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Artificial night lighting disrupts sex pheromone in a noctuid moth

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Abstract. 1. One major, yet poorly studied, change in the environment is the increase in nocturnal light pollution. Although this strongly alters the habitat of nocturnal species, the ecological consequences are poorly known. Moths are well known to be attracted to artificial light sources, but artificial light may affect them in other ways as well.

2. In this study, female Mamestra brassicae moths were subjected to various types of low-intensity artificial night lighting with contrasting spectral compositions (green-rich, red-rich, warm white) or to a dark control treatment and the effects on their sex pheromone production and composition were tested.

3. Artificial night lighting reduced sex pheromone production and altered the chemical composition of the pheromone blend, irrespective of spectral composition. Specifically, amounts of the main pheromone component Z11-16:Ac were reduced, while the deterring compounds Z9-14:Ac, Z9-16:Ac, and Z11-16:OH were increased relative to Z11-16:Ac when females were kept under artificial light. These changes may reduce the effectiveness of the sex pheromones, becoming less attractive for males.

4. These results show for the first time that artificial light at night affects processes that are involved in moth reproduction. The potential for mitigation through manipulation of the spectral composition of artificial light appears limited.

Key words. Disruption, light pollution, Mamestra brassicae, sex pheromone composition, sexual communication.

Introduction

As a result of the continuous rise in levels of artificial night lighting (Hölker et al., 2010a), nocturnal animals, representing the majority of the terrestrial fauna (Hölker et al., 2010b), are increasingly confronted with illumination of nightscapes (Cinzano et al., 2001). However, the ecological consequences of artificial night lighting, and possibilities for mitigation of negative effects, remain poorly studied to date (Gaston et al., 2012). Currently, artificial lighting of outdoor public spaces is undergoing a transition from traditional lamp types, such as high- and low-pressure sodium and fluorescence lamps (e.g. mercury vapour and metal halide lamps), towards (cold) white LED lamps (Stone et al., 2012). Depending on the lights that are replaced by the LEDs, this can result in an increased (compared with high- and low-pressure sodium; see Stone et al., 2012; Pawson & Bader, 2014) or a decreased effect (compared with mercury vapour or metal-halide lamps; see Van Grunsven et al., 2014) on insects. However, adjustment of spectral compositions is possible in LED lighting. This allows for application of lamps where harmful parts of the light spectrum are eliminated or strongly reduced in intensity (Gaston et al., 2012) in order to reduce their ecological impact (Poot et al., 2008).

Moths represent a largely nocturnal group of ~140 000 species (New, 2004; Bazinet et al., 2013) that are often attracted to sources of artificial light at night (Van Langevelde et al., 2011; Somers-Yeates et al., 2013). However, the effect of artificial light pollution on aspects of moth ecology is largely unknown.
Koert G. van Geffen et al. (2014, 2015) and should be investigated, as moth populations are declining in western Europe (Conrad et al., 2006; Groenendijk & Ellis, 2011; Fox et al., 2014). These declines are partly ascribed to nocturnal illumination (Conrad et al., 2006; Groenendijk & Ellis, 2011; Fox, 2013), with the proposed mechanism being phototaxis. In this paper, we focus on the influence of light on moth sex pheromone production, which is essential to attract potential mates.

Female moths produce and emit a sex pheromone that consists of a blend of mainly straight chain aldehyde, acetate, and alcohol components. The composition of the sex pheromone blend is highly species-specific: slight differences in relative amounts of different components allow respective males to differentiate between species and thus to identify conspecific females (Löfstedt et al., 1991; Symonds & Elgar, 2008). Males not only have receptors for attractive components, but also for deterring components, often produced by closely related, co-occurring species, to distinguish conspecific from heterospecific females (Den Otter et al., 1989; Christensen et al., 1994; Boo et al., 1995; Vickers & Baker, 1997; Evenden et al., 1999; Eliyahu et al., 2003; Gemeno et al., 2006; Eizaguirre et al., 2007).

The cabbage moth *Mamestra brassicae* (L.) (Noctuidae) is a common species in (sub)urban areas in western Europe. It is a habitat-generalist that is commonly found in cultivated and (sub)urban areas (Waring & Townsend, 2003). Adults of *M. brassicae* are frequently exposed to artificial light at night, as they are nocturnal and attracted to sources of artificial light (Waring & Townsend, 2003). In the Netherlands, populations of *M. brassicae* are declining, with a 50–75% reduction in the reported catches, primarily in light traps, corrected for observation intensity, over the period 1982–2013 (Ellis et al., 2013).

