



Visual cues of oviposition sites and spectral sensitivity of *Cydia strobilella* L.



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A B S T R A C T

We investigated whether the spruce seed moth (*Cydia strobilella* L., Tortricidae: Grapholitini), an important pest in seed orchards of Norway spruce (*Picea abies* (L.) Karst.), can make use of the spectral properties of its host when searching for flowers to oviposit on. Spectral measurements showed that the flowers, and the cones they develop into, differ from a background of *P. abies* needles by a higher reflectance of long wavelengths. These differences increase as the flowers develop into mature cones. Electroretinograms (ERGs) in combination with spectral adaptation suggest that *C. strobilella* has at least three spectral types of photoreceptor; an abundant green-sensitive receptor with maximal sensitivity at wavelength $\lambda_{\max} = 526$ nm, a blue-sensitive receptor with $\lambda_{\max} = 436$ nm, and an ultraviolet-sensitive receptor with $\lambda_{\max} = 352$ nm. Based on our spectral measurements and the receptor properties inferred from the ERGs, we calculated that open flowers, which are suitable oviposition sites, provide detectable achromatic, but almost no chromatic contrasts to the background of needles. In field trials using traps of different spectral properties with or without a female sex pheromone lure, only pheromone-baited traps caught moths. Catches in baited traps were not correlated with the visual contrast of the traps against the background. Thus, visual contrast is probably not the primary cue for finding open host flowers, but it could potentially complement olfaction as a secondary cue, since traps with certain spectral properties caught significantly more moths than others.

1. Introduction

Both insect pollinators and herbivores use a wide range of sensory cues to locate their plant resources, for example, emitted volatile compounds (Harrewijn et al., 1994; Renwick and Chew, 1994; Knolhoff and Heckel, 2014), temperature (Seymour and Schultze-Motel, 1997; Angioy et al., 2004; Lamprecht et al., 2013), shape (Rauscher, 1978; Mackay and Jones, 1989; Machial et al., 2012), and colour (Prokopy and Owens, 1983; Tsuji and Coe, 2014; Paris et al., 2015). The use of colour cues by insects has been studied intensely. Most commonly, insects seem to have a preference for broadband colours that appear yellow to humans (Prokopy and Owens, 1983; Mainali and Lim, 2010; de Oliveira et al., 2015) and are believed to represent a super-normal foliage-type stimulus for herbivores (Prokopy, 1972). Many pollinators, on the other hand, have an innate preference for blue (Kelber, 1997; Raine and Chittka, 2005; Burger et al., 2010; Yoshida et al., 2015). Finally, preferences for red have been shown in insects choosing red fruits as their larval habitat (Owens and Prokopy, 1984, 1986; Cornelius et al., 1999).

In this study, we investigate whether the spruce seed moth, *Cydia*

strobilella, relies on visual contrast to find its host. During late spring, these highly damaging, diurnal seed predators search for the flowers of Norway spruce, *Picea abies* (Stadnitskii et al., 1978; Seifert et al., 2000; Rosenberg and Weslien, 2005), Siberian spruce, *P. obovata*, or Jezo spruce, *P. jezoensis* (Bakke, 1963; Stadnitskii et al., 1978), with a peak activity around noon (Jakobsson et al., 2016). Previous literature states that *C. strobilella* flies from mid May to mid June (Bakke, 1963; Stadnitskii et al., 1978), but in southern Sweden the flight season often starts earlier (Svensson et al., 2013). After mating, which usually occurs on the day of emergence, *C. strobilella* females oviposit on the lower inner part of the scales of open spruce flowers (Bakke, 1963; Stadnitskii et al., 1978). As the flowers develop into cones, the scales close and harden making the inner parts inaccessible. This maturation process is accompanied by a colour change. To a human observer, open spruce flowers appear reddish violet, strongly contrasting against the green needle background. When the flowers close and develop into cones, the violet colour fades and the cones become increasingly brown and less obvious against the background. Could *C. strobilella* use such visual cues to guide oviposition?

Recently, the components of the sex pheromone released by *C.*

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strobilella females were identified (Wang et al., 2010), and even more recently, a blend of host volatiles including (+)- α -pinene, (-)- α -pinene, (+)- β -pinene, (-)- β -pinene and myrcene was shown to be attractive to the species (Jakobsson et al., 2016). A close and comparatively well-studied relative of *C. strobilella*, the apple moth, *Cydia pomonella*, is attracted to (*E,E*)-farnesene (Coracini et al., 2004; Light and Knight, 2005), the pear ester ethyl (*E,Z*)-2,4-decadienoate (Light et al., 2001; Il'ichev, 2004), (*E*)-4,8-dimethyl-1,3,7-nonatriene, and acetic acid (Knight et al., 2011), all of them host volatiles released by apple and pear. Electroretinograms (ERGs) of dark-adapted eyes revealed two sensitivity peaks in *C. pomonella*, a major one at about 580 nm and a slightly smaller one at about 365 nm (Pristavko et al., 1981). In field experiments with coloured traps, sex pheromone-baited green and orange traps caught most moths, indicating a preference for light of longer wavelengths (Knight and Miliczky, 2003), though previous studies had shown that the moths were attracted to light of short wavelengths between 310 and 380 nm (e.g., Tashiro et al., 1967; Pristavko et al., 1981; Steiner and Häuser, 2014).

