

MOTHS IN ILLUMINATED NIGHTS

Artificial night light effects on moth ecology

Koert G. van Geffen

Thesis committee

Promotor

Prof. Dr F. Berendse

Professor of Nature Conservation and Plant Ecology

Wageningen University

Co-promotors

Dr E.M. Veenendaal

Associate professor, Nature Conservation and Plant Ecology Group

Wageningen University

Dr R.H.A. van Grunsven

Post-doc, Nature Conservation and Plant Ecology Group

Wageningen University

Other members

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Prof. Dr M. Visser, Netherlands Institute of Ecology, Wageningen

Prof. Dr M.F. Wallis de Vries, Wageningen University

This research was conducted under the auspices of the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC).

MOTHS IN ILLUMINATED NIGHTS

Artificial night light effects on moth ecology

Koert G. van Geffen

Thesis

submitted in fulfilment of the requirements for the degree of doctor
at Wageningen University

by the authority of the Rector Magnificus

Prof. Dr M.J. Kropff,

in the presence of the

Thesis Committee appointed by the Academic Board

to be defended in public

on Wednesday 1 April 2015

at 1:30 p.m. in the Aula.

Koert G. van Geffen

Moths in illuminated nights – artificial night light effects on moth ecology,
116 pages.

PhD thesis, Wageningen University, Wageningen, NL (2015)

With references, with summaries in English and Dutch

ISBN 978-94-6257-230-0

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CHAPTER 1

MOTHS IN ILLUMINATED NIGHTS

- GENERAL INTRODUCTION -

1.1 ILLUMINATING THE PLANET

Artificial night lighting is a widespread form of environmental pollution. Satellite data and models of light proliferation show high levels of artificial night lighting particularly, but not exclusively, in developed and rapidly developing countries (Cinzano, Falchi & Elvidge 2001; Elvidge *et al.* 2001; Bennie *et al.* 2014, Fig. 1.1a). Artificial light pollution has dramatically altered nightscapes worldwide. An estimated two-third of the world human population live in areas where artificial night sky brightness is greater than 10% of the natural night sky brightness, which is the threshold set for a ‘polluted’ state (Cinzano, Falchi & Elvidge 2001). Artificial light at night masks natural monthly and seasonal rhythms of lunar sky brightness (Davies *et al.* 2013). For example, for approximately two-thirds of the inhabitants of the European Union, artificial night sky brightness is greater than on unpolluted full-moon nights (Cinzano, Falchi & Elvidge 2001), and as a consequence, the number of full-moon equivalent sky brightness hours is increased (Davies *et al.* 2013). Concerns about adverse aesthetic (Smith 2008), environmental (Longcore & Rich 2004; Rich & Longcore 2006; Hölder *et al.* 2010b) and human health (Navara & Nelson 2007) consequences are increasing (Hölder *et al.* 2010a), yet levels of light pollution increase worldwide on average with 6% per year (Hölder *et al.* 2010a), although trends differ remarkably between countries and regions (Bennie *et al.* 2014, Fig. 1.1b,c).

1.1.1 *Ecosystems in illuminated nights*

Of all terrestrial fauna, an estimated 60% is nocturnal (Hölder *et al.* 2010b). These animals have evolved under a fixed pattern of circadian rhythms, with distinct day and night periods, and being inactive in the former and active in the latter period. For millions of years, light has been a very reliable cue for the on- and offset of activity. However, the recent and continuous rise in night-time artificial light pollution severely interferes with the reliability of this cue which may have consequences for different aspects of animals’ ecology such as navigation, reproduction and intra- and inter-specific interactions (Longcore & Rich 2004). Research in this field is still in its infancy, so that the ecological consequences of light pollution are largely unknown. Nevertheless, recent studies have shown that artificial night lighting may affect many aspects of a wide range of both diurnal and nocturnal species (Rich & Longcore 2006).

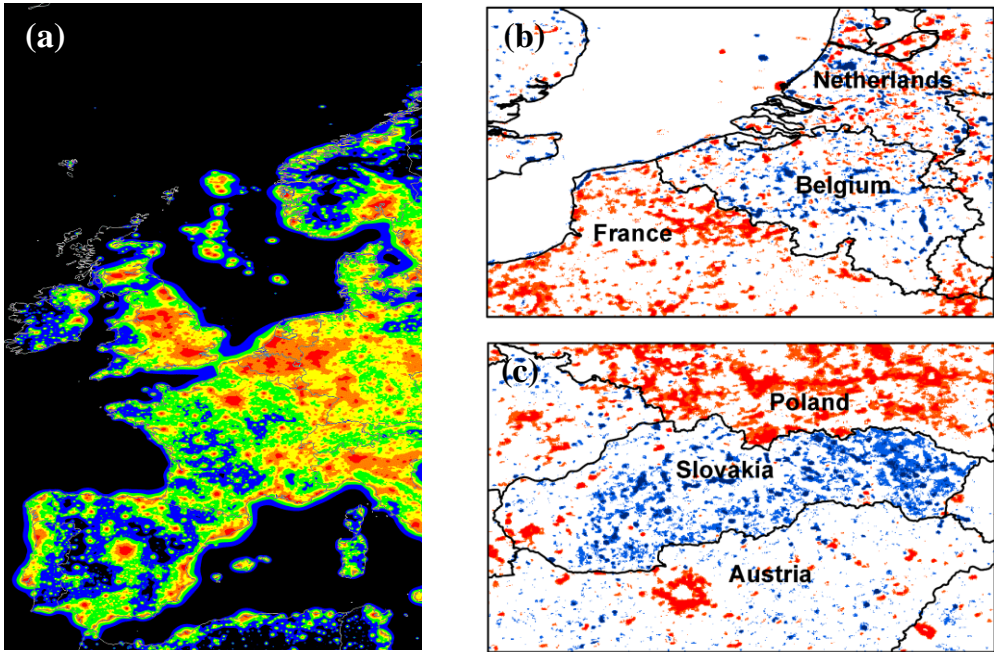


Figure 1.1 (a) Night-sky brightness in West Europe in 1993 (Cinzano, Falchi & Elvidge 2001). Colours correspond to ratios between the artificial sky brightness and the natural sky brightness of: <0.11 (black), 0.11-0.33 (blue), 0.33-1 (green), 1-3 (yellow), 3-9 (orange), >9 (red). Credit: P. Cinzano, F. Falchi (University of Padova), C. D. Elvidge (NOAA National Geophysical Data Center, Boulder). Copyright Royal Astronomical Society. (b) and (c) Change in night-sky brightness between 1995-2000 and 2005-2010 in two selected areas in Europe, showing contrasting trends in levels of light pollution (adapted from Bennie et al (2014)). Darkest red is increase in night time light levels of >5%, darkest blue is a decrease in night time light levels >5%, calculated as the difference between mean light levels in the periods 2005-2010 and 1995-2000.

Songbirds, for example, have been relatively well-studied with regard to light pollution (Spoelstra & Visser 2013). Female great tits (*Parus major*) breeding in illuminated nest boxes provided their chicks with food at a higher rate than females in dark control nest boxes (Titulaer *et al.* 2012). Males of four common European forest-breeding songbirds (*P. major*, *Cyanistes caeruleus*, *Turdus merula* and *Erithacus rubecula*) start singing earlier in vicinity of street lights than males in dark nights (Kempenaers *et al.* 2010). And artificial light at night can have strong effects on reproduction in songbirds. For example, male blue tits (*C. caeruleus*) that have a territory in an illuminated area being more successful in obtaining extra-pair mates than males with a territory in a dark area (Kempenaers *et al.* 2010). Moreover, already at very low levels of light at night (0.3 lux), artificial light can

alter the timing of the annual reproductive physiology in birds (Dominoni, Quetting & Partecke 2013), which may explain why female blue tits in illuminated night laid their first egg earlier than females in dark nights (Kempnaers *et al.* 2010).

Other species groups that are affected by light at night include nocturnal mammals such as bats (Rydell 2006; Jung & Kalko 2010; Stone, Jones & Harris 2012) and rodents (Bird, Branch & Miller 2004), soil-dwelling arthropods (Davies, Bennie & Gaston 2012), fish (Brüning, Hölker & Wolter 2011), amphibians (Buchanan 1993) and nocturnally active flying insects of a wide variety of taxa (Van Grunsven *et al.* 2014a).

1.2 RADIATION AND SPECTRAL COMPOSITION

For a good understanding of how artificial light affects ecosystems, some theoretical background in physics of light is required. Light is a specific type of electromagnetic radiation, which in physics refers to oscillations in electric and magnetic fields. Wavelengths and frequencies of these oscillations are highly variable, and radiation of different wavelengths lead to different phenomena. Light only differs from other types of electromagnetic radiation such as X-rays, radio waves and nuclear gamma radiation in its wavelength. Radiation wavelengths vary between 1 picometer (0.000000000001 meter, gamma radiation) through more than 1 kilometer (radio waves). The small set of wavelengths between about 380 and 780 nanometer (nm) is visible to the human eye and called ‘light’ (Fig. 1.2). The short wavelengths in light are blue, long wavelengths are red. Radiation with wavelengths shorter than 380 nm, but longer than 10 nm, is known as ultraviolet (UV), which is invisible to the human eye and therefore not ‘light’. Because many animal species, including birds, fish and invertebrates (including moths), are able to see part of the UV radiation, it may affect ecosystems in a similar way as light, despite that it is invisible to the human eye. Radiation with wavelengths that are longer than red light wavelengths, but shorter than 1 mm, is known as infra-red (IR) radiation, and includes thermal radiation. This part of the spectrum is exploited only by few species in the natural environment, for example by snakes, who use IR radiation for allocation of warm-blooded prey at night.

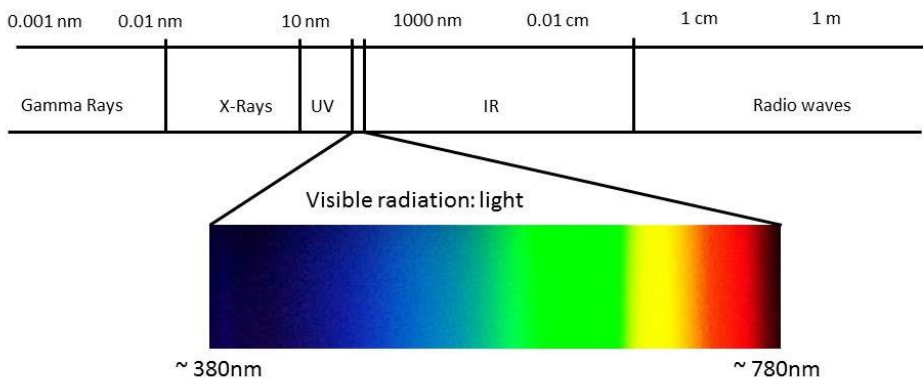


Figure 1.2 Overview of variation in wavelengths and a close-up on variation in wavelengths in the visible part of the spectrum (i.e. light)

1.2.1 Mitigation with spectral alterations

Artificial light sources vary in the set of wavelengths that are emitted. A white lamp emits radiation from a broad range of wavelengths, whereas a red lamp emits mainly longer wavelengths and a blue lamp mainly short wavelengths. With recent advances in LED (Light Emitting Diode) technology, it is now possible to exactly steer the spectral composition of a lamp. This opens opportunities for mitigation of potential negative effects of artificial light on ecosystems: it is now theoretically possible to create light without those wavelengths to which animals are most sensitive, and instead use wavelengths that they are less or insensitive to (Gaston *et al.* 2012). Although different species have different spectral sensitivities, and thus are able to see different part of the light spectrum, many species are unable to see red light (longer wavelengths). The upcoming LED-era therefore has opened opportunities to adjust spectral compositions of outdoor lighting devices in such a way that ecosystems are least affected. Indeed, examples of successful spectral mitigation of negative effects are already available in the literature. For example, attraction of nocturnally migrating bird species towards illuminated oil platforms at sea can be greatly reduced by application of light poor in long wavelengths (Poot *et al.* 2008), and attraction of insects towards artificial light sources with little or no short wavelength is reduced (Van Langevelde *et al.* 2011; Van Grunsven *et al.* 2014a).

1.3 MOTHS

With approximately 160,000 named species worldwide, Lepidoptera are a highly diverse order of insects. With 20,000 species, butterflies only represent a small part of this order; moths are the great majority with 140,000 species (New 2004). Butterflies are primarily diurnal (day active) and moths generally nocturnal (night-active). Moths are commonly subdivided into macro- and micro-moths however, this is highly subjective and these groups are not monophyletic. Macro-moths are in general the larger species, micro-moths the smaller species. However, as macro- and micro-moths are distinguished at family level, there are many examples of micro moth species being larger than certain macro-moth species. Macro-moths generally are the better-documented group, because they comprise those taxa that traditionally attracted most collectors' and hobbyists attention (New 2004). In the Netherlands, more than 2000 different species of moths have been recorded, 1100 of which are micro-moths and 900 species macro-moths. The macro-moths that occur in the Netherlands represent 19 different families, of which the Noctuidae and Geometridae are the largest (Waring & Townsend 2003)

Moths play a central role in many food webs. As larvae (caterpillar), moths are important herbivores on vegetation, act as a host for a wide variety of parasitoids, and are a bulk food source for insectivorous predators. In many moth species, adults feed on nectar produced by flowering plants, and act as pollinators. Also, adult moths are predated upon by a variety of animal groups including birds and bats.

1.3.1 *Moth attraction to light*

Moths have been studied relatively well with respect to their response to artificial night lighting (Frank 1988), because they are strongly attracted to sources of artificial light (Van Langevelde *et al.* 2011; Truxa & Fiedler 2012; Somers-Yeates *et al.* 2013). However, significant differences do exist among species and families (Merckx & Slade 2014; Van Grunsven *et al.* 2014b). Spectral composition of artificial light strongly influences the attractiveness of a lamp to moths, with in general strong attraction to lamps rich in short wavelength radiation (e.g. UV radiation, blue and green light), and little or no attraction to lamps with primarily long wavelength light (e.g. amber and red light) (Van Langevelde *et*

al. 2011), although differences in spectral sensitivity seems to exist between moth families (Somers-Yeates *et al.* 2013).

Moths are known to experience higher predation risks after being attracted to light, because bats and other nocturnal insectivorous predators exploit the relatively high prey density around night-time lights (Rydell 1992; Rydell 2006; Jung & Kalko 2010). This effect is further strengthened by the artificial light inhibition of the tympanate ultra-sound detection in moths (Svensson & Rydell 1998; Acharya & Fenton 1999), which in darkness is highly effective as a bat-defence mechanism (Roeder 1962; Conner & Corcoran 2011). Many other effects of artificial light on ecology of moths, such as reproduction, development and interactions with other trophic levels, have been suggested (Frank 1988; Frank 2006), but have rarely been studied. Hence, although we know that artificial light attracts moths and increases their mortality risk, the consequences of attraction for other aspects of moths ecology remain largely unknown. This is surprising as reproduction and development are also crucial for population viability.

1.3.2 *Potential role of artificial light in moth population declines*

Long-term, large-scale moth population monitoring programmes and moth trapping data have shown dramatic declines in moth populations in Western Europe (Conrad *et al.* 2006; Groenendijk & Ellis 2011, Fig. 1.3; Van Geffen *et al.* 2014). For example, in the United Kingdom, more than 20% of the 337 moth species studied showed a decline of >30% 10 yrs⁻¹ in the period 1968-2002 (Conrad *et al.* 2006). Comparable, in the Netherlands, populations of approximately one-third of the 626 studied species declined in the period 1980-2009 (Groenendijk & Ellis 2011). Multiple possible causes for these dramatic declines are suggested, including land-use changes, climatic changes and chemical pollution (Conrad *et al.* 2006; Groenendijk & Ellis 2011; Fox 2013), but the actual contribution of each these factors remains unknown and direct evidence for causal relationships are lacking. Light pollution is also often assumed to be one of the causal factors behind moth population declines (Conrad *et al.* 2006; Groenendijk & Ellis 2011; Fox 2013), mainly because moths are attracted to light. However, attraction as such may not necessarily lead to population declines, but insight in the consequences of artificial light on those aspects of moth ecology that are important for population size regulation remain poorly studied.

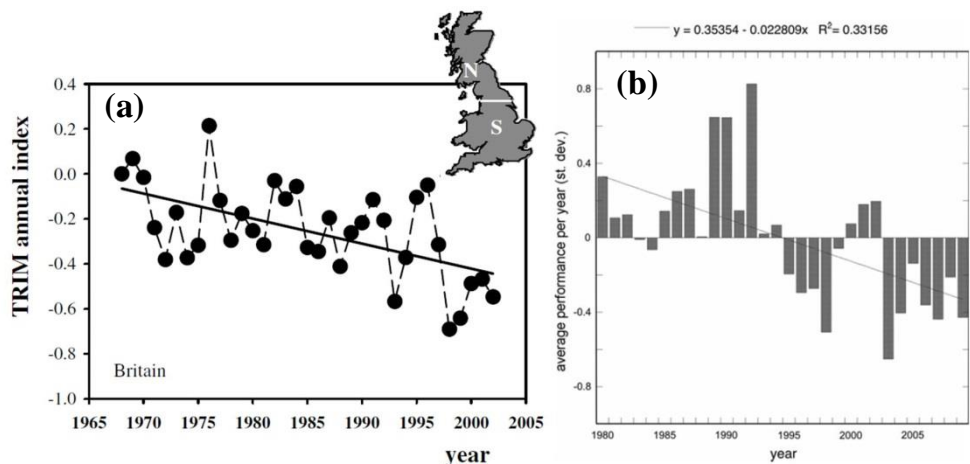


Figure 1.3 Moth population trends for (a) the United Kingdom over the period 1968-2002 (Conrad *et al.* 2006), and (b) the Netherlands over de period 1980-2010 (Groenendijk & Ellis 2011).

1.4 AIMS AND OUTLINE

In this thesis, I aim to gain insight in effects of variation in spectral composition in artificial light sources , on developmental, physiological and ecological processes that are important in the regulation and maintenance of moth population sizes. As such, this is the first attempt to systematically study mechanisms that may underlie potential artificial light induced moth population declines. Such information may help with interpretation and explanation of large-scale field observations and trends.

In the first experiment, **CHAPTER 2** in this thesis, I investigate effects of artificial night lighting on moth life history. We subjected 240 *Mamestra brassicae* (Noctuidae) caterpillars to different types of artificial light at night and tested how this affected their relative growth rate and other life-history traits such as age at pupal molt, pupal mass and duration of the pupal stage. The next two chapters focus on artificial light effects on various aspects of moth reproduction. **CHAPTER 3** deals with artificial light effects on female moth sex pheromone production, which is a crucial first step in the reproduction process in moths because males are only able to locate a con-specific female by the sex pheromone that is emitted by female moths. To test how artificial light at night affects sex pheromone production, we subjected female *Mamestra brassicae* moths to different types of artificial light at night and extracted their sex pheromone glands for chemical analyses of quantity

and composition of the sex pheromone blend. Because Chapter 3 was an experiment under controlled environmental conditions in a greenhouse, the next step was to test how artificial night lighting affects reproduction under natural conditions. Therefore, **CHAPTER 4** describes a field-based study on artificial light effects on moth reproduction, this time with the Geometrid moth *Operophtera brumata*. We trapped freshly emerged females of this moth species under different artificial night lighting regimes, and evaluated in the lab whether these females had mated, and how artificial light at night affected the proportion of mated females. In **CHAPTER 5**, I focus on caterpillar-plant interactions. For this, I designed an outdoor experiment, where *Plantago lanceolata* plants in pots were placed under different types of artificial light at night, and either subjected to herbivory by two *M. brassicae* caterpillars for two weeks, or kept caterpillar-free. I analysed how artificial night lighting influenced the two trophic levels, by monitoring caterpillar growth and measuring plant biomass and the amount of secondary metabolites in the leaves of the different plants. Finally, in **CHAPTER 6**, I review the findings of these studies in the context of our current knowledge on the impact of artificial light on moths. Furthermore, I discuss whether spectral alterations for mitigation of negative artificial light effects on moths is a promising tool, or over-estimated instead.