The sex pheromone of *M. brassicae* is well studied, which makes the species suitable as a model species. The pheromone consists of nine different compounds (Struble et al., 1980; Van de Veire & Dirinck, 1986; Den Otter & Van der Haagen, 1989; Renou & Lucas, 1994), most of which are common in co-occurring species (Table 1). The major constituent of the sex pheromone of *M. brassicae* is (Z)-11-hexadecenyl acetate (Z11-16:Ac). The attractiveness of this component is increased by small amounts of the two additional components, (Z)-11-hexadecenal (Z11-16:Ald) and (Z)-11-heptadecenyl acetate (Z11-17:Ac) (Van de Veire & Dirinck, 1986; Renou & Lucas, 1994). Three other compounds found in small amounts in the pheromone blend of *M. brassicae* are known to inhibit attraction of conspecific males: (Z)-9-tetradecenyl acetate (Z9-14:Ac), (Z)-9-hexadecenyl acetate (Z9-16:Ac), and (Z)-11-hexadecenol (Z11-16:OH) (Struble et al., 1980; Renou & Lucas, 1994). Finally, three additional compounds that are present in the pheromone blend but seem not to play a functional role in sexual communication are tetradecanal acetate (14:Ac), hexadecanal acetate (16:Ac), and (E)-11-hexadecenyl acetate (E11-16:Ac) (Renou & Lucas, 1994).

Sex pheromone production in moths is regulated by pheromone biosynthesis activation neuropeptide (PBAN) (Raina et al., 1989; Jacquin et al., 1994). *Mamestra brassicae* sex pheromone production occurs at night (Noldus & Potting, 1990), and release of PBAN is regulated by light (Tawata &}

<table>
<thead>
<tr>
<th>Species</th>
<th>Flight period</th>
<th>Habitat</th>
<th>Pheromone components</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mamestra brassicae</td>
<td>April–October</td>
<td>Cultured fields</td>
<td>Z11-16:Ac, Z11-16:Ald, Z11-17:Ac</td>
</tr>
<tr>
<td>Agrotis ipsilon</td>
<td>April–October</td>
<td>Cultured fields</td>
<td>Z11-16:Ac, Z11-16:Ald, Z11-17:Ac</td>
</tr>
<tr>
<td>Mamestra oleracea</td>
<td>April–October</td>
<td>Cultured fields</td>
<td>Z11-16:Ac, Z11-16:Ald, Z11-17:Ac</td>
</tr>
<tr>
<td>Mythimna unipuncta</td>
<td>May–November</td>
<td>Grasslands</td>
<td>Z9-14:Ac, Z11-16:Ac, Z11-17:Ac</td>
</tr>
<tr>
<td>Ochropleura plecta</td>
<td>April–October</td>
<td>Cultured fields</td>
<td>Z11-16:Ac, Z11-17:Ac, E11-16:Ac</td>
</tr>
</tbody>
</table>

*P* present in pheromone blend; *M* main pheromone component; *A* attractor component; *D* deterrent component.
Ichikawa, 2001). Therefore, the production of sex pheromone may be affected by artificial lighting.

In this study, we tested the effect of low levels of artificial night lighting with different spectral compositions on the amount and composition of the sex pheromone produced by *M. brassicaceae* females. Given the sensitivity of moths to short-wavelength radiation (Agee, 1973; Van Langevelde *et al.*, 2011), we expected the strongest reduction in sex pheromone production under light with higher levels of short wavelengths (e.g. green-rich and white light), and less reduction under light with lower levels of short wavelengths (e.g. red-rich), compared with moths in darkness.

**Materials and methods**

In a greenhouse, we constructed 20 open-top compartments of 50 × 50 × 60 cm³. Each compartment was randomly assigned to one of four artificial light treatments: green-rich (henceforth green), warm white (henceforth white), red-rich (henceforth red) (all 17 ± 1 lx, or 63, 53, and 96 mW m⁻², respectively) or no artificial light at night (65 ± 1 mlx). Treatments were divided over five randomised blocks.