The spectral sensitivities of photoreceptors in the compound eyes of winged insects (Pterygota) typically have maxima in the ultraviolet (~350 nm), blue (~440 nm) and green (~530 nm) part of the spectrum (Briscoe and Chittka, 2001), based on three visual pigments that were probably already present in the ancestral pterygote insect (Henze and Oakley, 2015). Many butterflies expanded this set of spectral sensitivities (Briscoe, 2008), with the current record being 15 types of spectral receptor in the compound eyes of the common bluebottle, *Graphium sarpedon* (Leptocerini, Papilionidae) (Chen et al., 2016). Moths, even though they are the most speciose group of Lepidoptera, have been studied less intensively (Briscoe and Chittka, 2001). Opsins for the typical three classes of visual pigments have been cloned from a few moth species or been identified in their genome (Chase et al., 1997; Velarde et al., 2005; Xu et al., 2013; Yan et al., 2014; Feuda et al., 2016). A survey of previously reported spectral sensitivity maxima (Supplementary Table S1) suggests that at least some species deviate from the pterygote ground plan by adding more receptor types (Belušić et al., 2017), which, in certain cases, extends the sensitivity range further towards longer wavelengths, with sensitivity maxima at up to 600 nm (Eguchi et al., 1982).

Here, we use ERG recordings in combination with spectral adaptation to determine the spectral sensitivities of the photoreceptors in the compound eyes of *C. strobilella*. Moreover, we measure the spectral reflectance of flowers and cones of *P. abies* at different developmental stages, as well as the reflectance of their natural background, the needles. Taking sensitivity and reflectance data into account, we model the contrast of spruce flowers and cones against the needles as presumably experienced by the moths. Finally, to examine how the predictions of our model agree with behaviour, we test how the moths respond to traps of different spectral properties in their natural habitat.

2. Methods

2.1. Moths

Male and female *C. strobilella* used for electrophysiological experiments originated from cones of *P. abies* collected in a spruce seed orchard near Gringelstad (55°55'40"N 14°06'17"E), Skåne Province, Sweden, in August 2012. The cones were stored in a climate chamber. During the first three months, the temperature was gradually lowered from 18 °C to 5 °C, and the light regime was adjusted from 12.5 h of light per day to 7 h of light per day. From January to August 2013, we triggered the emergence of the moths by first moving the cones to a climate chamber set to 10 °C and 12 h of light, and then, a week later, transferring them to another climate chamber set to 20 °C during a light phase of 16 h and 15 °C during the dark phase. We placed the cones in small wooden boxes (16 cm × 16 cm × 15 cm) with an inserted glass tube to facilitate collection of emerging moths moving towards the

outside light.

2.2. ERG recordings

To record ERGs, moths were glued upside down on a holder by fixing their wings, the antennae, the proboscis and the legs with a 1:1 mixture of beeswax and resin. A silver reference electrode was inserted into the abdomen through a hole in the body wall or, in later experiments, through the sexual tract or anus, which increased the viability of the insect in the setup. The site of entry was covered with electrode gel (Gel101, Biopac Systems Inc., Goleta, CA, USA). We advanced an electrolytically sharpened tungsten electrode through the ventral margin of the right compound eye into the retina using a piezo-driven micromanipulator (PM10 DC3-K, Märzhäuser, Wetzlar, Germany). A forked light guide (QR400-7-SR/BX, Ocean Optics, Dunedin, FL, USA) directed light from a 200 W xenon lamp (Cermax LX175 F ASB-XE-175EX, SP Spectral Products, Putnam, CT, USA) through its 400 μ m-wide central fibre into the darkened Faraday cage, in which the animal was placed. The light beam (16° divergence) illuminated the whole eye, when two shutters (VS25S2ZM1R1 and LS6ZM2, Uniblitz, Vincent Associates, Rochester, NY, USA) were open. The light spectrum could be altered using one of 22 narrow-band interference filters (dominant wavelengths 330–700 nm; 10–12 nm full-width at half-maximum; Melles Griot, Rochester, NY, USA). We ensured that the intensity in quanta $s^{-1} cm^{-2}$ (quantum flux) of each of the spectral flashes was the same by inserting neutral density filters (fused silica, Melles Griot) into the light path. Both the position of the light guide and the depth of the measuring electrode were adjusted to achieve maximal responses to light flashes of 40 ms duration separated by pauses of 5 ms.

For spectral adaptation, we presented long-wavelength light (dominant wavelength 600 nm, 20 nm full-width at half-maximum; “amber” LED A42182, Seoul Semiconductor, Ansan, Korea) continuously through the six outer fibres (each 400 μ m in diameter) of the light guide. The combined light beam had a divergence angle of 25° and provided intensities between 4×10^{14} and 7×10^{15} quanta $s^{-1} cm^{-2}$ at the position of the eye, depending on the operating current of the LED. Recorded ERGs were amplified (P15 AC amplifier, Grass Technologies, Warwick, RI, USA), sampled at 2000 Hz, digitized and saved using an NI PCIe-6251 data acquisition board and custom-made scripts in LabVIEW (both National Instruments Corporation, Austin, TX, USA).

For each moth, the experimental protocol was as follows: After at least 10 min of dark adaptation, we subjected the eye to a series of narrow-band spectral flashes from short to long wavelengths, with the dominant wavelength increasing in intervals of 10 or 20 nm. This series was then repeated in reverse order, i.e., from long to short wavelengths. Before and after each of the two spectral series, a response-intensity (V -log I) relationship using white light was established to assess the robustness of the recording and the dynamic range of responses. The whole procedure was carried out two to three times, before the eye was adapted to amber light for 15 min, and tested in the same way as under dark-adaptation. We increased the intensity of the adaptation light until we could no longer detect responses to long-wavelength flashes in the ERG. To assess the condition of the moth at the end of the experiment, we re-adapted the eye to darkness for 15 min, and checked whether the original responses could be recovered.