CHAPTER 2

ARTIFICIAL LIGHT AT NIGHT CAUSES DIAPAUSE INHIBITION AND SEX-SPECIFIC LIFE-HISTORY CHANGES IN A MOTH

Koert G. van Geffen, Roy H.A. van Grunsven, Jasper van Ruijven, Frank Berendse & Elmar M.

Veenendaal

Ecology and Evolution 2014, 4 (11): 2082-2089

ABSTRACT

Rapidly increasing levels of light pollution subject nocturnal organisms to major alterations of their habitat, the ecological consequences of which are largely unknown. Moths are well-known to be attracted to light at night, but effects of light on other aspects of moth ecology, such as larval development and life-history, remain unknown. Such effects may have important consequences for fitness and thus for moth population sizes. To study the effects of artificial night lighting on development and life-history of moths, we experimentally subjected *Mamestra brassicae* (Noctuidae) caterpillars to low intensity green, white, red or no artificial light at night and determined their growth rate, maximum caterpillar mass, age at pupation, pupal mass and pupation duration. We found sex-specific effects of artificial light on caterpillar life-history, with male caterpillars subjected to green and white light reaching a lower maximum mass, pupating earlier and obtaining a lower pupal mass than male caterpillars under red light or in darkness. These effects can have major implications for fitness, but were absent in female caterpillars. Moreover, by the time that the first adult moth from the dark control treatment emerged from its pupa (after 110 days), about 85% of the moths that were under green light and 83% of the moths that were under white light had already emerged. These differences in pupation duration occurred in both sexes and were highly significant, and likely result from diapause inhibition by artificial night lighting. We conclude that low levels of nocturnal illumination can disrupt life-histories in moths and inhibit the initiation of pupal diapause. This may result in reduced fitness and increased mortality. The application of red light, instead of white or green light, might be an appropriate measure to mitigate negative artificial light effects on moth life history.

INTRODUCTION

Over the past decades, increasing levels of artificial night lighting have led to large-scale alterations of night-scapes worldwide (Cinzano, Falchi & Elvidge 2001; Elvidge *et al.* 2001). Although there are remarkable differences in trends between countries and regions (Bennie *et al.* 2014), levels of artificial night lighting are currently still increasing annually with 6% on average (Hölker *et al.* 2010a). Although effects of artificial night lighting have been reported in diurnal species (Kempenaers *et al.* 2010; Titulaer *et al.* 2012), the ever-increasing levels of artificial night lighting may particularly affect nocturnal species, representing the majority of terrestrial fauna (Hölker *et al.* 2010b), as they are increasingly subjected to illumination of their habitat. Moths are a well-known example of organisms that are affected by artificial light at night, as they are strongly attracted to light, although significant differences exist among species and families (Van Langevelde *et al.* 2011; Truxa & Fiedler 2012; Somers-Yeates *et al.* 2013; Merckx & Slade 2014). Besides attraction, negative effects of artificial light at night on other aspects of moth ecology, such as survival, reproduction and development have been suggested (Frank 1988), but these have rarely been studied. For example, effects of artificial light on moth life history are unknown to date. In this respect, effects of artificial light on caterpillars is highly relevant as this is the life stage when important life history trade-offs, such as time to reach maturity and size at maturity, are faced. Unfavourable conditions during the larval stage are known to lead to lower caterpillar growth rates, advanced pupation and reduced pupal mass (Tammaru, Ruohomäki & Montola 2000). This in turn can affect important fitness measures such as female egg production (Honěk 1993; Tammaru, Esperk & Castellanos 2002), mate preference (Gage 1998; Van Dongen 1998; Iyengar & Eisner 2002; Xu & Wang 2009), longevity (Shirai 1995; Tammaru, Esperk & Castellanos 2002) and flight ability (important for gene spread; Shirai 1993; Shirai 1995; Merckx & Van Dyck 2006). In addition, natural light conditions are often used by organisms as a source of information about circadian and seasonal timing (Gaston *et al.* 2013). In many moth species, caterpillars use day length as the main environmental cue for the decision to initiate pupal diapause, where short days trigger diapause (e.g. Adkisson 1966; Huang *et al.* 2005; Xiao *et al.* 2010), ensuring that late generation caterpillars or pupae overwinter in diapause so that the imago life stage is synchronised with favourable seasons (Adkisson 1966). Light at night

can disturb the reliability of this cue (Gaston *et al.* 2013) by altering day length as perceived by caterpillars, which may lead to differences in timing or even inhibition of diapause.

In this study, we experimentally investigate the effects of various types of artificial night lighting, differing in spectral composition, on life history of *Mamestra brassicae* (L.) (Noctuidae). In Western Europe, this species has two generations per year of which the first generation pupates in summer and the second generation overwinters as a diapausing pupa (Steiner & Elbert 1998; Waring & Townsend 2003). As in many other moth species, caterpillars of *M. brassicae* are nocturnal.

We hypothesize that artificial light at night lengthens perceived day length, leading to a lower caterpillar growth rate, postponed pupation and reduced pupal mass. Furthermore, we expect that artificial light would lead to shorter duration of the pupal because it might interfere with day-length as a cue for diapause initiation and thus inhibit induction of diapause. Because caterpillar eyes (stemmata) are most sensitive to short-wavelength radiation (Ichikawa & Hideki 1982), we expect spectral composition of light to influence this process. Effects are expected to be more pronounced under green and white light (both containing short wavelengths), than under red light (comprising mainly long wavelength radiation). Insight in moth life-history under artificial night light with different spectral compositions may contribute to identification of mitigation opportunities, which are urgently needed given the rapid declines in moth populations in Western Europe, and the potential role of artificial light in this (New 2004; Conrad *et al.* 2006; Groenendijk & Ellis 2011; Fox 2013).

MATERIALS AND METHODS

Study species

We used *Mamestra brassicae* (Linnaeus 1785) as a model species. This species is common in (sub-)urban areas in Western Europe, but in the Netherlands, its populations are declining (www.vlindernet.nl/english.php and see Groenendijk & Ellis 2011; Vlinderstichting 2014), with 50-75% reduction in the number of reported catches (corrected for observation intensity) over the period 1982-2013 (Ellis *et al.* 2013). In Western Europe, *Mamestra brassicae* has two generations per year (Steiner & Elbert 1998; Waring &

Townsend 2003; Vlinderstichting 2014). Caterpillars of the first generation develop in late spring and early summer, experiencing long days (day lengths of approximately 16 hours), do not diapause and emerge as adult in late summer. The second generation caterpillars hatch late summer - early autumn. These caterpillars experience short days of approximately 11 – 12 hours, overwinter as diapausing pupae and emerge as adults the next spring. For the present study, eggs of *M. brassicae* were obtained from a mass rearing programme at the Entomology Department of Wageningen University (see Poelman *et al.* (2009) for rearing details).

Experimental set-up

In a greenhouse, we created 40 open-top compartments of 36x40x36cm (LxWxH). These compartments were divided over 10 blocks of four compartments. The four compartments in each block were randomly assigned to one of the four artificial light treatments (green, white, red or no (dark control) artificial light at night), resulting in $n=10$ compartments per light treatment in a randomized block design. In the compartments that were subjected to one of the artificial light treatments, we mounted LED lamps that were designed for this study by Philips Lighting (Eindhoven, the Netherlands). The spectral compositions of the red and green lamps (Fig. 2.1) were designed for their potential as ‘habitat-friendly’ street lighting, aiming to minimize adverse ecological effects of artificial light by varying the amount of shorter and longer wavelengths. Lamps were made using LEDs obtained from Farnell (Utrecht, the Netherlands): each green lamp 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), the white lamp comprised of three 60 lumen warm white LEDs (LXML-PWW1-0060), and red lamps were made with two 70 lumen red-orange LEDs (LXM2-PH01-0070) and one 60 lumen warm white LED. LEDs were mounted in a 15x8x5cm (LxWxH) black plastic housing. Light was mixed in a 10cm long, 3.5x3.5cm wide aluminium duct with a standard white diffusor (PPMA plate) at the end. Diffusers were adjusted to obtain light intensities of 7.0 ± 0.6 lux (measured with a LMT B360 illuminance meter (LMT, Berlin, Germany)). Although streetlight intensities are generally much higher, up to 60lux (Gaston *et al.* 2012), we applied low light intensities in order to mimic light levels in the surrounding of illuminated roads. Light levels in dark control treatments were 0.04 ± 0.006 lux. Lamps were turned on at 17.00h. (approximately

one hour before the onset of scotophase (night)) and off at 08.00h. (approximately one hour after the onset of photophase (day)). The day length in our experiment of approximately 11 hours is similar to what second-generation *M. brassicae* caterpillars are exposed to under natural conditions. Average day temperature (measured with i-buttons (Maxim, Sunnyvale, USA) for 14 and 10 nights in block 3 and 6, respectively) was $16\pm 2^{\circ}\text{C}$. Average night temperature was $15\pm 1^{\circ}\text{C}$ and was not affected by light treatment (One-Way ANOVA on average night temperatures $F_{3,3}=2.107$, $p=0.278$).

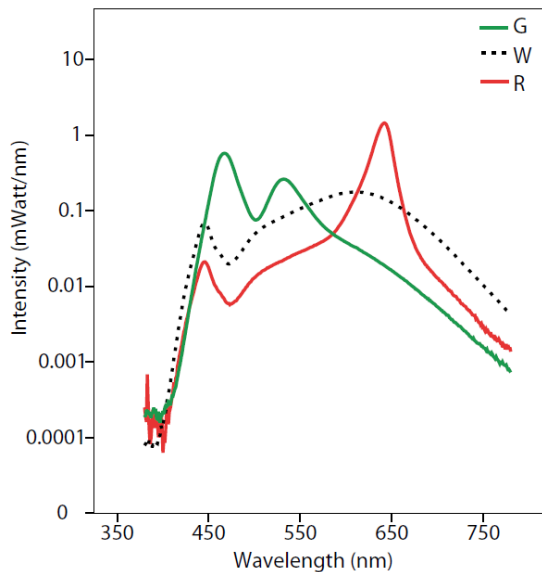


Figure 2.1 Visualisation of spectral compositions (intensities per wavelength) of the experimental lamps (G = green, W = white, R = red). Lines are averages of three lamps per type. The Y-axis is log scaled to enhance visibility of differences at low levels. Spectral measurements of lamps were performed at 25°C in a 2 meter diameter Ulbricht sphere equipped with a Cary Varian 17D digital spectrophotometer. Lamps were allowed to stabilize for 5 minutes 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.

A random subset of 240 twelve-day old (second instar) caterpillars was selected. Each caterpillar was individually housed in a 12.5cm high, 9.5cm diameter white plastic pot, which was closed off with transparent plastic foil to prevent escape. Pots were randomly divided over the different compartments (i.e. 6 pots per compartment). Plastic foil was replaced by white insect mesh (mesh size 1.35mm^2 , Harrod Horticultural, Lowestoft, UK) after 23 days for better ventilation. Each caterpillar was provided with artificial diet *ad*

libitum (see Supplementary Information for a list of ingredients) and a standardized piece of egg box for shelter. Care was taken that caterpillars were exposed to artificial light when feeding, i.e. that artificial diet was not under, or in the vicinity of, the egg box.

Caterpillars were weighed at the start of the experiment, and subsequently twice a week to determine growth rate. We checked for pupation daily. Because caterpillar growth follows an exponential pattern until maximum mass is obtained, relative growth rates until maximum mass (RGR) were calculated as $[\ln(\text{maximum mass}) - \ln(\text{start mass})] / \text{time}$ (Tammaru, Ruohomäki & Montola 2000). Pupal mass was recorded within 24 hours after pupation, after which pupae were sexed, covered with a 2cm layer of wood fibre and stored under a natural light-dark regime. Pots were checked daily for imago emergence, and emergence date was recorded.

Statistical analyses

RGR until maximum caterpillar mass, maximum caterpillar mass, age at molt to pupal stage (henceforth: age at pupation), pupal mass and the duration of the pupal stage (henceforth: pupation duration) were averaged for males and females within the same compartment to avoid pseudo replication. All resulting variables fulfilled assumptions of normality and homogeneity of variances. We used a general linear model with treatment (fixed), sex (fixed), block (random) and a sex*treatment interaction as factors. In case of a significant sex*treatment interaction, analyses were performed for the two sexes separately using a general linear model with treatment (fixed) and block (random) as factors. All analyses were performed in IBM SPSS Statistics version 20.

Because of technical failure of one red lamp on day 17 of the experiment, data from the six caterpillars in this compartment were excluded from the analyses. In other compartments, four caterpillars died in the beginning of the experiment and data obtained from these caterpillars were also excluded from all analyses. Of the remaining 230 caterpillars, 40 caterpillars (17%) fatally failed during pupal molt. This effect was independent of light treatment (general linear model on arcsine-transformed proportions $F_{3,35}=1.489$, $p=0.235$). We determined sex in 37 of these 40 caterpillars, and we used data on their RGR and age at pupation for analyses. However, because pupation failure generally was accompanied by physical damage of pupae and loss of body fluids, we did not use data for analyses on pupal mass. Because we were unable to sex the other three

caterpillars in which pupal molt fatally failed, data obtained from these caterpillars were excluded from both pupal mass and age at pupation analyses. Of the remaining 190 individuals, seven did not emerge from their pupae (1 green, 3 red and 3 dark) and thus were excluded from analyses on pupation duration. In total, we obtained pupation durations of 183 individuals.

RESULTS

Caterpillar RGR until maximum mass varied between 0.20 and 0.31 g day⁻¹, and did not differ between artificial light treatments ($F_{3,57}=0.844$, $p=0.476$, Fig. 2.2). The maximum mass of the caterpillars, however, varied between 0.76 and 1.43g, and was differentially affected by light treatment in males and females (sex*treatment $F_{3,57}=2.921$, $p=0.042$). When analysing sexes separately, light treatment had an effect on maximum caterpillar mass in males ($F_{3,24}=3.029$, $p=0.049$; Fig 2.3a), but not in females ($F_{3,24}=0.806$, $p=0.503$; Fig. 2.3b). In males, mass was significantly lower for caterpillars under white light than caterpillars under red light or the dark control, whereas caterpillars under green light were intermediate (Fig 2.3a).

Analyses on age at pupation and pupal mass revealed differences in artificial light effects on males and females as well (treatment*sex interactions for age at pupation ($F_{3,57}=2.845$, $p=0.046$) and pupal mass ($F_{3,56}=6.418$, $p=0.001$)). In sex-specific analyses, we found that male caterpillars that were subjected to white and green light pupated earlier than caterpillars in darkness ($F_{3,24}=6.260$, $p=0.003$). Caterpillars under red light were intermediate (Fig. 2.3c). Pupal mass in males was also affected by light treatment ($F_{3,24}=9.542$, $p<0.001$), with reductions of pupal mass of caterpillars subjected to white light compared to caterpillars that developed under red light or in darkness. Pupal mass of males in green light was significantly lower than that of males in red light, but not different from pupal mass of males in darkness or under white light (Fig 3e). In females, effects on age at pupation and pupal mass were absent ($F_{3,24}=0.392$, $p=0.760$ and $F_{3,23}=1.412$, $p=0.265$, respectively; Fig. 2.3d,f).

Pupation duration differed greatly between light treatments ($F_{3,61}=26.021$, $p<0.001$; Fig 2.4), irrespective of sex (sex*treatment $F_{3,61}=0.581$, $p=0.630$). Moths that were under green and white light as caterpillar emerged significantly earlier (after 73 (± 4) and 73 (± 5))

days on average (\pm s.e.), respectively) than moths that had experienced a dark treatment as caterpillars (139 (\pm 1.8) days, on average). Moths that had been under red light as caterpillars (118 (\pm 2) days, on average) (Fig. 2.4). By the time that the first moth from the dark control treatment emerged (after 110 pupal days), 85% of the moths that had been under green light as caterpillar and 83% of the moths that had been under white light as caterpillar had already emerged, whereas this was only 25% in moths that were under red light as a caterpillar. Moreover, 24%, 34% and 8% of the moths that had been as caterpillar under green, white and red light, respectively, emerged within half the time (55 days) needed for the fastest moth in darkness to emerge (Fig. 2.4).

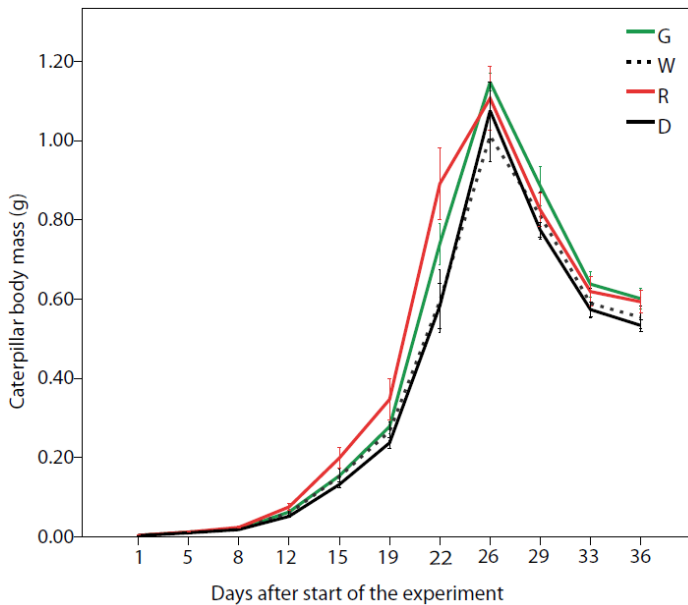


Figure 2.2 Growth curves of caterpillars (males and females combined) under various types of artificial light at night. G = green, W = white, R = red light at night and D = Dark control. Error bars are 1 standard error of the mean. The peaked shape is caused by the pre-pupation inactivity period after reaching maximum body mass.

