LED based trapping of whiteflies and fungus gnats:

From visual ecology to application

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Abstract

Yellow sticky card traps are used for monitoring and control of the greenhouse whitefly (*Trialeurodes vaporariorum*) and the black fungus gnat (*Bradysia difformis*) in greenhouses. The use of light emitting diodes (LEDs) has turned out as a promising approach to increase the efficiency and reliability of visual traps. Moreover, LEDs provide the possibility to study the visual behaviour and colour processing of insects. On the background of improving visual traps, the aim of this thesis was to investigate the colour choice behaviour of *T. vaporariorum* and *B. difformis*, thereby connecting basic experimental research with applicable aspects. Finally, an LED enhanced yellow sticky trap should be developed and evaluated.

In chapter 1 and 2, the visual behaviour of *T. vaporariorum* and *B. difformis* was studied with a number of LEDs from the ultraviolet (UV) and visible light range of the spectrum in combination with light scattering acrylic glass screens. Several choice assays with different LED colours and combinations were performed in a small-scale choice arena under greenhouse conditions. It was revealed, that *T. vaporariorum* possesses a yet undescribed photoreceptor sensitive for blue light and an inhibitory blue-green chromatic mechanism. This mechanism controls a 'wavelength-specific behaviour' used for host plant detection. Besides this chromatic processing, the behavioural response is distinctly intensity dependent. Based on subsequent modelling, photoreceptor peaks were estimated around 510 - 520 nm (green), 480 - 490 nm (blue) and 340 - 370 nm (UV). Consequently, *T. vaporariorum* possesses a trichromatic receptor setup. *B. difformis* shows two different, probably 'wavelength-specific', behaviours to UV radiation and green-yellow light, with UV being the most attractive stimulus. The two behaviours might be directly related to underlying photoreceptors, suggesting dichromatic vision in *B. difformis*. Moreover, the results show the superior attractiveness of especially UV LEDs compared to conventional yellow traps.

In chapter 3, LED enhanced yellow traps were constructed which combine yellow cards with specific edge lighting acrylic glass equipped with green high-power LEDs in a frame. Traps were equipped with cameras and an LED illumination system to generate transmitted and incident light images at dark night-time conditions which enabled the subsequent identification and counting of whiteflies and fungus gnats. The efficiency of these traps was compared with conventional yellow traps in small-scale tomato crop stands. The results show a significantly increased efficiency of the developed LED enhanced traps for whiteflies compared to yellow traps in experiments with high population densities and in choice situations with both trap types. A higher efficiency for fungus gnats was observed throughout. The obtained images allowed reliable counting of both pests, comparable with manual counting on traps.

Key words: LED trap, visual ecology, colour vision

Zusammenfassung

Gelbtafeln werden zur Überwachung der Gewächs-Weiße Fliege (*Trialeurodes vaporariorum*) und der Trauermückenart *Bradysia difformis* eingesetzt. Leuchtdioden (LEDs) haben sich als vielversprechende Möglichkeit zur Steigerung der Effizienz von visuellen Fallen erwiesen. Darüber hinaus bieten LEDs die Möglichkeit, das Sehverhalten und die Farbverarbeitung von Insekten zu untersuchen. Das Ziel dieser Arbeit war es, das Farbwahlverhalten von *T. vaporariorum* und *B. difformis* zu erforschen und experimentelle Grundlagenforschung mit anwendbaren Aspekten zu verbinden. Schlussendlich sollte eine LED-verstärkte Gelbtafel entwickelt und evaluiert werden

In Kapitel 1 und 2 wurde das visuelle Verhalten von *T. vaporariorum* und *B. difformis* mit einer Reihe von LEDs aus dem ultravioletten (UV) und sichtbaren Bereich des Spektrums in Kombination mit lichtstreuenden Acrylglasscheiben untersucht. In einer Wahlarena wurden mehrere Versuche mit unterschiedlichen LED-Farben und -Kombinationen durchgeführt. Es zeigte sich, dass *T. vaporariorum* einen noch unbeschriebenen, für blaues Licht empfindlichen Fotorezeptor aufweist, der in einer hemmenden Interaktion mit einem Grünrezeptor steht. Dieser Mechanismus steuert ein wellenlängenspezifisches Verhalten für die Erkennung von Wirtspflanzen. Diese chromatische Verarbeitung ist zusätzlich eindeutig intensitätsabhängig. Basierend auf einer anschließenden Modellierung wurden Fotorezeptorpeaks bei 510 - 520 nm (grün), 480 - 490 nm (blau) und 340 - 370 nm (UV) ermittelt. Folglich besitzt *T. vaporariorum* eine trichromatische Rezeptorkonfiguration. *B. difformis* zeigt zwei verschiedene, wahrscheinlich wellenlängenspezifische Verhaltensweisen gegenüber UV-Strahlung und grüngelbem Licht, wobei UV der attraktivere Reiz ist. Die beiden Verhaltensweisen könnten in direktem Zusammenhang mit zugrundeliegenden Fotorezeptoren stehen und lassen ein dichromatisches Sehvermögen vermuten.