Lamps were custom-made by Philips Lighting (Eindhoven, the Netherlands) using LEDs obtained from Farnell (Utrecht, the Netherlands). The spectral compositions of the lamps that were used in this study were specifically designed for potential future application in LED street lighting. Therefore, the lamps had a light quality that allows for colour distinction for the human eye. As a result, lamps were not monochromatic, but comprised wavelengths from the complete spectrum, although highly skewed for some lamps (Fig. 1). Spectral measurements of lamps were performed at 25 °C in a 2-m-diameter Ulbricht sphere (Agilent technologies, Santa Clara, California) equipped with a Cary Varian 17 D digital spectrophotometer. Lamps were allowed to stabilise for 5 min prior to measurements. A photocell was used to measure stability of the lamps and to correct spectral measurements for self-absorption by the lamp units. White lamps consisted of three 60-lumen warm white LEDs (LXML-PWW1-0060), red lamps of two 70-lumen red-orange LEDs (LXM2-PH01-0070) and one 60-lumen warm white LED, and green lamps consisted of one 90-lumen cool white LED (LXML-PWC1-0090), one 23-lumen blue LED (LXML-PB01-0023) and two 80-lumen green LEDs (LXML-PM01-0080). LEDs were mounted in a 15 × 8 × 5 cm³ (L × W × H) black plastic housing. Light was mixed in a 10-cm-long, 3.5 × 3.5 cm² wide aluminium duct with a standard white diffusor [Polymethyl methacrylate (PPMA) plate] at the end. Green and white lamps had two diffusors to obtain light intensities identical to the red lamps. Lamps were switched on 1 h before the onset of the scotophase (night, starting around 19.00 hours), and switched off 1 h after onset of the photophase (day, starting around 07.00 hours). Daylight was natural, average day and night temperatures were 20 ± 2 and 18 ± 2 °C, respectively. There were no differences in temperature between light treatments [GLM $F_{1,16} = 0.065$, $P = 0.978$; measured in each compartment for two nights with i-buttons (Maxim, Sunnyvale, California)].
compounds from Pherobank, Wageningen, the Netherlands) (Struble et al., 1980; Van de Veire & Dirinck, 1986; Den Otter & Van der Haagen, 1989; Renou & Lucas, 1994) was injected into the gas chromatograph after the gland extract injections for manual integration of peaks of all components in ChemStation (Agilent Technologies Deutschland GmbH, Böblingen, Germany). The amount of each compound was calculated relative to a 20 ng pentadecane internal standard (see Groot et al., 2010b).

Statistical analyses of pheromone experiment

Artificial light-induced differences in the amount of sex pheromone (in-transformed) were tested using nested ANOVA at the lamp level (the five moths in one compartment are not independent) with block as a random factor and a Tukey post hoc test. For all tests, homoscedasticity and normality of residuals were assessed visually.

As the amount of pheromone compounds can be highly variable, even between females within treatments, changes in pheromone composition are generally given in relative amounts (Heath et al., 1991; Teal & Tumlinson, 1997; Groot et al., 2005, 2009). However, relative amounts introduce the problem of interdependence; if the relative amount of one compound increases, the relative amount of another compound thus decreases. To overcome this interdependence, we performed log-contrast transformation (henceforth log-scaling), following Aitchison (1986) and Groot et al. (2010b), prior to analyses on artificial light-induced changes in pheromone blend composition. Differences in log-scaled amounts were analysed with standard parametric statistics. For this log-scaling, we scaled eight out of the nine compounds (14:Ac, Z9-14:Ac, Z11-16:Ald, Z9-16:Ac, E11-16:Ac, Z11-16:Ac, Z11-16:OH, and Z11-17:Ac) relative to the ninth compound (16:Ac) for each female separately, and took the logarithm of each ratio. We sacrificed 16:Ac for log-scaling, because: (i) male antennae are unable to detect this compound (Den Otter & Van der Haagen, 1989; Renou & Lucas, 1994), and hence loss of information on the variation in this compound was most likely not biologically important; (ii) it was the second most abundant pheromone compound, making the integration results reliable; and (iii) it had the lowest coefficient of variation of all pheromone compounds. Subsequently, we used a nested MANOVA to test for differences in sex pheromone blend composition. Variation in the relative amount of each of the log-scaled compounds was analysed with nested ANOVAs and Tukey post hoc tests.

Additionally, we calculated ratios between the main attractive component (Z11-16:Ac) and the three compounds that have been shown to decrease attractiveness of this main component: Z9-14:Ac, Z9-16:Ac, and Z11-16:OH (Struble et al., 1980; Renou & Lucas, 1994). We used nested ANOVAS with Tukey post hoc tests to test for differences in ratios under the different light treatments (the Z11-16:Ac/Z11-16:Ac ratio was ln-transformed). Statistical analyses were performed in spss version 20.