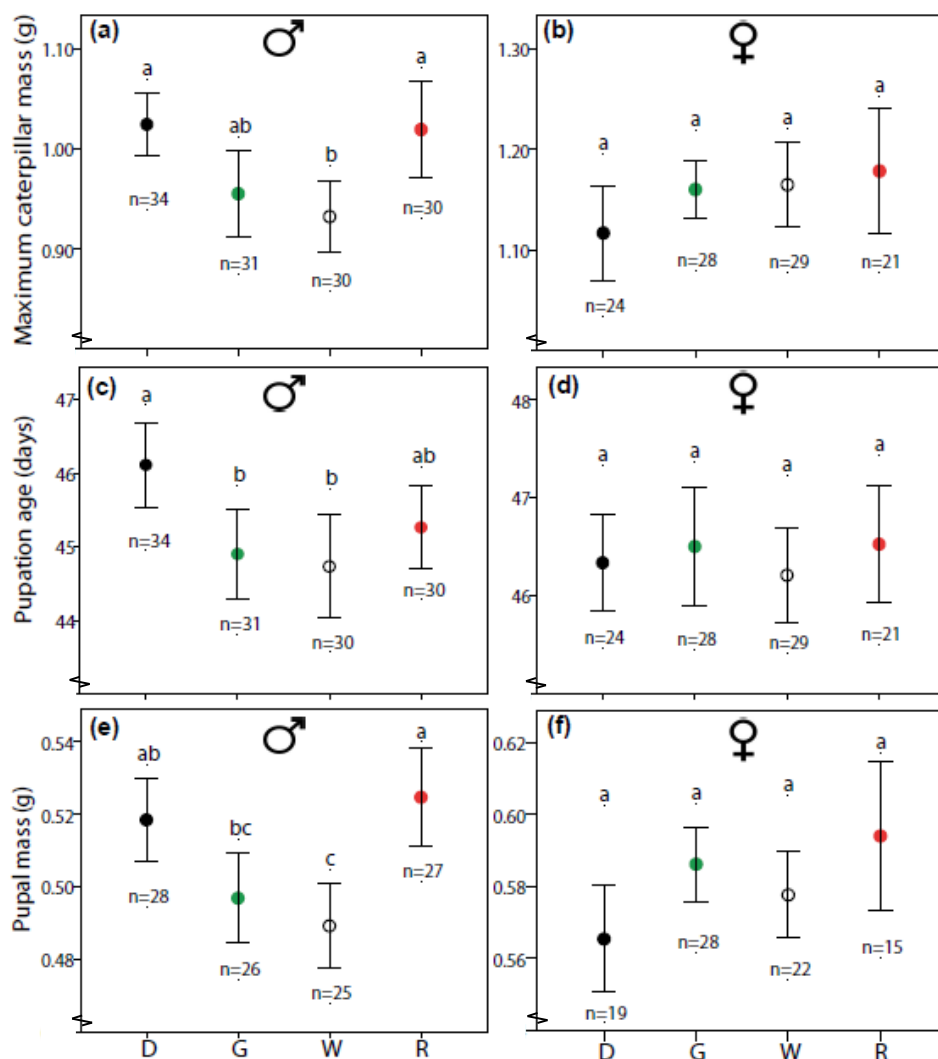


Figure 2.3 Maximum mass of male (a) and female (b) caterpillars, age at pupation of male (c) and female (d) caterpillars and pupal mass of male (e) and female (f) caterpillars subjected to different light treatments. Dots are averages, bars represent 95% confidence intervals. Different letters indicate significant differences between treatments (Tukey $\alpha = 0.05$), and replication (n) is shown below the bars. Differences in replication within sexes are due to pupation failure (see materials and methods). D = dark control, G = green light at night, W = white light at night and R = red light at night.

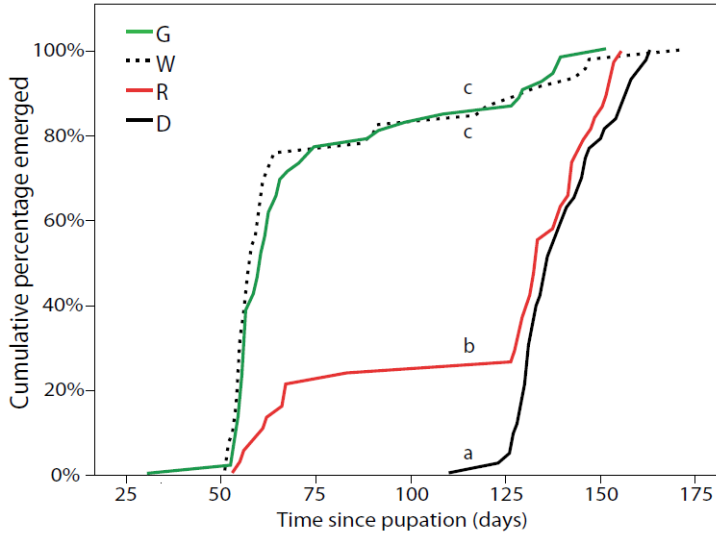


Figure 2.4 Duration of pupal stage of caterpillars that developed under the different light treatments ($n = 44$ (dark), 53 (green), 47 (white) and 39 (red)). Different letters indicate significant differences between treatments (Tukey $\alpha = 0.05$).

DISCUSSION

To date, studies on the effects of artificial night lighting on moths have almost exclusively focussed on attraction of moths to sources of artificial light (Van Langevelde *et al.* 2011; Somers-Yeates *et al.* 2013). However, given the rapid declines of moth populations in Western Europe (Conrad *et al.* 2006; Groenendijk & Ellis 2011) and the suggested role of light pollution in this (Groenendijk & Ellis 2011; Fox 2013), there is an urgent need to move beyond attraction and study potential effects of artificial night lighting on other aspects of moth ecology. Here, we show that artificial night lighting disturbs life history and seasonal timing of moths. Male caterpillars exposed to white light reached a lower maximum caterpillar mass than males under red light and in the dark control. Furthermore, male caterpillars under green and white light pupated earlier and with a lower pupal mass than males that were under red light or in dark controls. These effects of artificial lighting on male moth life history did not result from variation in relative growth rates, as these were unaffected by artificial night lighting. Although a shorter development time (i.e. early

pupation) may reduce the risk of death before reproduction (Benrey & Denno 1997; Nylin & Gotthard 1998), the concomitant reduction in mass may have negative consequences for males, such as decreased longevity, flight ability and sperm competition (Shirai 1993; Carroll 1994; Shirai 1995; Tammaru, Ruohomäki & Saikkonen 1996; Iyengar & Eisner 1999b), factors that may affect a males' fitness. In females, body mass is strongly correlated to egg production (Honěk 1993) and thus directly linked to fitness. Hence, the fitness consequences of early pupation at the cost of pupal mass are more severe for females than for males (Honěk 1993; Tammaru, Esperk & Castellanos 2002). As a consequence, the trade-off between early pupation and reduced mass differs between the two sexes. This which may explain why, in contrast to males, we did not find effects of artificial night lighting on age at pupation and pupal mass in females.

The initiation of pupal diapause in moths is strongly dependent on temperature and light conditions during the larval stage (Adkisson 1966; Roditakis & Karandinos 2001; Huang *et al.* 2005; He *et al.* 2009; Pavan *et al.* 2010; Xiao *et al.* 2010; Xu *et al.* 2014). We found very large differences in the duration of the pupal stage, with a vast majority of moths subjected to green and white artificial light as larva emerging earlier from the pupa than moths in darkness (Fig. 4) for both sexes. These differences are likely a result of suppression of diapause initiation by exposure to green and white light at night in the larval stage. Low intensities of artificial light have previously been shown to interfere with natural light-dark cycles as an information source about timing and location in a variety of other species groups (Gaston *et al.* 2013). Indeed, the low levels of artificial light used in our experiment are sufficient to interfere with day length as an environmental cue for seasonal timing of pupal diapause in moths. Under natural conditions, the inhibition of pupal diapause by artificial light at night can cause de-synchronisation of imago emergence, leading to mismatches with favourable seasons (Adkisson 1966) and thus high mortality as non-diapausing *M. brassicae* pupae are unlikely to survive the winter. Although, moths that were under red light also emerged on average earlier than moths in darkness, the pattern in emergence resembled that of moths in darkness (Fig. 4).

Spectral alterations have been suggested to provide a tool for mitigation of negative effects of artificial light on ecosystems (Van Langevelde *et al.* 2011; Gaston *et al.* 2012; Fox 2013). Our results show that negative effects of artificial light on moth life history and seasonal timing can be reduced by changing spectral compositions. Overall, compared to

white and green light, the effects of red light at night on moth life history and seasonal timing were less strong, or even absent. This can be explained by a low sensitivity of caterpillar stemmata to longer wavelengths such as red light (Ichikawa & Hideki 1982).

We conclude that low levels of artificial light at night affect key life history traits in moths and interfere with light as a cue for seasonal timing in moths, thereby disrupting the initiation of diapause. These effects can have important negative fitness consequences, which add up to other adverse effects of artificial light on moths such as attraction (Van Langevelde *et al.* 2011; Somers-Yeates *et al.* 2013) and the associated higher predation levels (Rydell 1992; Svensson & Rydell 1998; Acharya & Fenton 1999), and thus are likely to contribute to the observed widespread declines in moth populations in Western Europe (Conrad *et al.* 2006; Groenendijk & Ellis 2011; Fox *et al.* 2013). Furthermore, we show that the application of light that is poor in short-wavelength radiation (i.e. red light) can be used to at least partly mitigate negative effects of nocturnal illumination on moth life history and seasonal timing.

ACKNOWLEDGEMENTS

We thank Kwekerij Van Geffen V.O.F. for allowing us to perform this experiment in their greenhouse, the Entomology insect breeding staff for providing us with *M. brassicae* eggs, and Michiel Wallis de Vries for commenting on an earlier version of this manuscript. Margot Sauter performed a pilot study that was important for the design of this experiment. This study is part of the ‘Light On Nature’ project, which is funded by NWO-STW Grant 11110, Philips Lighting, and the Dutch Oil Company (NAM).

SUPPLEMENTARY INFORMATION

Ingredients of artificial diet for *Mamestra brassicae* caterpillars:

2 L water

70g agar (Sigma-Adlrlich Chemie GmbH, Steinheim, Germany) in 0.5L water

350g polenta (Windkorenmolen “de Vlijt”, Wageningen, the Netherlands)

125g yeast flakes (Natudis, Harderwijk, the Netherlands)

125g wheat germ (Natudis, Harderwijk, the Netherlands)

5g sorbic acid (Carl Roth GmbH, Karlsruhe, Germany)

4g Methyl 4-hydroxybenzoate, 99% (Acros Organics, Geel, Belgium)

20g ascorbic acid (Hinmeijer Chemic V.O.F., Haarlem, the Netherlands)

0.25 Streptomycin sulphate (Sigma-Adlrlich Chemie GmbH, Steinheim, Germany)

CHAPTER 3

ARTIFICIAL NIGHT LIGHTING DISRUPTS SEX PHEROMONE PRODUCTION IN A NOCTUID MOTH

Koert G. van Geffen, Astrid T. Groot, Roy H.A. van Grunsven, Maurice Donners, Frank Berendse &

Elmar M. Veenendaal

Ecological Entomology (2015), **IN PRESS**

ABSTRACT

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. In this study, we subjected female *Mamestra brassicae* moths 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 tested how this affected their sex pheromone production and composition. 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 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. Our 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.

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, Falchi & Elvidge 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, Jones & Harris 2012). Depending on the lights that are replaced by the LEDs, this can result in increased (compared with high and low pressure sodium, see Stone, Jones & Harris 2012; Pawson & Bader 2014) or a decreased effect (compared to mercury vapour or metal-halide lamps, see Van Grunsven *et al.* 2014a) 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 (Van Geffen *et al.* 2014; Van Geffen *et al.* 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, Herrebout & Menken 1991; Symonds & Elgar 2008). Males not only have receptors for attractive, 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, Lashbrook & Hildebrand 1994; Boo *et al.* 1995; Vickers & Baker 1997; Evenden, Judd & Borden 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 cultured and (sub-)urban areas (Waring & Townsend 2003). Since adults are nocturnal and attracted to sources of artificial light at night, *M. brassicae* moths are frequently exposed to sources of artificial light at night (Waring & Townsend 2003). In the Netherlands, populations of *M. brassicae* are declining, with a 50-75% reduction in the reported catches, corrected for observation intensity, over the period 1982 – 2013 (Ellis *et al.* 2013).

The sex pheromone of *M. brassicae* is well studied, this makes the species suitable as a choice 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, related 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 males are (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: tetradecanyl acetate (14:Ac), hexadecanyl 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 & 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. brassicae* 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 to moths in darkness.

MATERIALS AND METHODS

In a greenhouse, we constructed 20 open-top compartments of 50 x 50 x 60 cm. 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 lux, or 63, 53 and 96 mW/m² respectively) or no artificial light at night (65 ± 1 mlux). Treatments were divided over five randomized 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. Spectra of our study lamps are shown in Figure 3.1. Spectral measurements of lamps were performed at 25°C in a 2 meter diameter Ulbricht sphere equipped with a Cary Varian 17 D digital spectrophotometer. Lamps were allowed to stabilize for 5 minutes 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 15x8x5cm (LxWxH) black plastic housing. Light was mixed in a 10 cm long, 3.5 x 3.5cm wide aluminium duct with a standard white diffusor (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 one hour before the onset of the scotophase (night, starting around 19.00h.), and off one hour after onset of the photophase (day, starting around 07.00h.). Daylight was natural, average day and night temperatures were 20 ± 2 °C and 18 ± 2 °C, respectively. There were no differences in temperature between light treatments (GLM $F_{3,16}=0.065$, $P=0.978$; measured in each compartment for two nights with i-buttons (Maxim, Sunnyvale, USA)).

Mamestra brassicae pupae were obtained from a mass rearing program at the Laboratory of Entomology, Wageningen University, the Netherlands (Poelman *et al.* 2009). In this mass rearing, males and females are stored together in large deposition chambers in a controlled climate room at 20-22 °C, 50-70 % RH and 16:8 Light:Dark regime. Mating occurs in these chambers, after which females deposit eggs on filter paper. Caterpillars are reared on Brussels sprouts (*Brassica oleracea*) plants. Final instar caterpillars are placed on an artificial soil medium, in which they pupate. For our experiments, we obtained 100 ± 14 1 day-old female *M. brassicae* pupae from this mass-rearing, and placed them individually

in 13 cm high, 7.5 cm diameter white plastic containers. Each container was filled with a 0.5 cm layer of wood fibre and covered with 1.35 mm² white mesh. In each compartment (i.e. under each lamp) we placed five randomly selected containers. There were thus four light treatments with 5 compartments each and each compartment contained five pupae resulting in a nested design. Pupae were checked for emergence every day and freshly emerged moths were provided with a 1:10 sugar-water solution soaked piece of cotton wool for feeding.

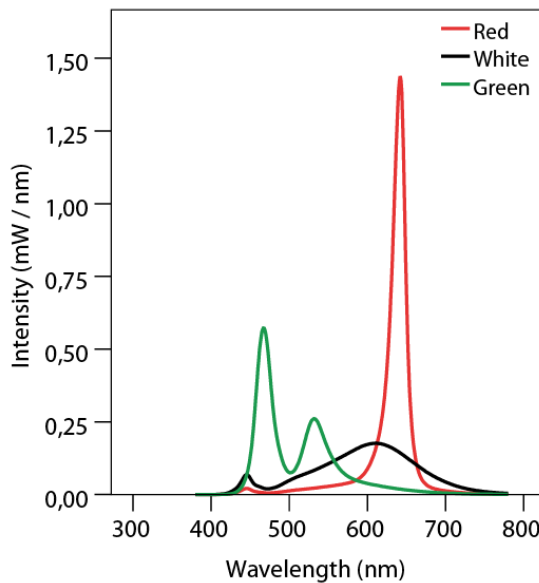


Figure 3.1 Visualisation of spectral compositions (intensities per wavelength) of the experimental lamps (G = green, W = white, R = red). Lines are averages of three lamps per type. The Y-axis is log scaled to enhance visibility of differences at low levels.

Each night, all three-night old females were collected for gland extraction. Extractions started at midnight (~5 hours after the onset of the scotophase), when females have produced sex pheromone for the coming night and start calling (Noldus & Potting 1990). Due to variation in emergence date, gland extractions were spread over 15 nights. Glands of 24, 22, 22 and 24 females from green, white, red and dark control treatments, respectively, were successfully extracted.

The pheromone gland extraction and Gas Chromatography analyses procedures followed have previously been described by Groot et al. (2010a). A multi-component reference blend containing the nine previously identified sex pheromone compounds of *M. brassicae* (Struble *et al.* 1980; Van de Veire & Dirinck 1986; Den Otter & Van der Haagen 1989; Renou & Lucas 1994) (14:Ac, Z9-14:Ac, Z11-16:Ald, 16:Ac, Z9-16:Ac, E11-16:Ac, Z11-16:Ac, Z11-16:OH and Z11-17:Ac; compounds from Pherobank, Wageningen, the Netherlands) was injected into the GC 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 (ln-transformed) were tested using nested ANOVA at the lamp level (the five moths in one compartment are not independent) with block as random factor and a Tukey post-hoc test. For all tests homoscedasticity and normality of residuals were assessed visually.

Since 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; Groot *et al.* 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 can be 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 taking the logarithm of each ratio. We sacrificed 16:Ac for log-scaling, because 1) male antennae are unable to detect this compound (Den Otter & Van der Haagen 1989; Renou & Lucas 1994), hence loss of information on the variation in this compound is most likely not biologically important, 2) it is the second-most abundant pheromone compound, making the integration results reliable, and 3) 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 3.2). Also, the composition of the sex pheromone blend was significantly altered by artificial light (Wilks' λ , $F_{24,186}=2.903$, $P < 0.001$; Fig. 3.3a,b). Specifically, we found a relative reduction of the major sex pheromone component, Z11-16:Ac, for females subjected to artificial light, as compared to darkness ($F_{3,16.4}=8.230$, $P =$

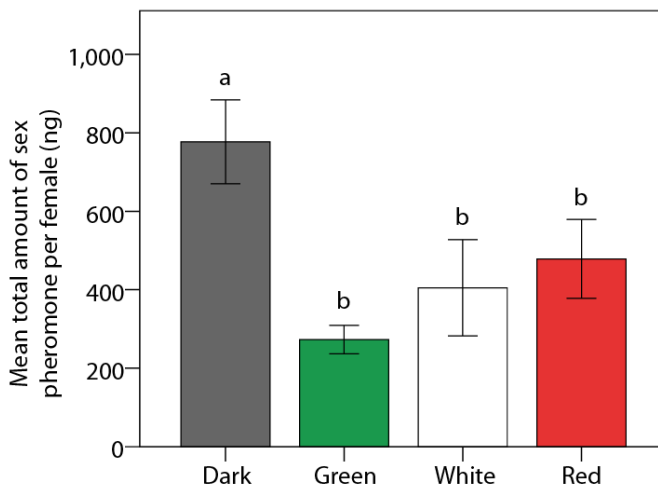


Figure 3.2 The amount of sex pheromone produced by *M. brassicae* females under different light treatments. Values are means (\pm SEM), different letters indicate significant differences (Tukey, $\alpha=0.05$).

0.001). Z9-16:Ac, a minor pheromone compound that may act as an deterrent (Renou & Lucas 1994) to 16:Ac ($F_{3,16.4}=5.123$, $P = 0.011$, Fig. 3.3a). 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 to darkness (1.4%) ($F_{3,16.5}=3.724$, $P = 0.032$, Fig. 3.4a). The ratios between the two other deterrents and the main attractive

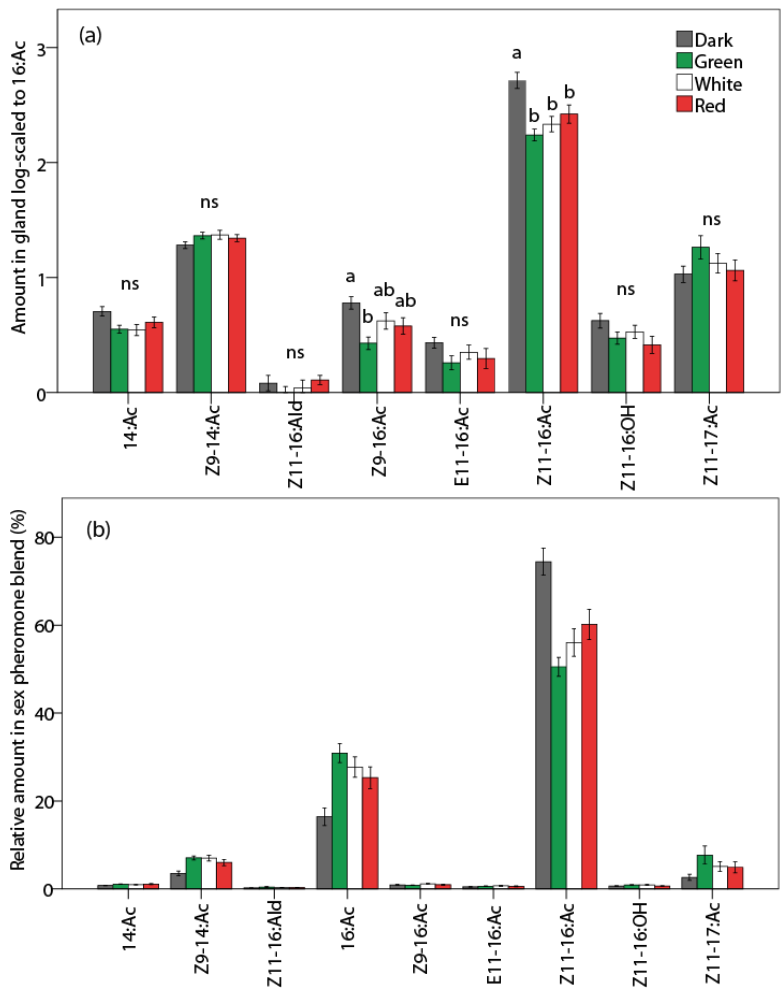


Figure 3 (a) Mean (\pm SEM) relative amounts of sex pheromone compounds, log-scaled to 16:Ac, in the sex pheromone blend of moths under the different light treatments. Letters indicate significant differences between treatments per component (Tukey, $\alpha=0.05$). (b) Mean (\pm SEM) un-transformed relative amounts of the nine sex pheromone compounds in the glands of females under different light treatments.