2.3. ERG analyses

All data were analysed using custom-made scripts in Matlab (R2013b, The MathWorks, Natick, MA, USA). To filter out high-frequency noise, we applied a sliding average with a window-width of 5 ms to the signal. Response amplitudes were then calculated as potential changes from the baseline at stimulus onset to maximal hyperpolarization, and converted into sensitivities based on the V -log I relationship obtained before and after each spectral series (for details

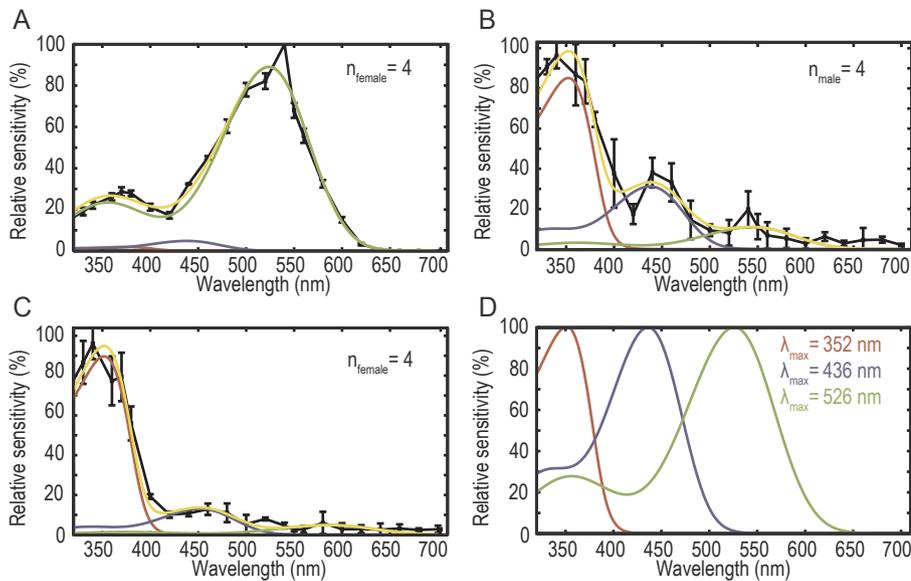


Fig. 1. Spectral sensitivity of the compound eyes of *Cydia strobilella* determined by ERGs. Examples of individual results after (A) dark adaptation (female individual), (B) adaptation to dim amber light (male individual), and (C) adaptation to strong amber light (female individual). (D) Normalized spectral sensitivity of the three types of photoreceptor. Results from ERGs are plotted in black (average \pm standard deviation, n gives the number of series that were averaged). Red, blue and green lines indicate the contributions of the UV-, blue- and green-sensitive receptor type, respectively, while the yellow line denotes the summed response of all receptors.

see Telles et al., 2014). We normalized all values to the maximal spectral sensitivity within each series, and averaged multiple series from the same individual for each experimental condition. Templates for the spectral absorbance of visual pigments (Govardovskii et al., 2000; Stavenga, 2010) were used to estimate the number and position of sensitivity maxima contributing to the ERG. In a first step, we fitted three such templates simultaneously to the mean spectral sensitivity curve of the dark-adapted eye applying a non-linear least squares approach. Amplitude and wavelength of the α -peaks of pigment absorbance were varied independently, whereas amplitude and wavelength of the β -peaks were calculated as functions of the respective values for the α -peaks, as proposed previously (Govardovskii et al., 2000; Stavenga, 2010). Curves that originated from stable recordings and showed minimal contributions of short-wavelength receptors were selected to determine the wavelength of maximal sensitivity (λ_{\max} of the α -peak) of a putative green receptor (Fig. 1A). Results obtained from different individuals were averaged. In a second step, we incorporated the mean λ_{\max} of the green receptor into the template formula, and analysed spectral sensitivity curves of the amber-adapted eye, in which the contribution of a putative ultraviolet (UV) receptor was maximal (Fig. 1C). The λ_{\max} values determined for the UV receptors of several individuals were averaged and also included in the model. In a third step, we estimated λ_{\max} of a putative blue receptor based on spectral sensitivity curves of the partially amber-adapted eye with comparatively high sensitivities in the middle range of wavelengths (Fig. 1B), and averaged the results.

2.4. Spectral properties of the Norway spruce

The spectral reflectance of flowers and cones of *P. abies* was measured on five different occasions during 2012 (2nd, 8th and 17th of May, and 1st and 19th of June). Based on the assumption that the colours of spruce needles do not change much during the season, they were only measured on May 2nd, 2012. On each occasion, we recorded the reflectance at three different positions around the base, the middle, and the top of a flower/cone (Fig. 2). The reflectance was measured from an angle of 45° using a light guide (1000 μ m in diameter, Ocean Optics, Dunedin, FL, USA) connected to a spectrometer (Maya, Ocean Optics) either outside in the shade on sunny days, or inside while illuminated by a 400 W xenon lamp (Gantz, Switzerland). As there were only small, non-systematic spectral differences between all nine measurements of the same flower/cone, we calculated a single average for each sample. For further analyses, the flowers/cones were divided into four