Results

The total amount of sex pheromone per moth varied between 154 and 2901 ng. Artificial light strongly reduced sex pheromone production ($F_{3,16,4} = 8.427$, $P = 0.001$), irrespective of spectral composition (Fig. 2). Also, the composition of the sex pheromone blend was significantly altered by artificial light (Wilks’ $\lambda, F_{24,186} = 2.903, P < 0.001$; Fig. 3a,b). Specifically, we found a relative reduction of the major sex pheromone component, Z11-16:Ac, for females subjected to artificial light, as compared with darkness ($F_{3,16,4} = 8.230, P = 0.001$). Z9-16:Ac, a minor pheromone compound that may act as a deterrent (Renou & Lucas, 1994), was reduced in females under green light compared with females in darkness when log-scaled to 16:Ac ($F_{3,16,5} = 5.123, P = 0.011$; Fig. 3a). However, relative to the amount of the main attractive component (Z11-16:Ac), the amount of Z9-16:Ac increased up to 1.6-fold (2.2%, under white artificial light) compared with darkness (1.4%) ($F_{3,16,5} = 3.724, P = 0.032$; Fig. 4a). The ratios between the two other deterrents and the main attractive pheromone component, Z11-16:Ac, also increased under artificial light: the ratio Z9-14:Ac/Z11-16:Ac was increased in females under artificial light (irrespective of spectral composition) compared with females in darkness ($F_{3,16,5} = 8.587, P = 0.001$; Fig. 4b), and the ratio of Z11-16:OH/Z11-16:Ac was significantly higher in females under green (1.8%) and white (1.7%) light than in females under red light (1.2%) and in darkness (0.9%) ($F_{3,16,2} = 6.819, P = 0.003$; Fig. 4c).

Discussion

We found that artificial light at night strongly reduced the total amount of sex pheromone produced by female M. brassicae, possibly through light inhibition of PBAN (Tawata & Ichikawa, 2001), the neuropeptide that regulates sex pheromone production (Raina et al., 1989; Jacquin et al., 1994). PBAN is produced in the suboesophageal ganglion, probably continuously, so that it accumulates during the photophase (Rafaeli, 1994), while its
release from the corpus cardiacum into the haemolymph is regulated by a circadian clock (Rafaeli, 1994), and coincides with the daily rhythm of sex pheromone production in *M. brassicae* (Iglesias *et al.*, 1999). How the release of PBAN is regulated by light is currently unknown (see also Groot (2014)).

Surprisingly, artificial light changed the chemical composition of the pheromone blend, reducing the relative amount of the main attractive component and increasing the relative amount of inhibitory compounds relative to the main attractive component. As the chemical composition of the sex pheromone blend is an important determinant of the attractiveness of sex pheromone signals, and key for recognition of conspecific females by males, slight changes in composition can reduce male response, or even change species specificity (Löfstedt *et al.*, 1978; Struble *et al.*, 1980). Species specificity is largely the result of strict differences in the relative contribution of different compounds in the pheromone blends. In the case of *M. brassicae*, many co-occurring species share important sex pheromone compounds (see Table 1). For example, Z11-16:Ac is the main attractive component in the blend of *Ochropleura plecta*, *Agrotis ipsilon*, and *Mythimna unipuncta*. These species all share the same habitat and flight period with *M. brassicae*. The difference between these species is that *O. plecta* and *M. brassicae* females also contain Z11-16:Ald as a (minor) attractive component in their pheromone blend, whereas it is absent, non-active, or deterrent in the other species (Table 1). *Agrotis ipsilon* and *M. unipuncta* have Z9-14:Ac and Z11-16:OH in their blends, while both compounds are deterrent for *M. brassicae* males, at least at higher doses (Descoins *et al.*, 1978; Struble *et al.*, 1980). In *M. brassicae*, this alcohol is most likely a precursor and not emitted from the gland (Bestman *et al.*, 1988), as this compound has been shown to be deterrent at levels as low as 0.1% (Descoins *et al.*, 1978). Hence, the greater than two-fold increase in the ratio of Z11-16:OH/Z11-16:Ac that we found may not affect the emission ratios, but, if so, even the smallest amount...
of Z11-16:OH will reduce the attraction of *M. brassicae* males. The other inhibitory compound for *M. brassicae*, Z9-14:Ac, is also produced by *M. brassicae* females. This compound has been shown to be attractive when present at 0.1% of the blend (Subchev *et al*., 1987), but inhibitory when 1–2% is added to the blend (Descoins *et al*., 1978; Struble *et al*., 1980). Thus, the nearly three-fold increase in this compound that we found under the different light conditions is likely to affect the attraction of *M. brassicae* males negatively. The small differences in pheromone blend composition require males to be highly sensitive to subtle changes in pheromone composition. However, actual testing of how the artificial light-induced compositional changes in sex pheromone blends affect the attraction of moths is an important next step to undertake.