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 to females in darkness ($F_{3,16.5}=8.587$, $P = 0.001$, Fig 3.4b), 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 3.4c).

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 sub-esophageal ganglion, likely continuously, so that it accumulates during the photophase (Rafaeli 1994), while its release from the corpus cardiacum into the hemolymph 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, Herrebut & Menken 1991; Symonds & Elgar 2008). 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 and related 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

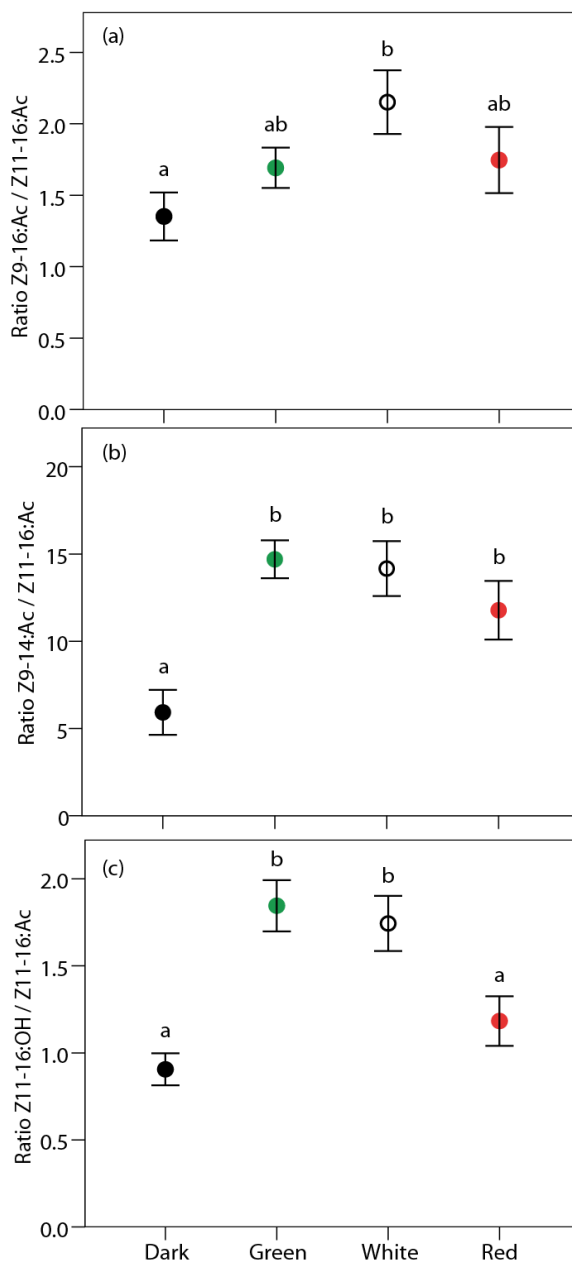


Figure 3.4 Ratio of (a) Z9-16:Ac / Z11-16:Ac, (b) Z9-14:Ac / Z11-16:Ac and (c) Z11-16:OH / Z11-16:Ac under the different light treatments. Dots represent means, bars are 1 SEM, letters indicate significant differences between treatments (Tukey, $\alpha=0.05$).

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, Erler & Vostrowsky 1988), as this compound has been shown to be deterrent at levels as low as 0.1 % (Descoins *et al.* 1978). Hence, the >2-fold increase in the ratio of Z11-16:OH / Z11-16:Ac that we found may not affect the emission ratios, but if so, then even the smallest amount of Z11-16:OH will already 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, Stanimovora & T.S. 1987), but inhibitory when 1-2% is added to the blend (Descoins *et al.* 1978; Struble *et al.* 1980). Thus, the nearly 3-fold increase in this compound that we found under the different light conditions is likely to negatively affect the attraction of *M. brassicae* males. The small differences in pheromone blend composition require males to be highly sensitive to subtle changes in pheromone composition. However, actual testing how the artificial light induced compositional changes in sex pheromone blends affects the attraction of moths is an important next step to be taken in the future.

The range of variation that we found in the pheromone composition of *M. brassicae* is similar or even higher to that found in the geographic variation of e.g. *Heliothis virescens*, *H. subflexa* (Groot *et al.* 2009), *Agrotis ipsilon* (Gemeno, Lutfallah & Haynes 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.* 2010) and shows that moth sex pheromone blends may be more plastic and variable than generally considered. In general, moth sex pheromones are hypothesized to be under stabilizing selection, because moth sexual communication is important for species recognition and should thus not vary, as this may directly decrease fitness. Our study is the second example showing that variation in the sex pheromone blend can be significant when varying only one environmental factor, in this case light. Recently, we also demonstrated that artificial light may lead to reduced mating

under field conditions in the geometrid moth *Operophtera brumata* (Van Geffen *et al.* 2015).

We need to point 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 correlation is found between the composition in the pheromone gland and the emitted blend (Du, Lofstedt & Lofqvist 1987; Heath *et al.* 1991; Bäckman, Bengtsson & Witzgall 1997; Svensson, Bengtsson & Lofqvist 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 2004a).

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). This indicates 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.* 2012) but is in line with a recent study by Pawson & 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.* 2014a), 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 to darkness, however, red light still often has significant negative effects (Van Geffen *et al.* 2014; Van Geffen *et al.* 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). Therefore, adjustment of spectral composition can possibly play a role in mitigation of 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 (Van Geffen *et al.* 2015). Such mating reductions can have important implications for moth populations in illuminated nights.

Table 3.1 Presence and role of the nine sex pheromone compounds of *M. brassicae* in the pheromone blends of related / co-occurring species, based on El-Sayed (2012) and references therein.

Species	Habitat	Flight period	14:Ac	Z9-14:Ac	Z11-16:Ald	16:Ac	Z9-16:Ac	E11-16:Ac	Z11-16:Ac	Z11-16:OH	Z11-17:Ac
<i>Mamestra brassicae</i>	Cultured fields	April - Oct	P	P D	P A	P	P D	P	P M A	P D	P A
<i>Agrotis ipsilon</i>	Cultured fields	April - Oct		P M A		P			P M A	P	
<i>Mamestra oleracea</i> *	Cultured fields	April - Oct							P	P	
<i>Mythimna pallens</i>	Grasslands, forest edges	May - Oct			P				P		
<i>Mythimna unipuncta</i> **	Grasslands	Aug - Nov		P A (0.01%) D (1%)	P D	P	P		P M A	P A	
<i>Ochropleura plecta</i>	Cultured fields	April - Oct		D	P A				P M A		
<i>Trichoplusia ni</i>	Habitat generalist	May - Oct		P A					P		

P: present in pheromone blend, M: main pheromone component, A: attractor: compound increases attractiveness of main component, D: deterrent: compound decreases attractiveness of main component. Information on habitat and flight period obtained from Waring and Townsend (2003).

* Synonym: *Lacanobia oleracea*

** Synonym: *Pseudaletria unipuncta*

composition is more plastic than previously assumed (Butlin & Trickett, 1997; Groot et al., 2010b; Löfstedt, 1993). Such shifts can have serious consequences for reproduction, because male response can be disrupted, which may lead to reduced mating (Van Geffen et al., 2015). Such mating reductions can have important implications for moth populations in illuminated nights.

ACKNOWLEDGEMENTS

We thank Kwekerij Van Geffen VOF for greenhouse space and Dennis van Veldhuizen for lab-assistance. Frank van Langevelde and Thijs Fijen contributed to earlier versions of this manuscript. This study is funded by NWO-STW grant 11110, Philips Lighting and the Dutch Oil Company.

CHAPTER 4

ARTIFICIAL LIGHT AT NIGHT INHIBITS MATING IN A GEOMETRID MOTH

Koert G. van Geffen, Emiel van Eck, Rens A. de Boer, Roy H.A. van Grunsven, Lucia Salis, Frank

Berendse & Elmar M. Veenendaal

Insect Conservation and Diversity 2015, **EARLY VIEW**

doi: 10.1111/icad.12116

ABSTRACT

Levels of artificial night lighting are increasing rapidly worldwide, subjecting nocturnal organisms to a major change in their environment. Many moth species are strongly attracted to sources of artificial night lighting, with potentially severe, yet poorly studied, consequences for development, reproduction and inter/intra-specific interactions. Here, we present results of a field-based experiment where we tested effects of various types of artificial lighting on mating in the winter moth (*Operophtera brumata*, Lepidoptera: Geometridae). We illuminated trunks of oak trees with green, white, red or no artificial LED light at night, and caught female *O. brumata* on these trunks using funnel traps. The females were dissected to check for the presence of a spermatophore, a sperm package that is delivered by males to females during mating. We found a strong reduction in the number of females on the illuminated trunks, indicating artificial light inhibition of activity. Furthermore, artificial light inhibited mating: 53% of females caught on non- illuminated trunks had mated, whereas only 13%, 16% and 28% of the females that were caught on green, white and red light illuminated trunks had mated respectively. A second experiment showed that artificial night lighting reduced the number of males that were attracted to a synthetic *O. brumata* pheromone lure. This effect was strongest under red light and mildest under green light. This study provides, for the first time, field-based evidence that artificial night lighting disrupts reproductive behaviour of moths, and that reducing short wavelength radiation only partly mitigates these negative effects.

INTRODUCTION

Light pollution is a widespread phenomenon, leading to loss of darkness in (sub-)rural areas around the world (Cinzano, Falchi & Elvidge 2001; Elvidge *et al.* 2001). Despite the continuous rise in levels of artificial light at night (Hölker *et al.* 2010a), the ecological consequences of light pollution remain largely unknown (Longcore & Rich 2004). A wide variety of both diurnal and nocturnal animals are likely to be affected by light at night, including birds (Kempenaers *et al.* 2010; Titulaer *et al.* 2012; Dominoni, Quetting & Partecke 2013), fish (Brüning, Hölker & Wolter 2011), mammals (Stone, Jones & Harris 2012) and invertebrates (Davies, Bennie & Gaston 2012). Of this latter group, moths represent a highly diverse, largely nocturnal species group, that are known to be attracted to

sources of artificial light at night (Van Langevelde *et al.* 2011; Truxa & Fiedler 2012; Somers-Yeates *et al.* 2013; Merckx & Slade 2014). This so-called ‘flight-to-light’ behaviour is a relatively well-studied phenomenon in moths, but artificial night lighting might also affect other aspects of nocturnal moth biology, such as development (Van Geffen *et al.* 2014) and mortality (Rydell 1992; Svensson & Rydell 1998; Acharya & Fenton 1999).

The Winter moth *Operophtera brumata* (L.) (Lepidoptera: Geometridae) is a univoltine species with adults emerging in November and December (Cuming 1961). In the Netherlands, *O. brumata* is a common species that occurs in (sub)urban parks and forests. Female *O. brumata* moths are brachypterous (i.e. have reduced wings and are unable to fly). After emergence from their pupae in late autumn, females climb trunks of their host trees, mainly oak (*Quercus robur*) and produce a sex pheromone (Roelofs *et al.* 1982) to attract males, which are able to fly. After mating, eggs are deposited near leaf buds in the crown of the host tree. Egg hatching is timed to coincide with bud burst (Visser & Holleman 2001). Winter moths caterpillars are important herbivores (Wesolowski & Rowínsky 2006) and a crucial early-spring bulk food source for breeding birds in northern European forests (Visser & Holleman 2001; Van Asch & Visser 2007). In the early summer, fully grown caterpillars spin a silk filament and descend to the ground where they pupate.

In this study, we experimentally test the effects of various types of artificial night lighting, differing in spectral composition, on mating in *O. brumata* under natural conditions. In two field experiments, we test whether and how artificial light at night 1) affects the number of females moving upwards on host tree trunks, 2) affects the proportion of mated females on host tree trunks, and 3) influences the male response to female sex pheromone. Because winter moth activity starts when light levels drop, we expected to find 1) reduced activity and thus reduced number of females moving upwards on illuminated trunks. Furthermore, given earlier lab-based indications for artificial light inhibition of sex pheromone release (Sower, Shorey & Gaston 1970) and male response to female sex pheromone (Shorey & Gaston 1964; Shorey & Gaston 1965), we expect 2) a strong reduction in the proportion of females that had mated under artificial light, compared to females on unlit trees, and 3) artificial light inhibition of male attraction to (synthetic) sex pheromone lures. In addition, given the sensitivity of moth eyes to short wavelength

radiation (i.e. UV radiation, green and blue light), we expected strongest effects under green and white light (both containing short wavelengths), and milder effects under red light (comprising mainly long wavelengths, to which moths are relatively insensitive) (Agee 1973; Van Langevelde *et al.* 2011). Insight in artificial light effects on reproduction, and the role of spectral composition therein, might provide tools for mitigation of potential negative effects of artificial light on moths, which is important given the rapid declines of moth populations in western Europe (Conrad *et al.* 2006; Groenendijk & Ellis 2011; Fox *et al.* 2014) and the suggested role of artificial night lighting therein (Fox 2013).

MATERIALS AND METHODS

Field site and lamps

For this study, we performed two experiments in an oak (*Quercus robur*) dominated forest in Wageningen, The Netherlands (51°58'57N, 5°39'20E). The forest had a dense *Rubus fruticosus* agg. understory and harboured an *O. brumata* population. We selected four ~30cm diameter *Q. robur* trees with a distance of 50m between trees. The four trees were randomly assigned to one of four artificial light treatments each night. These consisted of green/blue-ish (henceforth: green), white, red or no artificial light at night. The green and red treatments have a continuous spectrum but relatively large contribution of short wavelength light (green) or long wavelength light (red), see Supporting Information for spectral analysis. For the light treatments, we used custom made 20W Fortimo LED modules (Philips Lighting, Eindhoven, the Netherlands). Lamps were mounted on a pole at 1.5m above the soil surface at 2.5m distance from the trunk, switched on 1 hour before sunset and off 1 hour after sunrise. The dark control treatment consisted of a pole without a lamp. Light intensities (measured on the trunk with an illuminance meter (LMT B360, Berlin, Germany)) were 10 lux in the artificial light treatments, and <0.1 lux in the dark control.

Artificial light effect on mating

To test the effects of artificial light on mating frequency, we used funnel traps to catch female *O. brumata* that were climbing up on experimental trees. The funnel traps consisted

of a funnel with a 40cm entrance directed downwards, made of iron wire and white 15 denier tights (HEMA, Amsterdam, the Netherlands). This funnel is approximately 30cm long and in the top it ends in a 5cm long, 3.2cm diameter PCV pipe that connects the funnel with a 12.5cm high, 11.5cm diameter transparent collection bin, capped off with a white lid (see Fig. S4.2 in Supplementary Information). This funnel trap is highly effective in trapping *O. brumata* females. On each study tree we mounted two funnel traps; one trap on the illuminated side, and one on the opposite (shaded) side of the trunk. Traps were emptied every morning 1 hour after sunrise. All female *O. brumata* were collected, counted and taken to the lab to assess whether they had mated (see below). The experiment was repeated for eleven nights between 22 November – 3 December 2012, and sixteen nights between 19 November – 4 December 2013, leading to n=27 nights for all treatments. In order to control for eventual male preference for heavy females (Xu & Wang 2009), we recorded the mass of each female moth. The collected female moths were dissected and their reproductive system was stored in 70% ethanol for at least 1 day, after which the presence (female mated) or absence (female not mated) of a spermatophore in the *bursa copulatrix* was determined under a microscope. Because winter moths mate once per night (Van Dongen 1998), a maximum of one spermatophore was observed.

Artificial light effect on male response to sex pheromone

For testing the male response to female sex pheromone under different types of artificial light at night, we replaced the funnel traps on the illuminated side (L-side) of the four trunks by pheromone traps (type ‘Unitrap’, Pherobank BV, Wageningen, the Netherlands), which were loaded with a synthetic *O. brumata* pheromone lure (Pherobank BV, Wageningen, the Netherlands). We caught males in our pheromone traps for 7 nights between 5 – 13 December 2012 and 11 nights between 5 – 18 December 2013. Each morning, traps were emptied 1 hour after sunrise and the number of males was recorded. Males were released outside the forest to avoid catching ‘experienced’ males.

Statistical analyses

We used a Generalized Linear Model (GZLM) for Poisson distribution to test the effect of artificial light on the number of females in the funnel traps, with light treatment, tree side,

tree number, night, and light treatment * tree side as predictors and calculated the pairwise contrasts for the light treatment * tree side interaction. To test for difference in the relative number of mated females between the different light treatments, we applied a GZLM for binomial data, with light treatment, tree number, tree side, night and treatment * tree side as factors, and female body mass as a covariate. We calculated pairwise contrasts for light treatment. To test for artificial light effects on the number of males in the pheromone traps, we applied a GZLM for Poisson distribution using light treatment, night and light treatment * tree number as factors and the number of males as dependent variable. Pairwise contrasts were calculated for light treatment.

All statistical analyses were performed in IBM SPSS Statistics 20. Contrasts were corrected for false discovery rates using the Benjamini-Hochberg method (Waite & Campbell 2006).

RESULTS

We caught 365 female moths in total (109 in 2012 and 256 in 2013) in our funnel traps. Light treatment had a significant effect on the number of female moths caught in the traps (Wald $\chi^2 = 130.273$, $P < 0.001$), and there was a significant light treatment * tree side effect (Wald $\chi^2 = 30.719$, $P < 0.001$). We caught most females in traps on the dark control trees and in the trap on the shaded side of the tree that was subjected to red light (Fig. 4.1). Furthermore, traps on trees illuminated with green light caught significantly fewer females than those on white-illuminated trees, which in turn caught significantly fewer females than traps on red-illuminated trees (Fig. 4.1). In all light treatments, traps on the illuminated side of the trees caught significantly less females than traps on the shaded side of the trees. The numbers caught on the shaded sides of trees between different treatments followed the same pattern as with the illuminated sides; significantly fewer females were caught on the shaded side in the green light treatment than in the white treatment, which in turn caught significantly fewer than in the red treatment (Fig. 4.1).