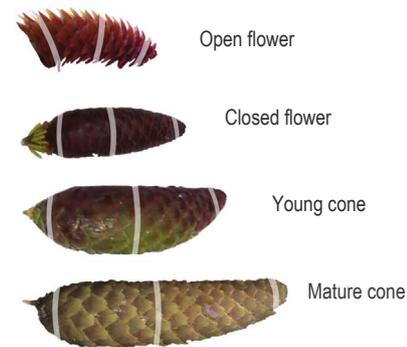


Fig. 2. Developmental stages of female flowers and cones of *Picea abies* used for spectral measurements taken along the areas marked by a white band in the figure. Flowers and cones are not shown to scale.

categories depending on their developmental stage (Fig. 2). The first category (“open flower”) contained flowers that were open or about to close their petals, the second category (“closed flower”) contained flowers that had almost completely or just closed their petals, the third category (“young cone”) contained young cones with closed petals/scales, and the last category (“mature cone”) contained cones that had matured.

2.5. Modelling achromatic contrast

We assume that the spruce seed moths rely on their abundant green-sensitive photoreceptors to encode achromatic contrast (“brightness”) (Osorio and Vorobyev, 2005). However, because there are indications that some insects use multiple spectral inputs for achromatic vision (Wardill et al., 2012), we also calculated the contrast for a channel to which all receptor types contributed equally. Quantum catches (Q_L) give the Michelson contrast, C , as:

$$C = \frac{(Q_L(\max) - Q_L(\min))}{(Q_L(\max) + Q_L(\min))} \quad (1)$$

where max and min denote the stimulus surface yielding the highest and the lowest quantum catch, respectively. Michelson contrast ranges from 0, no contrast, to 1, highest contrast possible. The contrast sensitivity of *C. strobilella* is not known, but assuming that it is similar to that of the honeybee, *Apis mellifera*, discrimination between surfaces should be possible at contrast values above 8% (Srinivasan and Lehrer,

1988).

2.6. Modelling chromatic contrast

We estimated colour contrast using the receptor noise limited model suggested by Vorobyev and Osorio (1998). In this model, we assume that colour discrimination is limited by receptor noise that is propagated into subsequent neural networks via colour opponent mechanisms. The limitation set by receptor noise is independent of how receptor outputs are compared in the post-receptor network, as long as all receptors are compared. The model yields predictions of chromatic contrast (“colour”) whereas achromatic contrast (“brightness”) is ignored. Here, we provide a short outline of the model, while comprehensive descriptions can be found in Vorobyev and Osorio (1998) and Kelber et al. (2003).

First we determine photoreceptor quantum catch:

$$Q_i = \int_{350}^{700} q_i(\lambda)R(\lambda)I(\lambda)d\lambda \tag{2}$$

where Q_i is the quantum catch of photoreceptor type i ($i = UV, blue, green$) for wavelengths λ between 350 and 700 nm, q_i stands for the sensitivity of photoreceptor type i (Fig. 1), R denotes the reflectance of spruce flowers/cones, traps or the background of needles (Fig. 3) and I is the illumination (standard daylight, d65; Wyszecki and Stiles, 2000).

We assume that intensity is coded log-linearly by photoreceptors (e.g. Kelber et al., 2003), so that the contrast for each receptor channel is:

$$\Delta f_i = \ln\left(\frac{Q_{i,stimulus 1}}{Q_{i,stimulus 2}}\right) \tag{3}$$

Receptor responses are combined in colour-opponent mechanisms that yield chromatic contrast ΔS :

$$\Delta S^2 = \frac{\omega_1^2(\Delta f_3 - \Delta f_2)^2 + \omega_2^2(\Delta f_3 - \Delta f_1)^2 + \omega_3^2(\Delta f_2 - \Delta f_1)^2}{(\omega_1\omega_2)^2 + (\omega_1\omega_3)^2 + (\omega_2\omega_3)^2} \tag{4}$$

where ω is the Weber fraction determined by noise in each receptor channel:

$$\omega = \frac{v_i}{\sqrt{\eta_i}} \tag{5}$$

As the noise, v , of a photoreceptor of spectral type i is related to its relative abundance in the receptive field, η , this equation accounts for signal enhancement given by pooling of receptor output. The relative abundance of the different photoreceptors (taking their relative numbers and sizes into account) and the absolute levels of receptor noise are not known for *C. strobilella*. Preliminary analyses of chromatic contrast for all possible combinations of abundances between 1:1:1 and 1:4:10 (for the UV:blue:green receptors; upper limit derived from data on the tobacco hornworm, *Manduca sexta*, cf. White et al., 2003) and absolute noise between 0.08 and 0.12 (cf. Vorobyev et al., 2001) indicated, without exceptions, lowest contrast for 1:1:10, 0.12 (receptor contrast, absolute noise), and highest contrast for 1:4:1, 0.08. We used these parameter settings to make a pessimistic and an optimistic estimate of chromatic contrast.

The unit of ΔS is JNDs (just noticeable differences), and the theoretical discrimination threshold is set to 1 JND. Animals may adopt different criteria ruling discrimination, leading to a behavioural threshold different from 1 JND. For example, honeybees usually do not discriminate stimuli if ΔS is lower than 2.3 JNDs (Vorobyev et al., 2001). We consider discrimination impossible for $\Delta S < 1$, uncertain for $\Delta S < 3$, and certain for $\Delta S > 3$.