The range of variation that we found in the pheromone composition of *M. brassicae* is similar to or even higher than that found in the geographic variation of, for example, *Heliothis virescens*, *Heliothis subflexa* (Groot *et al*., 2009), *Agrotis ipsilon* (Gemen* et al*., 2000), *Agrotis segetum* (Wu *et al*., 1999), and *Cydia pomonella* (Dumenil *et al*., 2014). However, the variation that we measured was due only to the different light conditions that the females had experienced for three nights. Such a plasticity in the pheromone blend has been reported only once before (Groot *et al*., 2010b) and shows that moth sex pheromone blends may be more plastic and variable than generally considered. In general, moth sex pheromones are hypothesised to be under stabilising selection, because moth sexual communication is important for species recognition and thus should not vary, as this may directly decrease fitness. Our study shows that varying only one environmental factor, in this case light, can change the sex pheromone blend significantly. Recently, we also demonstrated that artificial light may lead to reduced mating success under field conditions in the geometrid moth *Operophtera brumata* (Van Geffen *et al*., 2015).

It should be pointed out that we limited ourselves in this study to the sex pheromone composition present in the pheromone gland of individual females, as analysing pheromone release rates of individual females is technically very challenging. However, in general, a one-to-one correspondence is found between the composition in the pheromone gland and the emitted blend (Du *et al*., 1987; Heath *et al*., 1991; Bäckman *et al*., 1997; Svensson *et al*., 1997), indicating that the pheromone composition in the gland reflects the blend that is released. The only exception is Z11-16:OH, as alcohols serve as immediate precursors to their aldehyde and acetate derivatives (Tillman *et al*., 1999; Rafaeli, 2002; Jurenka, 2004).

Whereas compositional changes in the pheromone blend were strongest under green and white light, the reduction in pheromone quantity was also significant in females under red light (see Fig. 2). These results indicate that spectral alterations may mitigate negative effects of artificial light on moths, but only to a limited extent. This contrasts with the current perception that spectral alterations are a promising tool to reduce negative effects of artificial night lighting (Gaston *et al*., 2002), but is in line with a recent study by Pawson and Bader (2014), who demonstrated that shifts in colour temperature (i.e. slight changes in spectral composition) of white LEDs did not influence the number of insects that were attracted to the light. Other studies on moths, concerning attraction to artificial light (Van Langevelde *et al*., 2011; Somers-Yeates *et al*., 2013; Van Grunsven *et al*., 2014), development and life-history (Van Geffen *et al*., 2014), and reproduction under field conditions (Van Geffen *et al*., 2015), show that the negative consequences of artificial light may be mitigated at least partly by application of light that is poor in short-wavelength radiation. Compared with darkness, however, red light still often has significant negative effects (Van Geffen *et al*., 2014, 2015), despite the insensitivity of moth eyes to longer-wavelength radiation (Agee, 1973). Possibly, these negative effects of red light are driven by low amounts of short-wavelength radiation in the red light that is tested in these studies (Fig. 1). Completely removing short-wavelength light or using monochromatic light might effectively mitigate negative effects, but would result in very poor colour recognition and thus render a light source impractical for most applications. Therefore, adjustment of spectral composition could possibly play a role in mitigating the effects of artificial light, but the possibilities of mitigation with lights that are still of use for general application is limited compared with other modern light sources that do not emit UV.

We conclude that artificial light disrupts sex pheromone production in *M. brassicae* moths, both quantitatively and qualitatively. The significant differences in the pheromone blend after being in different experimental conditions shows that the pheromone composition is more plastic than previously assumed (Löfstedt, 1993; Butlin & Trickett, 1997; Groot *et al*., 2010b). Such shifts can have serious consequences for reproduction, because male response can be disrupted, which may lead to reduced mating success (Van Geffen *et al*., 2015). Such mating reductions could have important implications for moth populations on illuminated nights.

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