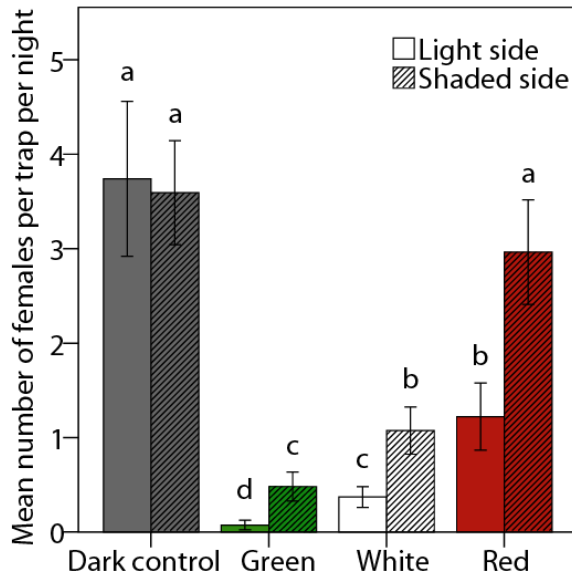


Figure 4.1 Mean number of females caught per night in traps on the illuminated (open bars) and shaded side (filled bars) of tree trunks under the different light treatments. Error bars represent 1 SEM, different letters indicate significant differences in the number of females between treatments ($\alpha=0.05$). $n=27$ nights per treatment.

In total, 339 females were successfully dissected (98 in 2012, 241 in 2013) for spermatophore analyses. Of the females caught in dark control traps, 53% (96 out of 180) had mated. Artificial light at night significantly reduced the proportion of mated females in our traps (Wald $\chi^2 = 17.214$, $P = 0.001$). This reduction was significant in all three light treatments, but was strongest under green light, where 13% (2 out of 15) of the females had mated (Fig. 4.2). With 28% (30 out of 106) mated, a significantly greater proportion of females in the red light treatment were mated than in green light. White light was intermediate with 16% (6 out of 38) mated, which is not significantly different from green or red light (Fig. 4.2). There was no significant treatment * tree side interaction (Wald $\chi^2 = 2.573$, $P = 0.462$), and no significant effect of tree number, tree side, night and female body mass on the relative number of mated females (Wald $\chi^2 = 2.237$, $P = 0.525$; Wald $\chi^2 = 0.000$, $P > 0.999$; Wald $\chi^2 = 13.106$, $P = 0.975$; Wald $\chi^2 = 0.007$, $P = 0.933$, respectively).

We caught 2194 males (576 in 2012, 1618 in 2013) in the pheromone traps. Light treatment affected the number of males in the traps (Wald $\chi^2 = 78.102$, $P < 0.001$), with control traps in darkness catching significantly more males than any other treatment (Fig.

4.3). Traps subjected to red or white light caught significantly fewer males than those illuminated with green light (Fig. 4.3).

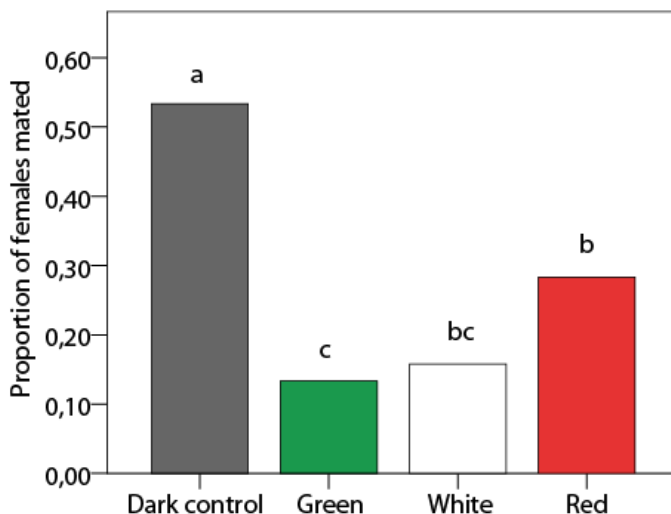


Figure 4.2 Proportion of mated females caught in the funnel traps under the different artificial light treatments (illuminated and shaded tree side combined). Different letters indicate significant differences between treatments ($\alpha=0.05$). $n=180$ (dark control), $n=15$ (green), $n=38$ (white), and $n=106$ (red).

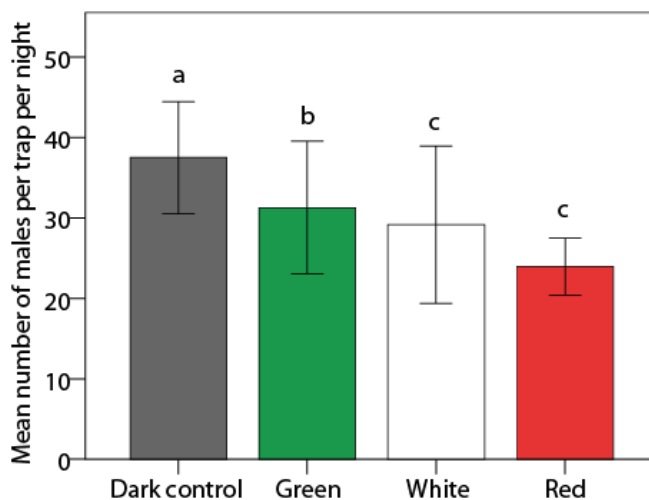


Figure 4.3 Mean number of males caught per night in the pheromone traps that were subjected to the different light treatments. Dots are means, bars represent 1 SEM, different letters indicate significant differences between the treatments ($\alpha=0.05$). $n=18$ nights for all treatments.

DISCUSSION

Large-scale, long-term studies have shown strong declines of moths over the past decades (Conrad *et al.* 2006; Groenendijk & Ellis 2011; Fox *et al.* 2014). Because many nocturnal moths are strongly attracted to sources of artificial light at night (Van Langevelde *et al.* 2011; Somers-Yeates *et al.* 2013; Merckx & Slade 2014), light pollution is often posited to be one of the causal factors of moth declines, although direct causal evidence for such a relationship is lacking to date (Fox 2013). In this study, we show that artificial night lighting strongly reduced 1) the number of female winter moths on tree trunks, 2) the proportion of mated females and 3) the number of males that were attracted to a synthetic female sex pheromone.

Compared to traps on dark control trees, traps on illuminated trees caught significantly less females, with the exception of traps on the shaded side of red-light trees. Because daily activity of *O. brumata* is initiated by the drop in light intensity at sunset (Alma 1970; Lorentzen 1974), and starts after light levels have dropped below a certain threshold (approximately 4 lux, see Lorentzen 1974), our artificial light treatments (~ 10 lux) likely caused suppression of *O. brumata* activity and thus the strong reductions in the number of females in the funnel traps on illuminated trunks. Because street light intensities are frequently much higher, up to 60 lux (Gaston *et al.* 2012), our findings may under-estimate the effect-size of artificial light in a street light situation. The number of females caught on the shaded side of the green and white illuminated trunks was low compared to that on shaded side of the red light and dark control trees. This indicates that the artificial light inhibition of activity extends over a spatial scale of at least several meters. However, the number of females caught on the shaded side of illuminated trunks was significantly higher than on the illuminated site, indicating that moths are less affected by artificial light on the shaded side. In red light, however, the funnel traps on the shaded side of the trees caught as many females as dark control traps, indicating that red light has a smaller effect size than white and green light. This is likely a result of the relative insensitivity of moth eyes to long wavelength radiation (i.e. red light) (Agee 1973).

Artificial light at night also largely inhibited mating: of the females caught in the dark control traps, 53% had mated, whereas this was significantly reduced to 13%, 16% and 28% in green, white and red light treatments, respectively. These results underline the

findings from earlier lab-based experiments on Noctuid moths: Sower *et al.* (1970) observed artificial light inhibition of calling behaviour (i.e. sex pheromone emitting behaviour). In addition, the male response to female sex pheromone might be inhibited by light at night, or the light might be a stronger attractant than to the conspecific sex pheromone (Shorey & Gaston 1964; Shorey & Gaston 1965). This is supported by our male response experiment, where we found the highest numbers of males in pheromone traps that were placed in dark control treatments, and significant reductions in the number of males in traps on illuminated trees. Of these traps, however, those subjected to green light caught more males than traps subjected to red and white light. Male moths are often attracted more towards green light (shorter wavelengths) than to red light (longer wavelengths) (Van Langevelde *et al.* 2011), which could lead to higher male density around the pheromone trap that was illuminated with green light.

Our experiments show that artificial light at night reduces female activity on the one hand, and reduces male responses to female sex pheromones. Hence, there is a negative synergistic effect of light at night with reductions in both female activity and in male responses to females. As such, this study is the first to demonstrate that artificial light at night negatively affects moth reproductive behaviour under natural conditions. Together with other negative effects of artificial light on moths, such as disturbance of life histories (Van Geffen *et al.* 2014) and increased predation risk (Rydell 1992), this inhibition of reproduction may at least partly contribute to the observed rapid moth declines in Western Europe (Conrad *et al.* 2006; Groenendijk & Ellis 2011; Fox *et al.* 2014) Furthermore, we show that a reduction in the amount of short wavelength only partly mitigates negative effects of artificial night lighting on reproduction in winter moths.

ACKNOWLEDGEMENTS

Marcel Visser commented an earlier version of this manuscript, for which we are grateful. This project is part of the ‘Light on Nature’ programme, which is funded by NWO-STW grant 11110, the Dutch Oil Company (NAM) and Philips Lighting.

SUPPLEMENTARY INFORMATION

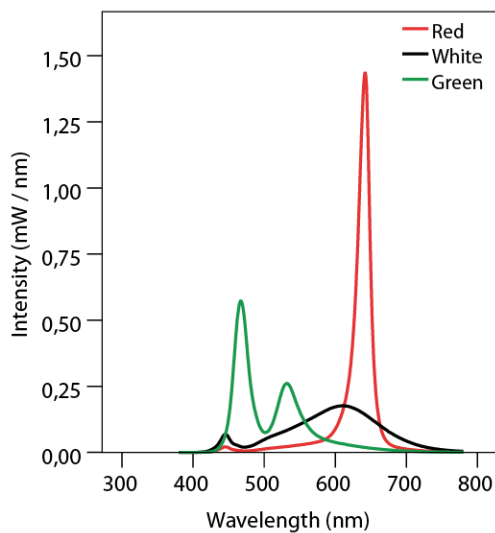


Figure S4.1 Visualisation of spectral compositions (intensities per wavelength) of the experimental lamps. Lines are averages of three lamps per type. Spectral measurements of lamps were performed at 25°C in a 2 meter diameter Ulbricht sphere equipped with a Cary Varian 17D digital spectrophotometer. Lamps were allowed to stabilize for 5 minutes 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.

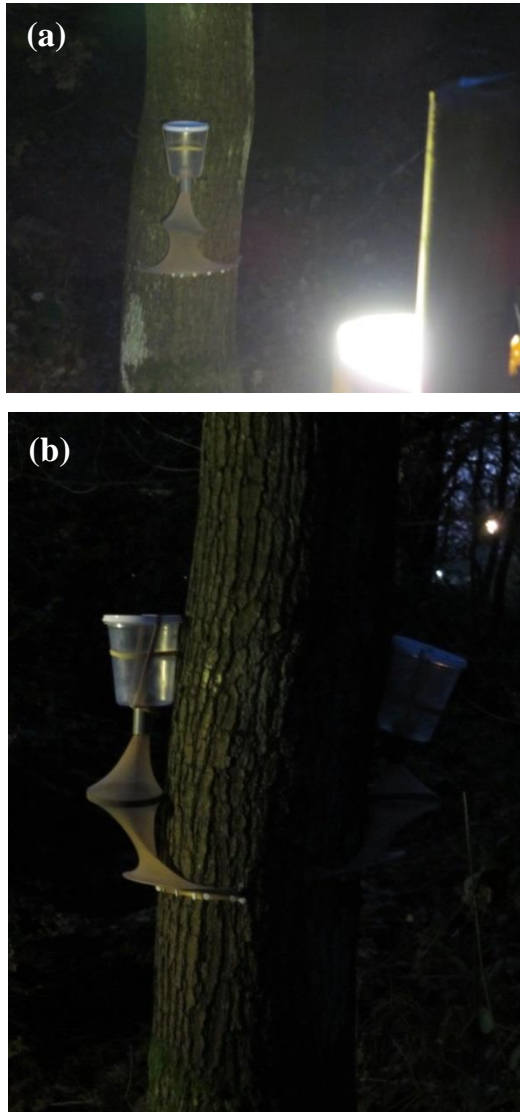


Figure S4.2 Picture of (a) front view of funnel trap on illuminated tree side with a white lamp, and (b) side view of funnel traps on illuminated and shaded tree side under white light.

CHAPTER 5

EFFECTS OF ARTIFICIAL NIGHT LIGHTING AND MOTH CATERPILLAR HERBIVORY ON PLANT BIOMASS AND SECONDARY METABOLITES

Koert G. van Geffen, Arjen Biere, Ciska Raaijmakers, Roy H.A. van Grunsven, Frank Berendse & Elmar
M. Veenendaal

ABSTRACT

Ecosystems in (sub-)urban areas around the world are increasingly subjected to artificial light at night, yet the consequences of light pollution for ecology of nocturnal species and the functioning of ecosystems remain poorly studied. Most studies of artificial light focus on effects on single species. Effects on (trophic) interactions between species remains however poorly studied, despite the fact that such interactions are important in shaping communities and ecosystems. Here, we tested how different types of artificial light at night (~5 lux), differing in spectral composition, affected biomass and leaf concentrations of secondary metabolites in *Plantago lanceolata* plants that were fed upon by *Mamestra brassicae* moth caterpillars under outdoor conditions. We show that in this model system artificial light at night, irrespective of spectral composition, did not affect the aboveground biomass, nor concentrations of the defensive secondary metabolites aucubin and catalpol in both herbivory-exposed and herbivore-free plants. Relative growth rates of caterpillars were also unaffected by light at night. As such, this study provides no evidence for direct or indirect (via herbivores) effect of artificial light on plants in outdoor conditions.

INTRODUCTION

Over the past decades, artificial night light pollution has dramatically altered night-scapes worldwide (Cinzano, Falchi & Elvidge 2001). Levels of light pollution continuously increase with approximately 6% per year (Hölker *et al.* 2010a), although trends differ strongly between regions (Bennie *et al.* 2014). Light pollution has potentially severe, yet largely unknown consequences for ecosystems (Longcore & Rich 2004; Rich & Longcore 2006; Hölker *et al.* 2010b). The ecological consequences of artificial light at night are being increasingly studied, but these studies have almost exclusively focussed on single species or species groups (e.g. Brüning, Hölker & Wolter 2011; Van Langevelde *et al.* 2011; Stone, Jones & Harris 2012; Titulaer *et al.* 2012; Van Geffen *et al.* 2014). Artificial light can potentially affect the interactions between species on different trophic levels. This remains poorly studied to date, despite the role of such interactions in shaping communities and ecosystems.

A very prominent trophic interaction that may affect ecosystem functioning is the interaction between plants and herbivores. One of the main defences of plants against

herbivory is the production of plant defence chemicals. Increased production of these chemicals may be induced by herbivory. For example, upon herbivory, the constitutive production of major groups of secondary metabolites, such as glucosinolates (Hopkins, Van Dam & Van Loon 2009; Textor & Gershenzon 2009), phenolics (e.g. Fields & Orians 2006) and alkaloids (e.g. Hol *et al.* 2004) are strongly up-regulated. Although the role of these induced secondary metabolites varies depending on the plant and herbivore species that is considered, in general, many of these compounds have feeding deterrent, digestibility reducing or toxic effects (Bowers & Puttick 1988; Li *et al.* 2000), reducing growth and / or reproduction of generalist herbivores (Awmack & Leather 2002). However the constitutive production (Marak, Biere & Van Damme 2003) or up-regulation of defences (e.g. Heil & Baldwin 2002) comes at a cost and plants must therefore optimize the amount of defence depending on their condition and their environment. and the plant must therefore optimize the amount of defence depending on their condition and their environment.

Prevailing light conditions can have a strong effect on the performance of plants. The flux of photosynthetic active radiation (PAR), the intensity of blue light and the red : far red (R : FR) ratio are types of light quantity and quality signals used by plants to sense their light environment and respond accordingly (Ballaré 2009; Ballaré 2014). Four different types of photoreceptors (phytochromes, cryptochromes, phototropins and photoreceptor FKF1) can be found in plant tissue, each sensitive to a certain part of the light spectrum and/or responsible for different physiological responses (Briggs 2006). Among the physiological responses that are influenced by the quantity (i.e. intensity) and quality (i.e. the spectral composition) of light in the environment, as sensed by these photoreceptors, is the production of plant defence chemicals (Roberts & Paul 2006). Shading by other plants for example causes a reduction in the R : FR ratio and the UV : PAR ratio (Roberts & Paul 2006; Ballaré 2009). Shade-intolerant plants respond to this by increasing the investment in growth at the cost of defence mechanisms (Ballaré 2009; Ballaré 2014). If a plant is exposed to artificial light, this may strongly reduce the reliability of light as a cue. Plants that are exposed to artificial light at night may receive higher amounts of PAR, blue light and a higher R : FR ratio compared to plants that are in darkness. Although most photoreceptors of plants are inactive at night and growth is reduced to a minimum (Casal 2013), phytochrome B (phyB) remains active. PhyB is a R : FR- and red-light

photoreceptor, and its activity is necessary for suppressing the induction of a shade response in unshaded plants in response to changes in the light environment at night (Casal 2013). Due to the phyB activity at night, variation in nocturnal light quality is sensed by plants, potentially causing physiological reactions. For example, exposure to light at night may, depending on the spectral composition of the light, substantially increase the R : FR ratio to which plants are exposed at night, which may lead to increased investment in chemical defences (Ballaré 2009). This may have implications for herbivores feeding upon these plants. Besides potential direct effects of artificial light on production of defence chemicals, artificial light may also have an indirect effect by affecting the feeding activity of herbivores on the plant (Roberts & Paul 2006; Shiojiri, Ozawa & Takabayashi 2006).

Here we study the effects of artificial light and herbivory by caterpillars of the Noctuid moth *Mamestra brassicae* on biomass and levels of secondary defensive metabolites of *Plantago lanceolata* plants. *Mamestra brassicae* (cabbage moth) is a common but declining (Ellis *et al.* 2013) generalist moth species in the Netherlands with two generations per year, occurring mainly in cultured areas (Steiner & Elbert 1998; Waring & Townsend 2003). *Plantago lanceolata* (ribwort plantain) is a generally perennial shade-intolerant herb with a worldwide distribution. In the Netherlands, it is very common in grasslands and road verges. The iridoid glycosides (IGs) aucubin and catalpol are important secondary metabolites of *P. lanceolata*. These IGs, and catalpol in particular, are induced by above- (Darrow & Bowers 1999; Fuchs & Bowers 2004) and belowground (Wurst *et al.* 2010; Bennett *et al.* 2013) herbivores. Aucubin and catalpol generally reduce feeding and delay development of generalist herbivores (Bowers & Puttick 1988; Reudler *et al.* 2011).

In this study we experimentally tested 1) how various types of low-level artificial light at night, differing in spectral composition, affects aboveground biomass and constitutive IG concentrations in *P. lanceolata*, 2) whether artificial light at night affects relative growth rates of *M. brassicae* caterpillars feeding upon *P. lanceolata* plants, and 3) how the combination of artificial light at night and caterpillar herbivory affects plant biomass and IG concentrations. We expected an interaction effect between artificial light treatment and caterpillar feeding on IG concentrations. This may either or both be caused by 1) the fact that many biotic and abiotic factors affect the ability of plants to induce secondary metabolites in response to herbivory, potentially including artificial light at night (see

above), or 2) caterpillars feeding may be reduced (either due to higher levels of constitutive IGs, or due to direct artificial light inhibition of caterpillar activity (Shiojiri, Ozawa & Takabayashi 2006; Shiojiri, McNeil & Takabayashi 2011)). To our knowledge, this is the first to study test how artificial light at night affects the interaction between plants and invertebrate herbivores.