2.7. Behavioural field trials

Traps were constructed using RAG (“delta”) traps (Csalomon, Hungary) with sticky inserts, and dressed with different colour filters

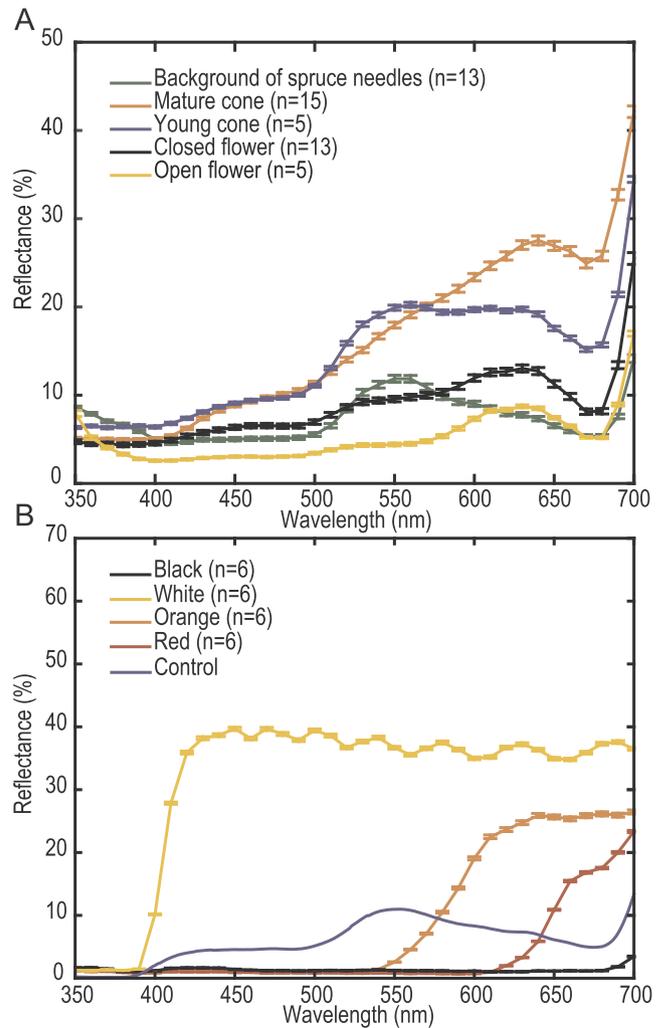


Fig. 3. (A) The average spectral reflectance of needles and different developmental stages of female flowers and cones of *Picea abies* (n-values indicate the number of measured flowers/cones). Error bars represent standard errors. (B) The average spectral reflectance of the traps used in the field trials to catch *Cydia strobilella* (n-values indicate the number of different positions measured on the same trap). Error bars represent standard errors. The reflectance of the control traps mainly depended on their background. Therefore, we calculated the curve for the control traps using the measured transmittance of the UV filters and the reflectance of spruce needles, which is what the moths perceive in the field.

(LEE, United Kingdom). For a typical trichromatic human, the resulting test traps appeared black (LF299, 1.2 ND), orange (LF158, deep orange), red (LF027, medium red), and white (LF129, heavy frost). Control traps only had a UV-absorbing filter that was used as the outermost layer in all traps to remove glare (LEE UV). Completely undressed traps were transparent except for black printed text. To enhance the colour of the test filters, and to cover the black print, all traps except the controls also had an innermost, scattering layer of white diffusing filter (LF129). The spectral reflectance of the final traps was measured using the equipment described for the Norway spruce in Section 2.4. with the following modifications: Illumination was provided by a HPX-2000-hp-DUV high power xenon light source (Ocean Optics), and both the light source and the spectrometer were connected to a back-scattering probe (600 μ m in diameter, Ocean Optics) with measurements taken at an angle of 90° to the measured surface. Since the control traps did not contain a white diffusing filter, their reflectance strongly depended on the underlying substrate. Therefore, we describe their spectral properties by the combination of the transmittance of the UV filter and the reflectance of spruce needles, which is what the insects perceive in the field.

Trapping experiments were performed in the spruce seed orchard near Gringelstad (see Section 2.1.) and in a spruce clone archive near Maltesholm (55°54'49"N 13°59'33"E), Skåne Province, Sweden, from May 3rd to 30th, 2013. In each location, five replicates were set up. Each replicate was placed in its own row of spruce trees and consisted of one trap of each colour and a control trap. The traps were placed four to five meters above the ground in a sunny and wind-protected, south-facing spot of a spruce tree at a minimum distance of 10 meters from other traps.

After the first half of the flight season, when no moths had been caught in the traps, one set of traps in Maltesholm was baited with a sub-optimal pheromone lure for *C. strobilella* (Wang et al., 2010). We used red rubber septa (11 mm × 5 mm, #224100-020 from Wheaton Science Products, Millville, NJ, USA) as dispenser, and applied 100 µl of pheromone solution, which contained 6 ng each of (*E,E*)-8,10-dodecadienyl acetate (*E8,E10*-12:OAc) and (*E,Z*)-8,10-dodecadienyl acetate (*E8,Z10*-12:OAc), i.e., a much lower dose compared to what has previously been used in field trials on this species (Wang et al., 2010; Svensson et al., 2013). *E8,Z10*-12:OAc was synthesized by Dr. Ilme Liblikas, University of Tartu, Estonia. The chemical purity was 80.4%, and the isomeric purity 88.1%. *E8,E10*-12:OAc was purchased from Bedoukian Research Inc (Danbury, CT, USA). The chemical purity was 97.7%, and the isomeric purity 98.3%. The differences in isomeric purities of the two acetates were compensated for to achieve a 1:1 ratio, which was confirmed by gas chromatography.

