on a glass side in a Faraday cage and a monochromatic light source.

Figure 2. Retinal spectral sensitivity of eyes of house flies, *Musca domestica,* as determined with a MonoScan2000, reporting the mean (+ SE) ratio to highest amplitude response (n = 10) to each band width tested.

Figure 1

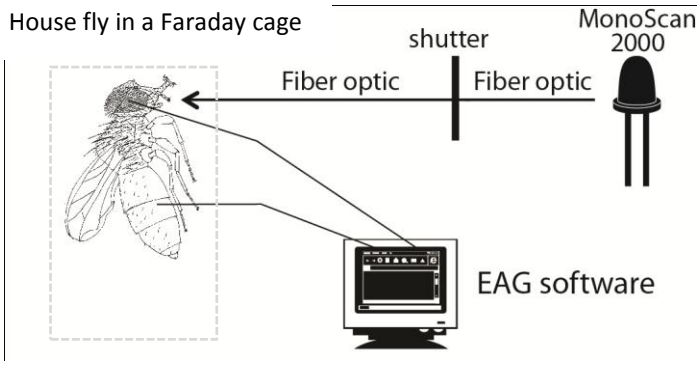
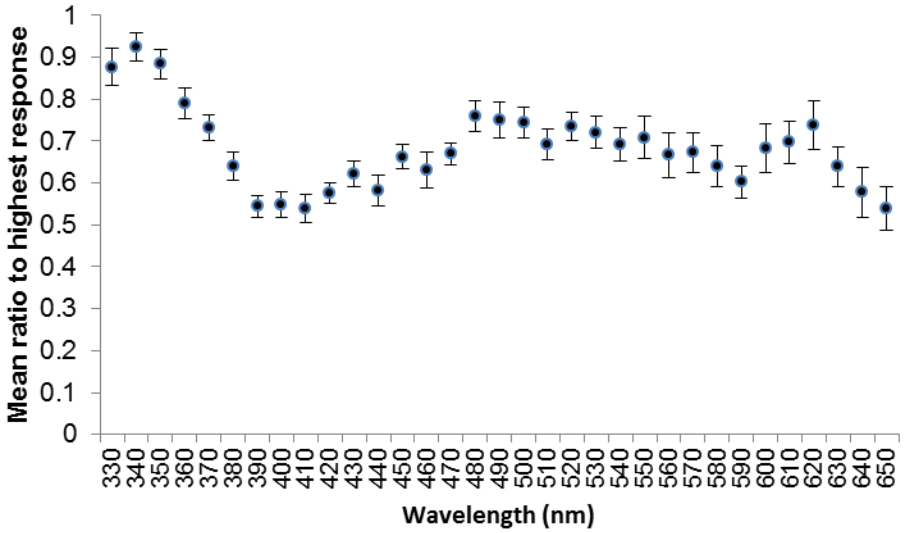

House fly in a Faraday cage
shutter
MonoScan 2000
Fiber optic
Fiber optic
EAG software

Figure 2


Mean ratio to highest response
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
330
340
350
360
370
380
390
400
410
420
430
440
450
460
470
480
490
500
510
520
530
540
550
560
570
580
590
600
610
620
630
640
650
Wavelength (nm)