MATERIALS AND METHODS

Plants, insects and microcosms

For this experiment, we grew *Plantago lanceolata* plants from seeds (Cruydhoeck, Nijebekoop, the Netherlands), which were sown in substrate (Special Care substrate, Horticoop, Bleiswijk, the Netherlands) in trays in a greenhouse. Seedlings were replanted in 18 cm high, 23 cm diameter pots 10 days after sowing, with one plant per pot. Pots were filled with a nutrient-rich field-soil (organic matter: 2.5%, available NH_4^+ 1.2 mg/kg, available NO_3^- 0.9 mg/kg, available PO_4^{3-} 0.9 mg/kg, pH: 5.5). Plants were further grown in the greenhouse in these pots for four weeks and watered when necessary.

In an experimental field near Wageningen University, the Netherlands (51°59'19"N, 5°39'28"E), we placed 48 aluminium cages measuring 1x1x1m, covered with 1.35mm² insect gauze 4m from each other. Cages were randomly assigned to one of our four artificial light treatments: green, white, red or no (dark control) artificial light at night (n=12 cages per light treatment). Cages were in three randomized blocks, each comprising 16 cages, four cages per light treatment. Lamps were custom-made by Philips Lighting (Eindhoven, the Netherlands). Spectral compositions of the lamps are provided in the supplementary information (Fig. S5.1). The R (641-710nm) : FR (711-780nm) ratios are 5.2, 5.9 and 58.4 for green, white and red light, respectively.

Four weeks after plants were planted in pots, we randomly placed four pots in each cage. From each set of four pots, we randomly selected two pots for caterpillar addition. For this, caterpillars of the Noctuid moth *Mamestra brassicae* (L.) were reared in a mass-breeding programme at the Wageningen University Laboratory of Entomology. Eggs were collected on 25 June 2014, caterpillars hatched 29-30 June. For details on the rearing programme, see Poelman et al. (2009). Caterpillars were reared on a diet of fresh Brussels sprouts (*Brassicae oleracea* var. *gemmifera*) leaves for 10 days. We randomly selected 96

third instar caterpillars for this experiment. In each pot that was assigned to a caterpillar treatment, we placed one caterpillar. On top of each pot (including the caterpillar-free pots), we placed a 16.5cm high frame covered with fine insect mesh (treat 200 μm , mesh 515 μm , 13.5 mesh / cm) to prevent caterpillars from escaping. Artificial night light intensities in the pots in the three light treatments were 5 ± 1 lux, whereas light levels in dark control pots were 70 ± 15 mlux. The experiments started 10 July, and plants were harvested on 24 July. Day-length in this time of the year is approximately 16 hours, nights are approximately 8 hours. One caterpillar died during the course of the experiment, data obtained from this caterpillar and the plant it fed upon were excluded from analyses.

Measurements

Prior to introduction, all caterpillars were weighed. Subsequently, twice per week, we searched for the caterpillars in all caterpillar-subjected pots and determined their body mass for relative growth rate calculations. Two weeks after introduction, caterpillars had approximately reached their maximum body mass and plants were harvested, dried at 50 °C for 72 h and weighed for aboveground biomass determination. For analyses of secondary metabolites, all leaves of each plant were first coarsely ground (1 mm) on a micro hammer mill (Culatti AG, Zurich, Switzerland), after which a random 1 gram subsample was ground in a Retsch MM200 ball mill (Retsch GmbH, Haan, Germany). Subsequently, a 25 ml subsample was extracted in methanol (Merck KGaA, Darmstadt, Germany) prior to HPLC analyses. The concentrations of aucubin and catalpol were analysed on a Dionex DX 500 with a GP40 gradient pump, a CarboPac PA1 guard (4 \times 50 mm) and analytical column (4 \times 250 mm), and an ED40 Electrochemical Detector for Pulsed Amperimetric Detection (PAD). NaOH (1M) and Milli-Q water were used as eluents (10:90%, 1 ml/min). Average retention times were 3.39 and 4.77 minutes for aucubin and catalpol, respectively. Concentrations were analysed using Peaknet Software Release 5.1 (DX-LAN module) (Marak, Biere & Van Damme 2002).

Statistical Analyses

Based on caterpillar body mass measurements, we calculated relative growth rates until maximum body mass of caterpillars following Van Geffen et al (2014). Because we replicated caterpillar and caterpillar-free treatments two times in each plot (i.e. two

replicates of each caterpillar treatment under each lamp), we used a Nested ANOVA where replicate (random factor) was nested in light treatment (fixed factor), and where block was a random factor. To test effects of artificial light and caterpillar treatments on aboveground plant biomass, % aucubin, % catalpol (ln-transformed to meet assumptions of normality and homogeneity of variances) and the ratio catalpol : total IGs, we performed nested ANOVA's where replicate (random factor) was nested in light treatment (fixed factor), and with caterpillar treatment as a fixed factor, block as a random factor and caterpillar * light as interaction factor.

RESULTS

Caterpillar relative growth rates varied between 0.07 and 0.31 g/day, but were unaffected by artificial light at night ($F_{3,3} = 2.674$, $P = 0.220$, Fig. 5.1). As expected, plants that were subjected to caterpillar herbivory reached lower biomass ($F_{1,177} = 41.974$, $P < 0.001$), but the effect of caterpillar feeding on plant biomass was unaffected by artificial light at night (caterpillar * light interaction: $F_{3,177} = 0.289$, $P = 0.834$, Fig. 5.2).

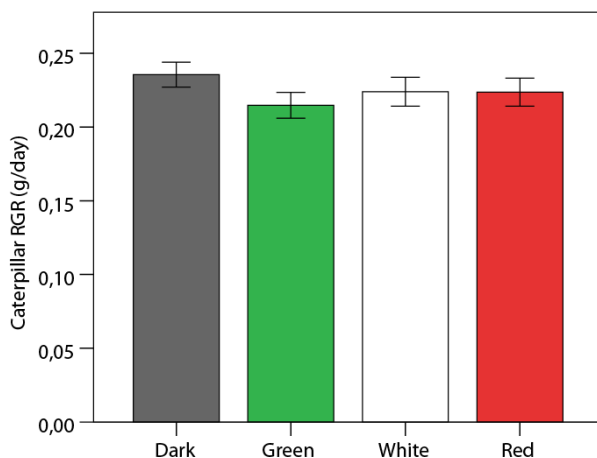


Figure 5.1 Caterpillar relative growth rates (RGR) under the different artificial light treatments. Error bars represent one standard error of the mean. Differences between treatments are not significant.

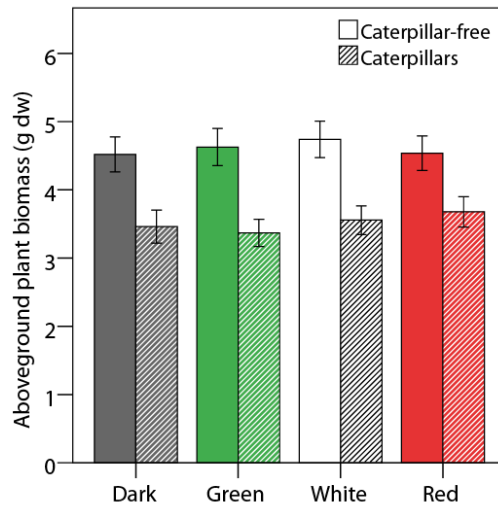


Figure 5.2 Aboveground biomass (gram dry weight) of *P. lanceolata* plants subjected to different artificial night light treatments and caterpillar treatments. Error bars represent one standard error of the mean.

Artificial light did not affect concentrations of aucubin in leaves ($F_{3, 2.997} = 0.414$, $P = 0.756$). Aucubin was not induced by caterpillar herbivory ($F_{1,177} = 1.349$, $P = 0.247$), and there was no interaction between caterpillar herbivory and light on aucubin concentrations ($F_{3,177} = 0.897$, $P = 0.117$, Fig. 5.3a). In contrast to aucubin, concentrations of catalpol were increased in caterpillar-exposed plants compared to caterpillar-free plants ($F_{3,177} = 22.090$, $P < 0.001$), but this caterpillar-induced increase in catalpol was equal across all light treatments (light*caterpillar $F_{3,177} = 0.305$, $P = 0.822$, Fig. 5.3b). The same holds for the ratio catalpol : total IGs: caterpillar herbivory led to significantly higher ratios ($F_{1,177} = 29.443$, $P < 0.001$), but this effect was similar across all light treatments (light*caterpillar $F_{3,117} = 0.777$, $P = 0.508$, Fig. 5.3c).

DISCUSSION

The results from this study provide no evidence for direct or indirect artificial light effects on plants and moth caterpillars feeding upon these plants. Recently, Van Geffen et al. (2014) showed that growth rates of *M. brassicae* caterpillars feeding on an artificial diet were unaffected by artificial light at night. In some moth species, the diel periodicity of caterpillar feeding activity is entrained by plant volatile emissions responding to light,

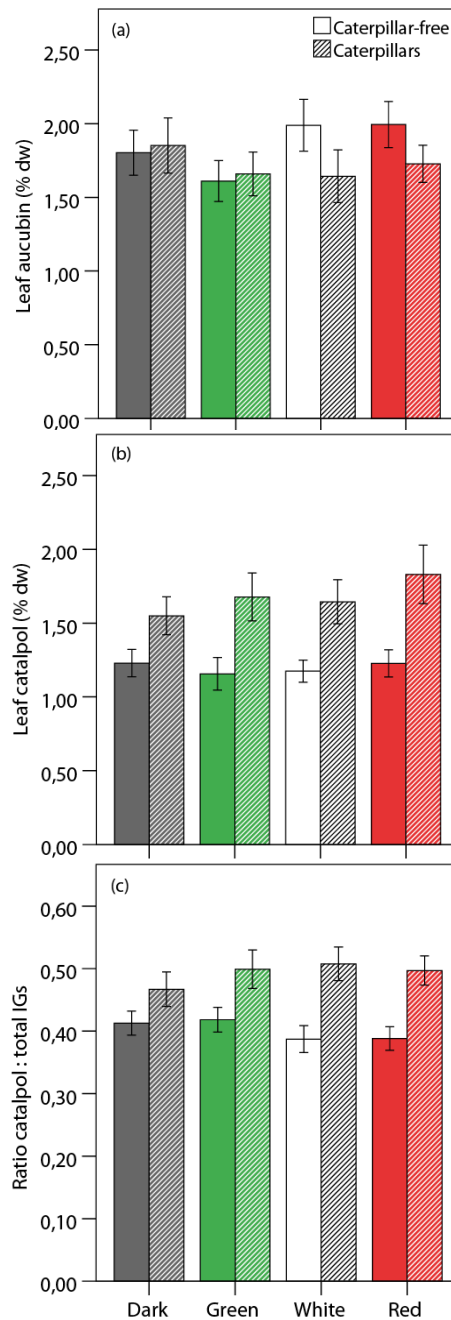


Figure 5.3 Aucubin (a) and catalpol (b) concentrations and the catalpol : total IGs ratio (c) in leaves of *P. lanceolata* under different artificial light and caterpillar treatments. Error bars represent one standard error of the mean.

instead of the light itself (Shiojiri, Ozawa & Takabayashi 2006; Shiojiri, McNeil & Takabayashi 2011). Although in the current study caterpillars fed on plants, in contrast to our earlier study (Van Geffen *et al.* 2014), we also found that relative growth rates of caterpillars that were feeding on plants in illuminated nights were similar to relative growth rates of caterpillars that fed on plants in darkness. Hence, there is a direct nor indirect effect of light at night on caterpillar performance.

Earlier studies on effects of artificial night lighting on plants in outdoor conditions are rare (Briggs 2006). There is scattered evidence that artificial light may affect flowering, phenology and vegetative growth of plants under natural conditions (Matzke 1936; Cathey & Campbell 1975a; Cathey & Campbell 1975b). However, here, we showed that in semi-natural conditions, growth of *P. lanceolata* was not affected by different artificial night lighting treatments during a two-week period. Although artificial light at night may allow for photosynthesis, the low light intensities used in our experiment (~5 lux) are negligible relative to sunlight intensities (varying between ~ 1.000 to over 100.000 lux), and as a result, the potential increase in biomass due to artificial light are probably negligible as well (Briggs 2006).

Although the intensity of artificial light at night is very low compared to daylight, the timing of the light is unnatural, since there is no other light at night apart from low levels of light from celestial objects (moon, stars), the quality of the light experienced by plants at night is (largely) dependent on the characteristics of the artificial light source. Constitutive IG levels were similar under all different light treatments, despite the fact that plant investment in chemical defence often depends on light properties such as the R : FR ratios (e.g. Izaguirre *et al.* 2006). Despite that phyB photochromes are active at night, and thus changes in R : FR ratios are sensed by plants, the differences in light conditions at night may have been completely overruled by high daylight intensities (Briggs 2006). Furthermore, upon herbivory, aucubin, one of the major secondary defence chemicals in *P. lanceolata*, was not induced by caterpillar herbivory in any of the light treatments in our experiment. In contrast, catalpol, often has more strongly negative effects than aucubin on both herbivores (Reudler *et al.* 2011) and their natural enemies (Nieminen *et al.* 2003; Reudler *et al.* 2011), was induced by caterpillar herbivory. This is in line with several other studies that show that mainly catalpol is induced in response to herbivory (Fuchs & Bowers 2004; Bennett *et al.* 2013). However, artificial light did not affect the induction of either of

the IGs (Fig. 5.3a,b), nor the qualitative shifts in IG composition (Fig. 5.3c) despite the suggested role of light quality and quantity in the synthesis of defence chemicals (Ballaré 2009; Ballaré 2014). Because light at night did not influence levels of constitutive and induced IG production, caterpillars under the different light regimes were not differentially affected indirectly via light-induced alterations of chemical defence.

Effects of artificial night lighting on inter-specific and inter-trophic interactions have remained poorly studied to date. Some examples of such interactions that are affected by light at night include the interaction between hunting bats and moths, where sources of artificial light at night causes clustering of prey and exploitation of these high prey densities by some bat species (Rydell 1992; Rydell 2006). This effect is further strengthened by artificial light inhibition of the tympanate bat-avoidance mechanism that allows moths to detect and avoid hunting bats (Svensson & Rydell 1998; Acharya & Fenton 1999). Also, artificial light at night can change the functional composition of invertebrate communities, with reported increases in relative abundance of predators under streetlights (Davies, Bennie & Gaston 2012). Such community changes may have important consequences for inter-trophic interactions in illuminated ecosystems. However, despite that both plants (Briggs 2006; Raven & Cockel 2006) and moths (Frank 1988; Frank 2006; Van Geffen *et al.* 2014; Van Geffen *et al.* 2015) can be directly affected by artificial light at night, our experiment does not provide evidence that the interaction between the two is affected by artificial night lighting with various spectral compositions.

ACKNOWLEDGEMENTS

We thank Jan van Walsum and Frans Möller for practical assistance and Unifarm for providing greenhouse space for growing plants. This study is part of the ‘Light On Nature’ project, which is funded by the NAM, Philips Lighting and NWO-STW grant 11110.

SUPPLEMENTARY INFORMATION

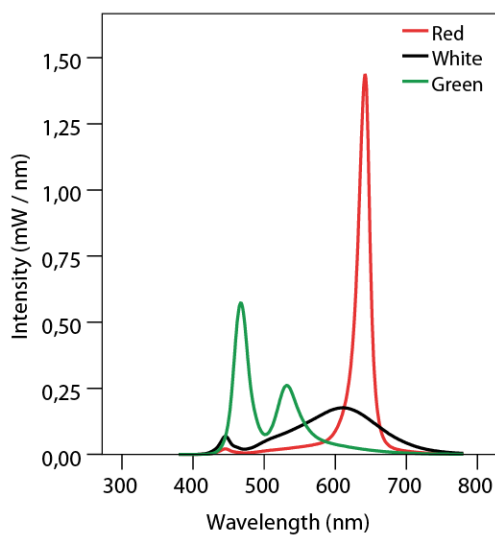


Figure S5.1 Visualisation of spectral compositions (intensities per wavelength) of the experimental lamps. Lines are averages of three lamps per type. Spectral measurements of lamps were performed at 25°C in a 2 meter diameter Ulbricht sphere equipped with a Cary Varian 17D digital spectrophotometer. Lamps were allowed to stabilize for 5 minutes 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.

CHAPTER 6

MOTHS IN ILLUMINATED NIGHTS

- SYNTHESIS -

The rapid loss of dark skies at night over the past decades has exposed nocturnal organisms to a major change of their habitat (Cinzano, Falchi & Elvidge 2001; Elvidge *et al.* 2001). Despite being a widespread and continuously growing phenomenon (Hölker *et al.* 2010a; Bennie *et al.* 2014), the ecological consequences of artificial light at night remain poorly studied (Longcore & Rich 2004). As a consequence, options for mitigation of potential negative effects, such as reductions in lighting intensities and alterations of spectral compositions are largely unexplored (Gaston *et al.* 2012). In this thesis, I studied the effects of three types of artificial light, differing in their spectral composition, on moths. Moths are known to be strongly attracted to sources of artificial light at night, and studies on artificial light effects on moths are strongly biased to this so-called ‘flight-to-light’ behaviour (Van Langevelde *et al.* 2011; Somers-Yeates *et al.* 2013; Merckx & Slade 2014; Van Grunsven *et al.* 2014b). In my thesis, I aimed to move beyond attraction, and study aspects of moth ecology that are important for population fitness. More specifically, I studied effects of artificial light on moth life history, various aspects of reproduction and interactions between a moth caterpillar and its host plant. Potential artificial light effects on these aspects of moth ecology can affect moth populations sizes, and insight in the responsible mechanisms is of utmost importance given the rapid population declines of many moth species in Western Europe (Conrad *et al.* 2004; Conrad *et al.* 2006; Groenendijk & Ellis 2011; Fox 2013; Fox *et al.* 2014).

In this synthesis chapter, I review the findings that I presented in the previous chapters in the light of present literature on artificial light effects on moths and explore the implications for moth populations. Also, I assess whether spectral alterations can be used to mitigate artificial light effects, and provide recommendations for future research.

From attracted to affected

The attraction of moths to sources of artificial light at night is a well-studied phenomenon. Moths are particularly strongly attracted to light rich in short wavelength radiation, and only slightly or not at all attracted to light with predominantly longer wavelengths (Van Langevelde *et al.* 2011; Somers-Yeates *et al.* 2013). In contrast, few studies have attempted to examine how artificial night lighting affects other aspects of moth ecology, such as development, reproduction and interactions with other trophic levels, which are all key aspects of population viability. In the studies I presented in this thesis, I aimed to fill this

knowledge-gap, and indeed demonstrated that effects of artificial light at night extend far beyond attraction. Light at night affects important aspects of moth behaviour and development in various ways in different life stages. The results from the studies in this theses indicate that the strong focus on attraction of moths does not give full insight in the potential consequences of artificial night lighting for moths, and that many important aspects other than attraction may be severely disrupted by artificial night lighting.

In the first chapter of this thesis, I studied for the first time the effects of various types of low level artificial night lighting on early life stages of moths. By subjecting caterpillars of the Noctuid moth *Mamestra brassicae* to three types of artificial light at night, and comparing several of their important life history traits with those of caterpillars that were reared in dark nights, I found differences in response to artificial light between males and females. Sex-dependent responses of moths to artificial light have previously been demonstrated in attraction to light; males are more strongly attracted to artificial light than females (Altermatt, Baumeier & Ebert 2009). In line with this, I found that artificial light at night affects the development and life-history of male moths more strongly than that of female moths. I showed that male caterpillars that were under white and green light pupated earlier and with a lower weight than males that were reared in dark nights. The outcome of this classical trade-off between development time and body mass (Nylin & Gotthard 1998) can have a strong impact on the fitness of individual moths in later life stages. Early pupation may reduce a caterpillars' susceptibility to parasitism, but the concomitant reduction of pupal (i.e. adult body) mass potentially reduces the flight capacity of males, and thus a males' capacity to spread its genetic material. Also, in some moth species, such as the Eribid moth *Utetheisa ornatix*, females are known to prefer heavy males for mating (Iyengar & Eisner 1999a). The development and life-history of females moths, in contrast, was little or not affected by the different artificial night lighting regimes.