The traps were checked once per week and then rotated by one position within their replicate. All caught tortricid moths were boiled in an 8% KOH solution to remove adipose tissue. After the removal of soft tissue, the genitalia were compared with the drawings by Razowski (2003) to determine the species and sex.

The verified catches were log (x + 1) transformed to adjust for a skewed data distribution including zeros, and tested for significance using a linear mixed-effects model followed by Tukey's HSD (Honest Significant Difference) post hoc test. Differences caused by positional effects and differences due to day were compensated for by treating them as random factors in the statistical model. All statistics were performed in R (R development core Team, 2014) using the nlme package (Pinheiro et al., 2014) and the multcomp package (Hothorn et al., 2014). Trap catches were tested for correlation with chromatic and achromatic contrast using the hmisc package (Harrell, 2016)

3. Results

3.1. ERG analyses

We determined the spectral sensitivity of *C. strobilella* by ERGs recorded from their compound eyes. Since differences between males and females were within the accuracy of the measurements, we pooled data for both sexes. Recordings from the dark-adapted retina showed that the dominant receptor type was green-sensitive (Fig. 1A) and had its maximal sensitivity at $\lambda_{max} = 526 \pm 5$ nm ($n_{male} = 8$, $n_{female} = 11$). Spectral adaptation experiments under amber illumination revealed additional blue-sensitive receptors ($\lambda_{max} = 436 \pm 4$ nm, $n_{male} = 3$, $n_{female} = 2$) and UV-sensitive receptors ($\lambda_{max} = 352 \pm 3$ nm, $n_{male} = 0$, $n_{female} = 6$) (see Fig. 1B and C for individual examples). In males, an independent sensitivity peak in the UV could be isolated under spectral adaptation, but the signal-to-noise ratio was too low to fit the template for the respective visual pigment reliably to the data. Therefore, we only used results from females to determine the λ_{max} -value of the UV-sensitive receptor type.

3.2. Visual contrast of *P. abies* flowers and cones against needles

Differences in reflectance between open flowers and needles were most pronounced at wavelengths between 500 and 600 nm (Fig. 3A, compare the green and the yellow curve). As the flowers closed and

Table 1

Achromatic contrasts in Michelson units calculated for *Cydia strobilella*. (A) *Picea abies* flowers and cones against a background of green *P. abies* needles. Objects with positive values appear brighter than the background, while objects with a negative value appear darker than the background. (B) Traps used in the behavioural field trials against *P. abies* needles. We assume that the moths rely on their green-sensitive photoreceptors to encode achromatic contrast. However, we also calculated achromatic contrasts for a channel to which all receptor types contributed equally, because some insects seem to use multiple spectral inputs for achromatic vision.

(A)					
Developmental stage	Open flowers	Closed flowers	Young cones	Mature cones	
Contrast for green receptors	-33%	1%	29%	27%	
Contrast for equal contributions of all receptor types	-31%	1%	26%	24%	
(B)					
Trap type	Black	White	Orange	Red	Control
Contrast for green receptors	-73%	63%	-45%	-81%	-6%
Contrast for equal contributions of all receptor types	-69%	65%	-51%	-77%	-10%

matured into cones, the spectral reflectance increased at all wavelengths, but particularly above 500 nm (black and blue curves). Mature cones differed from young cones by a further increase in reflectance at wavelengths above 600 nm (compare blue and orange curves). This development caused major changes in achromatic contrast (“brightness”) as experienced by the moths. Open flowers were darker than the spruce needles, but once the flowers had closed they no longer presented an achromatic contrast to the background (Table 1A). Matured cones in later stages were instead brighter than the needles.

For the moths, the chromatic (“colour”) contrast of flowers and cones against the needle background increased continuously with flower and cone maturation (Table 2A). Mature cones offered sufficient chromatic contrast for detection even with our most pessimistic estimates (see methods). However, open spruce flowers, the developmental stage that the adult moths are targeting, were spectrally similar to the background and would only present a slight chromatic contrast visible to *C. strobilella* assuming optimistic parameters.

3.3. Behavioural field trials

Reflectance spectra for all four test traps and the control trap are shown in Fig. 3B. We name the test traps based on human visual perception. All but the control traps had an achromatic contrast against the

Table 2

Chromatic contrasts in JNDs (Just Noticeable Differences) calculated for *Cydia strobilella*. (A) *Picea abies* flowers and cones against a background of green *P. abies* needles. (B) Traps used in the behavioural field trials against *P. abies* needles. Since important characteristics of sampling and neural processing are unknown for *C. strobilella*, chromatic contrast is given for both the most pessimistic (min) and the most optimistic (max) estimate. For details see Methods.