Although female moths may be relatively insensitive to artificial light during their larval development, they are highly sensitive to light at night in the imago life stage. Previously, Sower et al. (1970) observed that female *Trichoplusia ni* (Noctuidae) moths that were exposed to light at night were 'calling' (i.e. emitting sex pheromone) significantly less than moths in darkness. However, observing calling behaviour does not give information on the quantity and quality of the sex pheromone that is emitted. Therefore, I tested how light affected the production of sex pheromone in *M. brassicae*. I demonstrated

that under controlled experimental conditions, the production of sex pheromone by female *M. brassicae* moths is strongly reduced by artificial light at night. This sex pheromone is produced *de novo* each night, and the production is regulated by the Pheromone Biosynthesis Activating Neuropeptide (PBAN) (Raina *et al.* 1989; Jacquin *et al.* 1994). The release of PBAN has been shown to be highly light-sensitive: PBAN production starts below a certain light level threshold (Jacquin *et al.* 1994; Tawata & Ichikawa 2001; Závodská *et al.* 2009). This light-regulation likely underlies the artificial light inhibition of sex pheromone production. In addition to the reduction in sex pheromone quantity, the chemical composition of the pheromone blend – which in case of *M. brassicae* comprises 9 components (e.g. Struble *et al.* 1980; Den Otter & Van der Haagen 1989; Arn, Tóth & Priesner 1992; Renou & Lucas 1994) – is subjected to important shifts. The relative amount of Z11-16:Ac, the pheromone component that is the major responsible for attraction of males (Renou & Lucas 1994), was reduced in the blend of females that were subjected to artificial light at night. In contrast, the relative amounts of some minor compounds (Z11-16:OH, Z9-16:Ac, Z11-17:Ac) that are known to reduce attraction of males (Renou & Lucas 1994), was increased in the blends of the light-subjected females. The change in pheromone quality, i.e. a shift in relative amounts of the different compounds, suggests a change in activity of one or more of the enzymes involved in the biosynthetic pathway (Morse & Meighen 1987; Knipple *et al.* 2002; Jurenka 2004b; Lassance *et al.* 2010; Vogel *et al.* 2010).

A valid question then was how these lab-based findings on the effect of artificial light on sex pheromone production would affect the reproduction of moths in field-conditions. To test this, I performed a series of experiments with the Geometrid moth *Operophtera brumata*. I subjected freshly emerged female moths to the four different artificial light at night treatments, and analysed whether or not these females were mated after one night. The results from this study match very well with that from the sex pheromone study on *M. brassicae*: more than half of the moths that were in the dark control treatment were mated, whereas under artificial light, and particularly under white and green light, mating incidence was reduced strongly, to only 13% under green light. Furthermore, I tested whether artificial light influenced male *O. brumata* attraction to synthetic sex pheromone lures, and found that sex pheromone lures in darkness attracted more males than lures that were illuminated at night. Laboratory studies have indeed shown that light interferes with

the male response to female sex pheromone. This may either be a result of direct competition between the light and calling (sex pheromone emitting) females (Shorey & Gaston 1965), or a result of the fact that artificial light physiologically inhibits reception of sex pheromone compounds and thus a behavioural response by males (Shorey & Gaston 1964). However, lures near green light attracted significantly more moths than lures near red light. This might be a result of the stronger attraction of males towards short wavelength light sources (Van Langevelde *et al.* 2011; Somers-Yeates *et al.* 2013). This may have led to stronger clustering of males around the green lamp than around the red lamp, and thus a larger sampling population surrounding green lamps.

Trophic interactions

While effects of artificial light on individual species or species groups are increasingly studied (Buchanan 1993; Kempnaers *et al.* 2010; Brüning, Hölker & Wolter 2011; Davies, Bennie & Gaston 2012; Stone, Jones & Harris 2012; Titulaer *et al.* 2012; Dominoni, Quetting & Partecke 2013), the consequences of artificial night lighting for inter-specific and inter-trophic interactions remain largely unknown, despite the fact that such interactions are important for shaping communities and ecosystems. Artificial night lighting has been shown to interrupt with moth-bat interactions. Not only do many bat species exploit high moth densities by hunting mainly around sources of artificial light at night (Rydell 1992; Rydell 2006), the tympanate ultrasound detection system in moths, evolved to avoid hunting bats, is paralysed by artificial night lighting (Svensson & Rydell 1998; Acharya & Fenton 1999), leading to higher predation risk under artificial light than in darkness. Other than this, nothing is known about artificial light effects on interactions between moths and either higher or lower trophic levels. Our study on the effects of artificial light on the interaction between plants and moth caterpillars demonstrated that direct effects on the performance of both herbivore and the plant are absent in our model species *M. brassicae* and *Plantago lanceolata*. Caterpillar relative growth rate was unaffected by artificial night lighting. Also, artificial light did not influence the aboveground biomass of both caterpillar-subjected and caterpillar-free plants, indicating that artificial light at night has no direct or indirect (via caterpillars) effect on plant biomass. In our experiment, the production of two important iridoid glycosides (defence chemicals), aucubin and catalpol, was unaffected by artificial light, both with and without

caterpillar feeding. This is surprising because the artificial light sources differed strongly in light properties such as red : far red ratio. This ratio is by Phytochrome B (PhyB) photoreceptors, which are also sensitive at night (Casal 2013). PhyB is known to affect production of secondary metabolites (Roberts & Paul 2006). This illustrates that, despite that light has potentially strong direct effects on both plants (Briggs 2006; Raven & Cockel 2006) and moths (e.g. chapter 2, 3 and 4 of this thesis), the interaction between the two is not affected by light at night. This surprising result demonstrates that the biology of individual species may be more strongly influenced by artificial night light lighting directly (chapter 2, 3 and 4), rather than indirectly via alterations of trophic interactions.

Extrapolation to other moth species

In most of the experiments described in my thesis (chapter 2, 3 and 5), the Noctuid moth *M. brassicae* was used as a model species, which raises the valid question to what extent the results apply for other moth species. Unfortunately, relatively little is known about individual species' responses to artificial light. The only current insight in inter-specific variation in moth responses to artificial night lighting comes from recent studies on attraction of moths to artificial light. These studies have shown significant differences in responses of moths to artificial light on a family-level. For example, both Van Grunsven et al. (2014b) and Merckx & Slade (2014) showed in mark-release-recapture experiments strong differences in attraction of moths from major moth families: Eribid moths are much more strongly attracted towards artificial light than Noctuid and Geometrid moths. These differences in attraction between families leads to higher light-subjection of Eribidae than (for example) Noctuidae, and possibly, developmental, physiological and behavioural responses of Eribidae are much stronger than those demonstrated in my experiments with a Noctuid moth.

I performed two experiments on the artificial light effect on moth reproduction processes. First, a lab-based experiment to test how artificial light affects the production of sex pheromone in female moths (chapter 3), and second, a field-based experiment to test how light at night affects mating occurrence (chapter 4). The former experiment was performed with *M. brassicae*, the latter with the Geometrid *Operophtera brumata* (winter moth). Despite the phylogenetic, behavioural, and ecological differences between these two species, the results of these two studies are very similar: artificial light at night has a strong

negative effect on various aspects of the reproduction process. This similarity in artificial light response between these two totally different moth species, strongly suggests that the possibility that the effects of artificial light that I demonstrated in *M. brassicae* may apply for many more (and even phylogenetically un-related) moth species. Clearly, testing effects of artificial light on other moth species is the only possibility for validation of this hypothesis.

Implications for moth populations

Altogether, negative effects of artificial light through attraction of moths (Van Langevelde *et al.* 2011; Somers-Yeates *et al.* 2013; Merckx & Slade 2014; Van Grunsven *et al.* 2014b), development, life-history (Chapter 2) and reproduction (Chapter 3 and 4) may add up, leading to a significant pressure on moth populations in illuminated nights. For example, the population size of the main study species in this thesis, *M. brassicae*, has decreased over the past 30 years (Fig. 6.1, Ellis *et al.* 2013; Vlinderstichting 2014). To date, we can only speculate about the consequences of increasing levels of nocturnal artificial illumination for moth populations (Frank 1988; Frank 2006), but in the near future, extensive monitoring studies such as the moth-monitoring in the Light on Nature project may provide further insight in the consequences of artificial night lighting differing in spectral composition for contrasting night-active moth groups (Spoelstra *et al.* in press).

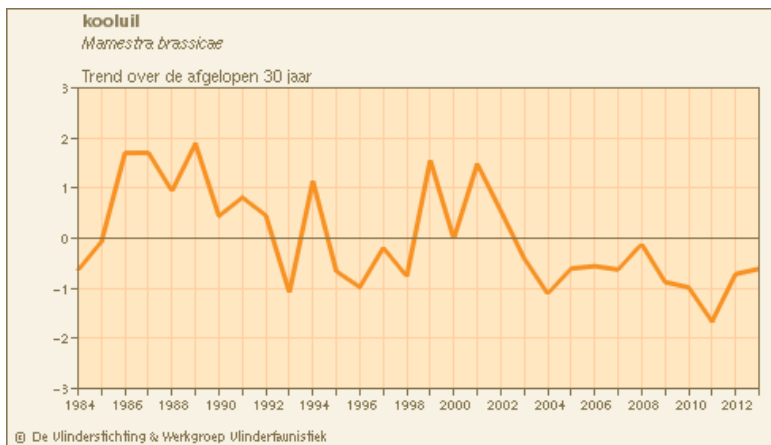


Figure 6.1 Population trend of the main study species in this thesis, *M. brassicae*, in the period 1984 – 2013. Obtained from www.vlindernet.nl (Vlinderstichting 2014), for information on trend-line calculation, see Groenendijk & Ellis (2011).

Mitigation potential of spectral alterations

Because moth eyes are highly sensitive to short wavelength radiation, and less sensitive to longer wavelengths (Agee 1973), application of light that is poor in short wavelengths has often been proposed as a tool for mitigation of potential negative effects of artificial light on moths (Frank 1988; Van Langevelde *et al.* 2011). Indeed, studies on moth attraction to artificial light show that long-wavelength lighting attracts less moths than short wavelength lights (Van Langevelde *et al.* 2011; Somers-Yeates *et al.* 2013). The studies in this thesis show the same trend: green and white light (both containing relatively much short wavelength radiation) have particularly strong negative effects, for example on vital aspects of moth ecology such as development and reproduction. In general, green and white light differ only slightly in their effects on moths. Possibly, the differences in the spectral composition are overruled by the relative large proportion of short wavelengths in the spectra of both lamp types. This is in line with Pawson & Bader (2014), who show that changes in colour temperatures (i.e. small changes in spectral compositions) of white LED lamps do not influence the number of insects that are attracted. Compared to green and white light, effects of red light are less pronounced. However, in contrast to what is often suggested, it does not fully mitigate negative effects. Under red light at night, in approximately 20% of the second generation *M. brassicae* caterpillars, pupal diapause was not induced (chapter 2 in this thesis). This would be fatal under natural conditions because adult moths would emerge from their pupae mid-winter. Under red light, the production of sex pheromone in female *M. brassicae* moths was significantly reduced, and contained lower relative amounts of the main attracting component Z11-16:Ac, and, relative to this main attractor, high amounts of attraction inhibiting component Z9-14:Ac (chapter 3 in this thesis). And female moths of the Geometrid *O. brumata* were mated significantly less under red light than in darkness. Also, traps that were loaded with this species' synthetic sex pheromone lure attracted less males when placed in vicinity of a red light, compared to darkness (chapter 4 in this thesis).

These results show that sensitivity of moths to light at night, almost irrespective of spectral composition, is present in more aspects of moth ecology and thus potentially higher than previously thought. This underlines the urgent need for conservation, and where possible, restoration of darkness at night.

Future research

Currently, research on moth responses to artificial light at night are dominated by experimental testing of attraction to light (Merckx & Slade 2014; Van Grunsven *et al.* 2014a; Van Grunsven *et al.* 2014b). With a new model on insect attraction to artificial light sources, we are now able to make very reliable predictions on attraction of different insect taxa to a light source with a known spectral composition (Donners *et al.* in prep). Field testing of insect attraction to light sources has therefore become a well investigated domain. We know how moths are attracted to light. The challenging question at hand is how this will affect their populations. The two-step approach that we followed in our Light On Nature project, i.e. monitoring moth populations in areas that were previously dark before they were experimentally illuminated on the one hand, and experimentally testing underlying mechanisms on the other hand, may provide a first step to unveiling the moth population size consequences of artificial night lighting.

Artificial light effects on moths are dependent on spectral composition of light. In the studies presented in this thesis, we demonstrated that effects of short-wavelength radiation on moth development, reproduction and interactions with other trophic levels are most pronounced, whereas in most cases, the effects of red light are milder, albeit not absent. Future research opportunities lie in testing alternative long-wavelength spectra which further reduce negative effects of red light at night, yet fulfil norms for application in public areas and for street lighting.

Final conclusion

The studies in this thesis show that artificial light at night affects many aspects of the ecology of moths. Combined with long-term monitoring of moth populations in experimentally illuminated, but previously dark field sites of the ‘Light on Nature’ project, this improves our insight in the contribution of artificial night light to the rapid declines in moth populations and the mechanisms underlying this potential relationship. By studying the effects of lamps with different spectral compositions, tools may be provided for reducing potential negative effects of artificial light. As such, this thesis has highlighted previously unknown mechanisms of artificial light effects on moths, this will contribute to conservation of moths in illuminated nights.

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SUMMARY

Daily variation in light levels is the most important and the most commonly used cue for onset of activity of virtually all organisms on the planet. Almost all terrestrial species on earth have evolved to be active in a certain part of the day, and as a result are adapted to certain light conditions. Diurnal species are active under high light intensities (the period known as the photophase, i.e. daytime), nocturnal species are active in low light intensities (the period known as the scotophase, i.e. night), and crepuscular species are in between, active in twilight (i.e. dusk and dawn). During the course of evolution, light intensity has been a very reliable cue for the on- and offset of activity of all these species, but recently, the night is no longer dark per definition. Mankind illuminates the night with artificial light sources, which has led to world-wide large-scale alterations of night-scapes. Levels of light pollution continuously rise, currently with approximately 6% per year on average. However, so far, the ecological consequences of this major global change phenomenon have remained largely unknown.

Moths are a highly diverse species group with ~120.000 species worldwide, most of which are nocturnal. Like other nocturnal organisms, moths are subjected to illumination of their previously dark habitat. Although moths are well-known to be strongly attracted to artificial light sources, particularly light sources rich in short-wavelength radiation, the effects of artificial light on other aspects of their ecology, including development, reproduction and interactions with other species, remain covered in darkness. Given the rapid declines in moth population sizes in Western Europe, insight in the effects of artificial light at night on moth ecology is urgently needed. Understanding the mechanisms behind such effects might help to develop tools for mitigation of potential negative impacts (for example by spectral alterations of artificial light). Therefore, in this thesis, I aimed to move beyond attraction and study effects of various types of artificial light, differing in spectral composition, on aspects of moth ecology that are important for maintaining and regulating population sizes: development and life history, reproduction, and inter-specific interactions.

First, I showed that artificial light at night during caterpillar development affects several life history characteristics of *Mamestra brassicae* moths. Male caterpillars developed faster, leading to earlier pupal molt and a lower pupal weight. These effects can

have important implications for their fitness in later life stages, for example because a lower pupal mass (which is directly linked to adult body mass) makes males less attractive to females and reduces their flight ability (and thus gene spread ability). Effects of artificial light on the timing of pupal molt and on the pupal mass in females were absent, probably because body mass in female moths is directly linked with egg production, and therefore buffered against changes induced by environmental conditions. The strongest effects of artificial light at night during the caterpillar stage were observed in the duration of the pupal stage. Moths of both sexes that were under artificial light as a caterpillar, particularly green and white light, had a much shorter pupal stage than moths from dark control treatments. The moths that were in dark controls, and a part of the moths that were under red light, underwent pupal diapause. This diapause was induced by the short daylengths in our experiments, which mimicked conditions to which second generation caterpillars are exposed under natural conditions. These second generation caterpillars develop in autumn under short days and overwinter as diapausing pupae. Caterpillars subjected to artificial light experience day lengths longer than they are in reality, so that caterpillars are not triggered to initiate pupal diapause. Under natural conditions, this inhibition of diapause would lead to high mortality because adult moths would emerge from pupae in winter.

An important theme in this thesis is the artificial light effect on mating and reproduction. Under natural conditions, reproduction starts with female moths that produce a sex pheromone, which often consist of a highly species-specific blend of chemicals, to attract con-specific males for mating. I tested the effects of various types of artificial light at night on sex pheromone production in female *Mamestra brassicae* moths. I showed that artificial light at night strongly affects the quantity and quality of the sex pheromone blend. Females under artificial light produced less sex pheromone than females in darkness. Moreover, the chemical composition, which is essential for species recognition, changes due to artificial light. The relative amount of the most important compound of the sex pheromone blend, Z11-16:Ac, was reduced under artificial light. On the other hand, the amount of several minor components that act as deterrents, Z9-14:Ac, Z9-16:Ac and Z11-16:OH, relative to the main attractive compound, increased under artificial light. Hence, females exposed to illumination at night produced less sex pheromone, with a much less attractive chemical composition, than females in dark nights.

The next step was to test whether this effect would also lead to reduced mating under field conditions. For this, I used a species that is phylogenetically unrelated and has a very different ecology: the winter moth *Operophtera brumata* (Geometridae). Adults of this species emerge in forests at the end of November and beginning of December. Females have reduced wings and are unable to fly. Directly after emergence from their pupae in the soil, these females climb tree trunks (particularly oak (*Quercus* spp.)) trunks, where they mate. Females climb further to deposit eggs near leaf buds of the tree. To test how artificial light affected the amount of mating in this species, we experimentally illuminated oak trunks, and trapped female *O. brumata* moths that were climbing these trunks. Females were counted and we checked their *bursa copulatrix* for presence of a spermatophore, which is a sperm bag delivered by a male during mating. Presence of a spermatophore is evidence of mating, absence means a female has not mated. First, we found a strong artificial light induced reduction of activity of females, with lowest number of females in traps on trees that were subjected to green light. Furthermore, the proportion of mated females was strongly reduced under artificial light. This effect is likely a combined effect of artificial light inhibition of sex pheromone production (see above), and artificial light reduction of the number of males attracted to the produced sex pheromone. We demonstrated this latter effect in *O. brumata* by subjecting pheromone traps, loaded with synthetic *O. brumata* sex pheromone, to different artificial light treatments. This showed that male moth attraction to female sex pheromone is reduced by artificial light.

In the final experiment, I tested how artificial light at night influenced the interaction between moth caterpillars and plants. I grew *Plantago lanceolata* plants from seeds and placed them outside under the four different artificial light regimes. Half of the plants were subjected to herbivory by caterpillars of *M. brassicae*, the other half of the plants were caterpillar-free. I monitored the growth of the caterpillars until they reached maximum body mass. After that, I harvested the plants for biomass analyses and measurements of secondary metabolites in the leaves. I showed that light at night did not affect the relative growth of caterpillars and the aboveground biomass production of plants. Moreover, both constitutive and induced levels of aucubin and catalpol, two important iridoid glycosides in *P. lanceolata*, were unaffected by artificial light at night, despite the suggested role of light quality and quantity in the production of these defence chemicals. These results show that the interaction between a moth caterpillar and a plant are not affected by low levels of

artificial night lighting in outdoor conditions. This indicates that direct effects of artificial light at night on the biology of individual species (such as development and reproduction of moths) are probably much more important than indirect effects on the interactions between different species from different trophic levels.