(A)					
Developmental stage	Open flowers	Closed flowers	Young cones	Mature cones	
Min	0.7	1.3	1.8	2.3	
Max	2.2	6.7	7.3	9.3	
(B)					
Trap type	Black	White	Orange	Red	Control
Min	1.4	7.1	2.7	1.5	7.6
Max	7.4	34.9	8.5	6.6	33.1

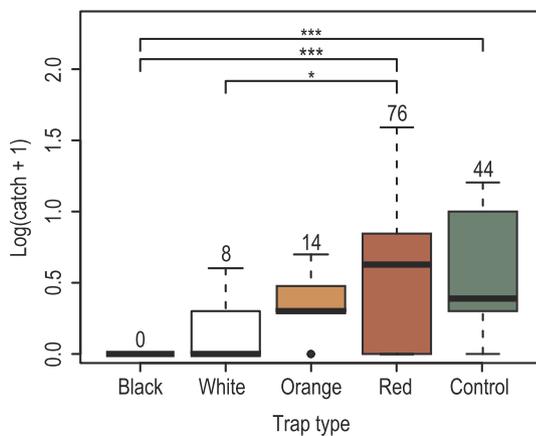


Fig. 4. Box plots displaying trap catches of *Cydia strobilella* in RAG delta traps modified to test the importance of visual contrast in host finding. All traps included in this analysis additionally contained a lure with a suboptimal dose of the female sex pheromone of *C. strobilella*. We used five traps of each type that were checked for moths twice, one week apart. The total catch of each trap type is displayed above the respective box. Upper and lower edges of each box represent the upper and lower quartiles. The inner line of the boxes shows the median and the whiskers outside the boxes indicate the highest and lowest trap catch within a distance of 1.5 times the interquartile range. One outlier is plotted as an individual data point. Trap catches were tested for significance using a linear mixed-effects model followed by Tukey's HSD (Honest Significant Difference) post hoc test. Stars indicate statistically significant differences based on $P < 0.001$ (***), and $P < 0.05$ (*).

spruce needles that should be easily detectable by the moths (Table 1B). With regard to chromatic contrast against the needles, control traps and white traps provided the greatest contrast to the moths, followed by the orange and red traps, in turn followed by the black traps, which had the lowest chromatic contrast of all traps (Table 2B).

Whereas none of the unbaited traps caught any *C. strobilella*, the traps with the additional sex pheromone lure caught 142 individuals in total, all males. Of these, 76 were caught in the red traps, 44 in the control traps, 14 in the orange traps, eight in the white traps and none in the black traps. Black traps caught significantly fewer moths than red traps (ANOVA: $F = 5.89$, $P < 0.001$) and control traps ($P < 0.01$). White traps also caught significantly fewer individuals than red traps ($P < 0.05$) (Fig. 4). We found no significant correlation between trap catches and the visual contrast of the trap to the needles (Fig. 5).

4. Discussion

To oviposit, female *C. strobilella* search for open spruce flowers. Males are trying to find females to mate, and therefore open flowers should be attractive locations for them as well. Our measurements show that the reflectance of open flowers and spruce needles differs strongest at wavelengths between 500 and 600 nm (Fig. 3A). The green-sensitive receptors of *C. strobilella* have their main sensitivity peak at 526 nm (Fig. 1), and are thus well suited to detect the achromatic (“brightness”) contrast between open flowers and needles (Table 1A). Interestingly,

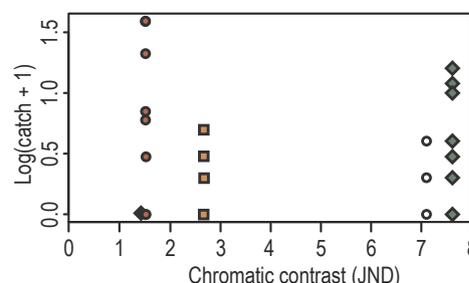
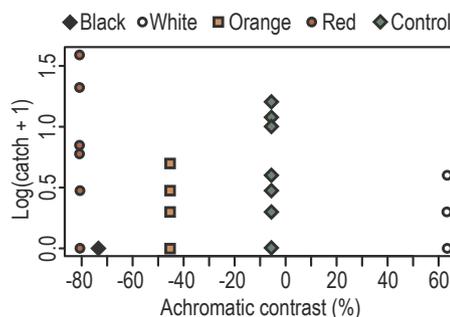


Fig. 5. Trap catches of *Cydia strobilella* (same data as in Fig. 4) plotted against achromatic and chromatic contrasts of modified RAG delta traps. Black diamonds represent black traps, open circles represent white traps, orange squares represent orange traps, red circles represent red traps, and green diamonds represent control traps only covered by a UV-absorbing filter. Each data point indicates the catch in a single trap during one week ($n = 10$ for each trap type; please note that several data points are identical).

achromatic contrast does not only offer a strong visual signal to detect open flowers but also to discriminate them from folding or folded cones. For the moths, open flowers appear darker than the needles, while closed flowers are similar to the background and cones are brighter. Since the reflectance changes mainly at wavelengths above 500 nm, the contributions of the UV- and blue-sensitive receptors for detecting achromatic contrast are negligible.

Our findings for chromatic (“colour”) contrast are different. Because the sensitivity curves of the green receptor and the other two receptor types hardly overlap at wavelengths above 500 nm, chromatic contrast between open spruce flowers and needles is low even with optimistic modelling (Table 2A). An additional long-wavelength receptor would substantially improve the detectability of open flowers against the background. Discrimination values increase by a factor of three, when adding a fourth receptor type with a sensitivity peak at 600 nm to the model.