Together, the studies presented in this thesis demonstrate, for the first time, that effects of artificial light at night on moths extend beyond attraction. Vital components of the fitness of moths, such as development and reproduction, are negatively affected by light at night. However, almost all results also demonstrate that effects of red light at night are relatively weak, or even absent. This indicates that spectral alterations by means of reducing the amount of short wavelength radiation, may be a helpful tool in reducing negative effects of artificial light on moths. However, given for example significant reductions of sex pheromone production under red light compared to dark conditions, conservation of darkness remains the best option.

SAMENVATTING

De dagelijkse variatie in lichtniveaus is de belangrijkste en meest gebruikte *trigger* voor veel organismen op aarde om actief te worden of juist om te rusten. Vrijwel alle soorten op land zijn op zo'n manier geëvolueerd dat ze actief zijn in een bepaald deel van het etmaal, en als gevolg daarvan zijn aangepast aan de specifieke lichtniveaus die gebruikelijk zijn in dat deel. Dag-actieve soorten zijn vooral actief wanneer lichtniveaus relatief hoog zijn (gedurende de dag), nacht-actieve soorten zijn actief wanneer lichtniveaus juist laag zijn ('s nachts), terwijl er ook soorten zijn die daar precies tussenin actief zijn, dus in de schemering. Gedurende de evolutie is lichtintensiteit altijd een zeer betrouwbare maat geweest voor het aanbreken van dag en nacht, en dus voor het dagelijkse begin en einde van activiteit voor al deze soorten. Echter, een op evolutionaire tijdschalen zeer recente ontwikkeling is dat de nacht niet langer per definitie donker is. De mensheid verlicht de nacht met kunstmatige lichtbronnen, wat wereldwijd heeft geleid tot grootschalige veranderingen in het nachtelijke landschap. De niveaus van deze 'lichtvervuiling' nemen nog altijd toe met gemiddeld 6% per jaar. Echter, de ecologische gevolgen van lichtvervuiling zijn grotendeels onbekend en blijven dus gehuld in duister.

Nachtvlinders zijn een zeer soortenrijke groep insecten met ~120.000 verschillende soorten wereldwijd, waarvan de grote meerderheid nachtactief is. Net als andere nacht-actieve organismen, hebben nachtvlinders te maken met kunstmatige verlichting van hun voorheen donkere leefomgeving. Hoewel het bekend is dat nachtvlinders sterk door kunstlicht worden aangetrokken, en dan vooral lichtbronnen met veel straling in de korte golflengtes, blijven de effecten van nachtelijk kunstlicht op andere aspecten van de nachtvlinder-ecologie, zoals ontwikkeling, voortplanting en interacties met andere soorten, onbekend. Gelet op de snelle achteruitgang van nachtvlinderpopulaties in west Europa, die vaak deels wordt toegeschreven aan de toename in lichtvervuiling, is het erg belangrijk om goed inzicht te krijgen hoe nachtelijk kunstlicht de ecologie van nachtvlinders beïnvloedt, en wat de mogelijkheden zijn om eventuele negatieve effecten van licht te verminderen. Daarom was het doel van dit promotieonderzoek om verder te kijken dan alleen naar de aantrekking van nachtvlinders door kunstlicht. Dit heb ik gedaan door te testen wat de effecten zijn van verschillende soorten nachtelijk kunstlicht, met verschillende spectrale

samenstellingen, op die aspecten van de ecologie van nachtvlinders die bepalend zijn voor veranderingen van de populatieomvang: ontwikkeling, voortplanting en inter-specifieke interacties (interacties met andere soorten).

Allereerst heb ik aangetoond dat nachtelijk kunstlicht tijdens het rupsenstadium belangrijke 'life history' parameters beïnvloedt bij de nachtvlinder *Mamestra brassicae*. Mannetjes rupsen ontwikkelden zich sneller, wat leidde tot eerdere verpopping, maar ook tot een lager pop-gewicht. Deze effecten kunnen belangrijke gevolgen hebben voor de fitness in latere stadia. Bijvoorbeeld, mannetjes met een lager popgewicht (en dus later een lager lichaamsgewicht) zijn minder aantrekkelijk voor vrouwtjes dan zwaardere mannetjes, en hebben een kleinere vliegcapaciteit (en zijn dus minder goed in staat om genetisch materiaal te verspreiden). Deze effecten van kunstlicht op het moment van verpoppen en popgewicht waren echter afwezig in vrouwelijke rupsen. Dit komt waarschijnlijk doordat het popgewicht in grote mate bepaalt hoeveel eitjes een vrouwtjes nachtvlinder kan leggen. Een lager popgewicht heeft dus zeer sterke en directe gevolgen voor de fitness van vrouwtjes. Deze eigenschap is daarom dus beter beschermd tegen veranderingen in externe omstandigheden. Het sterkste effect van nachtelijk kunstlicht tijdens het rupsenstadium bleek te zitten in de duur van het popstadium. Zowel mannetjes als vrouwtjes die als rups blootgesteld waren aan nachtelijk kunstlicht, hadden een sterk verkorte duur van het popstadium. Nachtvlinders die als rups in de donkere controle of onder rood licht hadden gezeten, gingen in diapauze tijdens het pop-stadium. Deze diapauze werd getriggerd door de korte daglengtes in ons experiment. Hiermee werd de situatie nagebootst waaraan tweede generatie *M. brassicae* rupsen onder natuurlijke omstandigheden zijn blootgesteld. Deze tweede generatie rupsen ontwikkelen zich in de herfst onder korte daglengtes en overwinteren als pop in diapauze. Rupsen die aan kunstlicht werden blootgesteld, ervoeren juist een lange dag (zoals onder natuurlijke omstandigheden eerste generatie rupsen dat doen; deze ontwikkelen in de zomer in lange dagen) doordat ze ook 's nachts in het licht zaten. Hierdoor werden ze niet getriggerd om in diapauze te gaan. Onder natuurlijke omstandigheden leidt deze inhibitie van de diapauze ertoe dat volwassen nachtvlinders midden in de winter uit hun pop komen, met hoge mortaliteit tot gevolg.

Een belangrijk thema in dit proefschrift is het effect van kunstlicht op de voortplanting. Onder natuurlijke omstandigheden begint de voortplanting met de productie van een seksferomoon door vrouwtjes nachtvlinders. Dit seksferomoon is een zeer soort-

specifieke mix van chemicaliën waarmee vrouwtjes hun soortgelijke mannetjes aantrekken voor de paring. Ik heb de effecten van verschillende soorten nachtelijk kunstlicht op de seksferomoon productie van *M. brassicae* getest, waarbij duidelijk is geworden dat kunstlicht een sterk effect heeft op de hoeveelheid en chemische samenstelling van het seks feromoon. Vrouwtjes onder kunstlicht produceerden minder seksferomoon dan vrouwtjes in het donker. Bovendien beïnvloedde kunstlicht de chemische samenstelling van het feromoon, die van essentieel belang is voor mannetjes om hun vrouwelijke soortgenoten te herkennen. Zo was bijvoorbeeld de belangrijkste aantrekkende component, Z11-16:Ac, relatief weinig aanwezig in het feromoon van vrouwtjes onder kunstlicht. Daartegenover staat dat onder kunstlicht de hoeveelheid van enkele kleine componenten die afstotend werken, namelijk Z9-14:Ac, Z9-16:Ac en Z11-16:OH, toenamen in verhouding tot de belangrijkste aantrekkende stof. Vrouwtjes onder kunstlicht produceerden dus minder seks feromoon dan vrouwtjes in het donker, waarvan bovendien de samenstelling minder aantrekkelijk is.

De volgende stap was testen of dit effect in het veld daadwerkelijk zou leiden tot minder reproductie. Hiervoor gebruikte ik een phylogenetisch en ecologisch totaal andere soort: de wintermot *Operophtera brumata* (Geometridae). Imago's van deze soort komen eind november, begin december massaal uit hun pop in loofbossen. Vrouwtjes hebben gereduceerde vleugels en kunnen niet vliegen. Meteen na de ontpopping klimmen de vrouwtjes omhoog langs boomstammen (voornamelijk eik (*Quercus* spp.)), waar ze worden bevrucht door mannetjes die wel kunnen vliegen. Vrouwtjes klimmen door tot in de kroon van de boom, waar ze eitjes leggen dichtbij of op de bladknoppen. Om te testen hoe kunstlicht de reproductie van deze soort beïnvloed, heb ik verschillende eikenstammen verlicht, en omhoogklimmende vrouwtjes op deze stammen gevangen. Deze vrouwtjes werden geteld en in het lab werd voor ieder vrouwtje bepaald of ze gepaard had of niet, door in de *bursa copulatrix* te kijken of er een spermatofoor aanwezig was. Een spermatofoor is een spermazakje dat tijdens de paring door mannetjes wordt overgedragen aan het vrouwtje. Aanwezigheid van een spermatofoor is bewijs dat het vrouwtje gepaard heeft, afwezigheid is bewijs dat ze niet gepaard heeft. Uit dit experiment kwam allereerst dat kunstlicht de activiteit van vrouwtjes sterk verminderde. We vingen de minste vrouwtjes in valletjes op bomen die werden beschenen met groen licht. Daarnaast was het aandeel gepaarde vrouwtjes significant lager in vallen op de verlichtte bomen dan in vallen

op de donkere bomen. Dit effect is waarschijnlijk een gecombineerd resultaat van remming van de seksferomoon productie (zie hierboven), en vermindering van het aantal mannetjes dat op een seksferomoon producerend vrouwtjes af komt als gevolg van het kunstlicht. Dit laatste heb ik bij *O. brumata* aangetoond door feromoonvallen met een synthetisch seks feromoon in het bos te plaatsen nabij verschillende lampen, waaruit bleek dat vallen in het donker meer mannetjes vingen dan valletjes in het licht.

In het laatste experiment heb ik onderzocht hoe kunstlicht de interactie tussen nachtvlinderrupsen en planten beïnvloedt. Ik heb hiervoor *Plantago lanceolata* [smalle weegbree] planten opgekweekt en vervolgens buiten blootgesteld aan de vier verschillende lichtbehandelingen. De helft van de planten werden blootgesteld aan herbivorie door een *M. brassicae* rups, de andere helft van de planten bleven rups-vrij. Ik heb vervolgens de groei van de rupsen gemonitord tot ze hun maximale lichaamsgewicht hadden bereikt. Hierna heb ik de planten geoogst voor biomassabepalingen en voor het meten van secundaire metabolieten in het blad. Deze metingen toonden aan dat nachtelijk kunstlicht geen invloed had op de groei van de rupsen en op de bovengrondse biomassa productie van de planten. Bovendien bleken de hoeveelheden aucubine en catalpol, de twee belangrijkste chemische afweerstoffen in *P. lanceolata*, niet beïnvloed te worden door nachtelijk kunstlicht, ondanks het feit dat lichtkwantiteit en -kwaliteit hier wel degelijk een invloed op kunnen hebben. wordt beïnvloedt door nachtelijk kunstlicht onder min of meer natuurlijke omstandigheden. De directe effecten van kunstlicht op individuele soorten (zoals op de ontwikkeling en voortplanting van nachtvlinders) lijken dus veel belangrijker te zijn dan de effecten op de interacties tussen soorten.

Tezamen tonen de studies in dit proefschrift aan dat de effecten van kunstlicht op nachtvlinders veel verder gaan dan aantrekking alleen. Cruciale componenten van de fitness van nachtvlinders, zoals ontwikkeling en reproductie, worden negatief door kunstlicht beïnvloed. Bijna alle resultaten wijzen er echter op dat de effecten relatief klein of zelfs afwezig zijn onder rood licht. Dit betekent dat aanpassingen van de spectrale samenstelling van het licht, en dan met name het verminderen van de korte golflengte straling, een goed hulpmiddel kan zijn om negatieve effecten van kunstlicht op nachtvlinders te verminderen. Maar er zijn ook resultaten die aantonen dat zelfs deze aanpassing van het gebruikte lichtspectrum niet voldoende is. Het herstel en behoud van donkere nachten is daarom nog altijd de beste optie.

ACKNOWLEDGEMENTS

I would like to thank everybody that practically, mentally or accidentally, helped me finishing my PhD.

Special thanks to all those that supervised me and my project, and to all my colleagues. Very special thanks to Jan van Walsum, Frans Möller, Hennie Halm, Anne-Marie van den Driessche, Gerda Martin and Petra Kloppenburg for their understanding and gezelligheid.

HAJJE WAR!



SHORT BIOGRAPHY

Koenraad Gijsbertus (Koert) van Geffen werd op 3 december 1985 geboren in een schuur in Kerkwijk. Koert is een tuinderszoon en groeide buiten al spelend en werkend op, met als droom geitenboer of tuinder te worden. Na de basisschool begon hij aan de HAVO aan SG Cambium in Zaltbommel, waar hij in 2003 slaagde. Hij begon met een BSc Dier- en Veehouderij aan de HAS Den Bosch, waar hij echter zijn agrarische ambities uit het oog verloor. Na zijn diplomeren in 2007 studeerde Koert verder aan de Vrije Universiteit in Amsterdam, waar hij de MSc Ecology volgde. Hier raakte hij geïnteresseerd in systeemecologie, waar interacties tussen bodem, plant en lucht in een veranderende wereld centraal staan.

Na twee fundamentele onderzoeksstages – één naar de link tussen houteigenschappen en houtafbraaksnelheid van tropische bomen, en één naar de invloed van macro-detritivoren op afbraak van sub-arctisch bladmateriaal – werd het tijd voor wat meer toegepast onderzoek. Koert begon eind 2010 als AiO binnen het LichtOpNatuur project, met dit proefschrift als eindresultaat.

Na het afronden van zijn promotietraject heeft Koert de wetenschap vaarwel gezegd. Hij heeft een eigen maalderij opgezet in korenmolen Sara Catharina (anno 1846) in Kerkdriel waar hij eeuwenoud molenaarsambacht combineert met moderne RVS apparatuur om bijzonder meel te produceren voor de bakkerijsector (zie www.instormensterk.nl). Daarnaast verhuurt Koert zichzelf als freelancer in de agrarische sector, met name sierteelt tuinbouw.

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AFFILIATIONS OF CO-AUTHORS

FRANK BERENDSE

Wageningen University, Nature Conservation and Plant Ecology Group,
Droevendaalsesteeg 3a, P.O. box 47, 6700AA Wageningen, the Netherlands

ARJEN BIERE

Netherlands Institute of Ecology, Department of Terrestrial Ecology, Droevendaalsesteeg
10, P.O. Box 50, 6700AB Wageningen, the Netherlands

RENS A. DE BOER

Wageningen University, Nature Conservation and Plant Ecology Group,
Droevendaalsesteeg 3a, P.O. box 47, 6700AA Wageningen, the Netherlands

MAURICE DONNERS

Philips Research, High-Tech Campus 34, 5656AE Eindhoven, the Netherlands

EMIEL VAN ECK

Wageningen University, Nature Conservation and Plant Ecology Group,
Droevendaalsesteeg 3a, P.O. box 47, 6700AA Wageningen, the Netherlands

ASTRID T. GROOT

Institute for Biodiversity and Ecosystem Dynamics, University of Amsterdam, P.O.
Box 94248, 1090 CE Amsterdam, the Netherlands

Max Planck Institute for Chemical Ecology, Department of Entomology, Hans-Knöll-
Strasse 8, 07745 Jena, Germany

ROY H.A. VAN GRUNSVEN

Wageningen University, Nature Conservation and Plant Ecology Group,
Droevendaalsesteeg 3a, P.O. box 47, 6700AA Wageningen, the Netherlands
Netherlands Institute of Ecology, Department of Animal Ecology, Droevendaalsesteeg 10,
P.O. Box 50, 6700AB Wageningen, the Netherlands

CISKA RAAIJMAKERS

Netherlands Institute of Ecology, Department of Terrestrial Ecology, Droevendaalsesteeg
10, P.O. Box 50, 6700AB Wageningen, the Netherlands

JASPER VAN RUIJVEN

Wageningen University, Nature Conservation and Plant Ecology Group,
Droevendaalsesteeg 3a, P.O. box 47, 6700AA Wageningen, the Netherlands

LUCIA SALIS

Netherlands Institute of Ecology, Department of Animal Ecology, Droevendaalsesteeg 10,
P.O. Box 50, 6700AB Wageningen, the Netherlands
University of Groningen, Chronobiology Unit, Nijenborgh 7, 9747AG Groningen, the
Netherlands

ELMAR M. VEENENDAAL

Wageningen University, Nature Conservation and Plant Ecology Group,
Droevendaalsesteeg 3a, P.O. box 47, 6700AA Wageningen, the Netherlands

PE&RC Training and Education Statement

With the training and education activities listed below the PhD candidate has complied with the requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)



Review of literature (4.5 ECTS)

- Moths in illuminated nights (2014)

Post-graduate courses (5 ECTS)

- I GIS; PE&RC (2011)
- Life history theory; RSEE: Research School Ecology and Evolution (2013)

Laboratory training and working visits (4.5 ECTS)

- Moth sex pheromone production; Institute of Biodiversity and Ecosystem Dynamics, University of Amsterdam (2012-2013)
- Male moth responses to female sex pheromone in wind tunnel; Institute of Biodiversity and Ecosystem Dynamics, University of Amsterdam (2013)

Invited review of (unpublished) journal manuscript (2 ECTS)

- Plant and Soil: collembola contributions to leaf litter decomposition (2011)
- Journal of Plant Ecology: tropical wood decomposition (2012)
- Journal for Nature Conservation: reducing effects of illumination of cultural heritage sites on moths (2014)

Deficiency, refresh, brush-up courses (1.5 ECTS)

- Basic statistics (2011)

Competence strengthening / skills courses (1.2 ECTS)

- PhD Competence assessment (2011)
- Kamer van Koophandel: zo start u een eigen bedrijf (2014)
- Kamer van Koophandel: startersdag (2014)

PE&RC Annual meetings, seminars and the PE&RC weekend (2.1 ECTS)

- PE&RC Day: extreme life (2012)
- NAEM: Netherlands Annual Ecology Meeting; two day attendance each year (2011-2013)

Discussion groups/local seminars/other scientific meetings (4.7 ECTS)

- Discussion meetings STW (2011-2014)
- Discussion group: Ecology Theory and Application (2011-2013)

International symposia, workshops and conferences (6.1 ECTS)

- Future of butterflies; poster presentations (2012)
- Terry Callaghan tribute symposium; oral presentation ; Lund, Sweden (2012)
- Mini-symposium on artificial light effects on ecosystems; oral presentation; NIOO (2012)
- 1st Conference on Artificial Light at Night (ALAN); oral presentation; Berlin, Germany (2013)

Lecturing / supervision of practical's / tutorials (3 ECTS)

- Working colleges landschapsgeografie; (2011-2014)
- Fietspraktica ecologie (2012-2014)

Supervision of MSc students

- Margot Sauter: caterpillars under artificial light
- Dechen Lham: moth attraction to artificial light
- Matthijs Kolpa: moth matings under artificial lightThijs Fijen: moth feeding in illuminated nights
- Rick Wessels: moth-parasitoid interactions under artificial light

FUNDING

This research is supported by the NAM, Philips Lighting and the Dutch Technology Foundation STW, which is part of the Netherlands Organisation for Scientific Research (NWO) and partly funded by the Ministry of Economic Affairs (project number 11110), and co-funded by Philips Lighting and the NAM.