We got significantly lower catches of *C. strobilella* in black traps than in red traps (Fig. 4), and both traps only differed in reflectance at wavelengths above 600 nm (Fig. 3A). This could be interpreted as an indication for a red-sensitive photoreceptor type in *C. strobilella*. However, while the sensitivity of the moths’ green receptors is much lower between 600 and 650 nm than at 526 nm, they will still absorb some light. A recent study has shown that green rice leafhoppers, *Nephotettix cincticeps*, with spectral sensitivities ($\lambda_{max} = 354$ nm, 449 nm and 527 nm) similar to *C. strobilella*, were able to detect red and even near-infrared light in a Y-maze using their green receptors (Wakakuwa et al., 2014). To test for red receptors in *C. strobilella*, we additionally compared orange and red traps, both of which reflect light that mainly activates the moths’ green receptors. The differences in catches between these two traps could point to more than one type of long-wavelength receptor, but they were statistically not significant ($P = 0.43$).

All spectral sensitivity curves that we obtained for from the compound eyes of *C. strobilella* could be fitted well assuming three types of photoreceptor with sensitivity maxima in the UV, blue and green part of the spectrum (Fig. 1). Yet, we have to consider the possibility that we did not pick up the responses of a rare or small photoreceptor type, especially if it was located far from our electrode. Lepidopterans typically possess stacked receptors with one cell contributing only to the proximal part of the retina (Briscoe, 2008; White et al., 2003; Belušić et al., 2017). In the African armyworm moth, *Spodoptera exempta*, the basal cell is maximally sensitive to wavelengths of 560 nm (Langer et al., 1979; Meinecke and Langer, 1984). If *C. strobilella* like *Adoxophyes (reticulana) orana* (Hämmerle and Kolb, 1987), another tortricid moth, has a small proximal photoreceptor, this cell would be a prime candidate for a red-sensitive receptor we could have missed. Thus, our physiological data do not indicate an additional type of long-wavelength receptor in *C. strobilella*, but we cannot exclude its existence either. Neither are our catch results conclusive.

The chromatic contrast for a UV-blue-green trichromatic visual system increases as the flowers mature and turn into cones (Table 2A). Moths that feed on seeds of *P. abies* and are active later in the season, such as the cloaked pug, *Eupithecia abietaria*, or the spruce coneworm,

Dioryctria abietella, might take advantage of the higher chromatic contrasts of mature cones against the needles. However, to our knowledge no data on the spectral sensitivities of these species are available, nor has the importance of visual cues for host finding been tested in them.

None of our traps caught any *C. strobilella* before the pheromone bait was added. This may not be surprising since some insects use visual signals predominantly as close range cues after having been attracted by olfactory cues from a longer distance (Hidaka, 1972; Campbell and Borden, 2006; Fukaya et al., 2006). However, even at short-range, it seems unlikely that male *C. strobilella* employ visual contrast as a primary cue to distinguish open flowers from spruce needles. The chromatic contrast for open flowers is low, at least if we assume UV-blue-green trichromacy (Table 2A), and we found no correlation between trap catches and chromatic contrast, nor between trap catches and achromatic contrast (Fig. 5). Our black and red traps offered similar contrasts to the needle background (Tables 1B and 2B), but black traps caught no individuals at all, while red traps caught 71 individuals (Fig. 4). These results are unlikely if contrast per se is a crucial cue used for host finding. Yet, spectral identity may have some importance. The moths could perceive black and red as different hues (directions of colour loci in colour space), and saturations (distance of loci from the “grey” point), although the chromatic contrast (distance between loci) between the background and the red or black traps is the same (Wyszecki and Stiles, 2000). In other words, the photons absorbed above 600 nm by the green (and potential other long-wavelength) receptors may be sufficient to give red traps a different chromatic appearance from black traps, and this could attract *C. strobilella*. How such spectral preferences may complement olfactory cues and aid moths in the search for oviposition sites could be an interesting topic for future studies.

For many insects, visual information like colour has to be combined with other cues, often olfactory, to trigger attraction (Ockenfels and Schmidt, 1992; Rojas and Wyatt, 1999; Tasin et al., 2011). Furthermore, olfactory cues including plant volatiles can alter innate colour preferences differently in males and females (Yoshida et al., 2015). In this study, we used a sex pheromone lure in combination with spectral cues, which only attracted males. To prevent damage in spruce seed orchards more efficiently, it would be desirable to catch female *C. strobilella*. We recently identified an attractive blend of host volatiles (Jakobsson et al., 2016), which was used to design trap lures mimicking *P. abies* flowers based on olfaction. Similar to pheromone-baited traps, these traps were able to catch males. Combining the lure based on host volatiles with traps of spectral properties that caught significantly more moths than others in our field trials might result in higher trap catches of males and possibly also attract females.

5. Conclusion

In this study, we have shown that *C. strobilella* has at least three spectral types of photoreceptor with maximal sensitivities at 352 nm (UV), 436 nm (blue), and 526 nm (green), respectively. Surprisingly, trap catches in field trials indicate that the reflectance of wavelengths above 600 nm (red) can attract *C. strobilella*. The achromatic contrast of open spruce flowers against the needle background is high and offers a potential signal to distinguish suitable oviposition sites from closed flowers and cones. Chromatic contrast, on the other hand, is low, at least if colour vision in *C. strobilella* is trichromatic based on UV, blue and green receptors. Nevertheless, our results suggest that neither chromatic nor achromatic contrasts are used to detect open flowers from a great distance, since only traps that were additionally baited with female sex pheromone attracted moths. Trap catches in the pheromone-baited traps were not correlated with the visual contrast of the traps against the needles, indicating that it is not a primary short-distance cue for males either. Still, the different responses to black and red traps suggest that visual preferences could function as a complement to olfaction in attracting moths.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.jinsphys.2017.06.006>.

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