In Kapitel 3 wurden LED-verstärkte Gelbtafeln konstruiert, bei denen Gelbtafeln mit speziellem Acrylglas kombiniert wurden, das die Kantenbeleuchtung mit in einem Rahmen montierten grünen Hochleistungs-LEDs in ermöglichte. Die Fallen wurden mit Kameras und einem LED-Beleuchtungssystem zur Erzeugung von Durchlicht- und Auflichtbildern bei Dunkelheit ausgestattet. Diese ermöglichten die anschließende Identifikation und Zählung von Weißen Fliegen und Trauermücken. Die Effizienz dieser Fallen wurde mit herkömmlichen Gelbtafeln in kleinen Tomatenbeständen verglichen. Die Ergebnisse zeigen eine deutlich gesteigerte Effizienz der LED-verstärkten Fallen für Weiße Fliegen in Experimenten mit hoher Populationsdichte und in Wahlsituationen mit Gelbtafeln. Eine höhere Attraktivität für Trauermücken wurde durchgehend beobachtet. Die gewonnenen Bilder ermöglichten eine zuverlässige Zählung beider Schädlinge, vergleichbar mit der manuellen Zählung auf Fallen.

Schlagwörter: LED Falle, visuelle Ökologie, Farbsehen

1 General Introduction

1.1 Background and general research approach

The visual trapping of pest insects for plant protection issues has always been of great interest in horticultural greenhouse production. Yellow sticky card traps are used worldwide as a standard tool in integrated pest management (IPM) for the monitoring of greenhouse pests such as whiteflies, aphids, fungus gnats and thrips (Pinto-Zevallos and Vänninen, 2013; Cloyd, 2010; Ohnesorge and Rapp, 1986). High densities of yellow traps or large yellow roller traps can also be used for mass trapping as a direct control measure (Lu et al., 2012; Sampson et al., 2018). Especially leaf-feeding insects are attracted to yellow traps while some flower-feeding insects also prefer other colours such as the western flower thrips which is commonly trapped with blue traps (Prokopy and Owens, 1983; Natwick et al., 2007). The basic observation, that many herbivorous insects such as whiteflies and aphids are attracted to yellow was already referred long time ago (Lloyd, 1921; Moericke, 1955; Moericke et al., 1966). The explanation of this phenomenon and in particular the underlying visual ecology regarding behavioural and physiological mechanisms are not fully understood for various insects, even today. Therefore, the development of visual trapping methods is not very diverse and mainly limited to yellow or blue coloured traps and more or less simple trap designs (Shimoda and Honda, 2013). However, the consequent implementation of IPM strategies and biological plant protection measures which are tailored for different pests in diverse cropping systems increasingly requires specific and efficient monitoring systems and control measures.

With the upcoming of the light emitting diode technology (LED), some improved approaches which combine coloured traps with LEDs have arisen (Chen et al., 2004; Stukenberg et al., 2015; Otieno et al., 2018). On the background of the progressing development of LEDs in terms of efficiency, specificity and last not least cost efficiency, and their ongoing implementation in horticultural lighting technology (Yeh and Chung, 2009), more efficient visual trapping devices as an alternative to the common sticky card traps can be developed in the future (Johansen et al., 2011). Moreover, it is of great interest to reduce the workload for monitoring and to improve its accuracy and the timing of plant protection measures. Therefore, the implementation of (semi-)automatic image acquisition and analysis methods for the assessment of yellow traps or even on-site detection of pests is under increasing development (Qiao et al., 2008; Xia et al., 2012).

Insect colour vision, visual behaviour and physiology is generally well researched for some model organisms like honeybees and butterflies (Chittka, 1996; Kelber, 2001), but the knowledge is still relatively limited for economically important pest insects. Most of the applied horticultural and agricultural studies with coloured traps focus on comparing trap efficiencies and are not able to explain the observed colour preferences or to support understanding of the

underlying visual ecology. Studies with coloured traps and LED equipped traps do not compare key factors like reflection, emission, light quality (wavelengths pattern) and intensity independent from each other (Johansen et al., 2011). In order to understand the colour preferences and to identify factors that could be further improved by light enhancement, it is crucial to gain more basic knowledge on the visual ecology and physiology of relevant herbivorous insects. If basic and applied research are combined, we have the possibilities to get a step ahead today.

1.2 LEDs and their potential for colour vision research and visual traps

LEDs are already used to study specific plant responses and alteration of light induced defence mechanisms against pest insects (Rechner et al., 2016; Acharya et al., 2016; Massa et al., 2008). Moreover, they are increasingly important for indoor plant cultivation and greenhouse lighting (Yeh and Chung, 2009). They provide the possibility to generate narrow-bandwidth radiation which can be dimmed and individually composed. They are available in the ultraviolet (UV) range and for the main colours in the visible range of the electromagnetic spectrum. LED light is generated by electroluminescence based on different semiconductor materials and mixtures which do not allow to adjust any desired wavelength at equal efficiency. Especially in the green-yellow range (550-560 nm), the so called “green gap”, no efficient LEDs are available up to date (Laubsch et al., 2010). Nevertheless, almost every colour is available which allows to specifically analyse the spectral sensitivity of the target insects in narrow wavelength ranges. They offer a convenient tool to create different spectral light qualities and intensities and one can investigate the insect’s behaviour towards it independently from surrounding light conditions. This makes them interesting in colour vision research because they allow to adjust precise and individual light setups for investigating various questions on behavioural responses (Tokushima et al., 2016). Their potential for visual trapping as compared to broadly reflecting coloured traps lies in greater attractiveness and flexible adaptation to certain conditions or species (Stukenberg et al., 2015; Otieno et al., 2018). Moreover, LED light devices take up little space, have long lifetime and low power consumption and they may enable pest monitoring in places where conventional light sources are impractical. Additionally, the operation with low voltage makes the technique quite safe for the application in greenhouses (Yeh and Chung, 2009).

1.3 Insect photoreceptor optics

Visual detection plays a key role for insects’ navigation, orientation and localization of host plants, prey and mates (Prokopy and Owens, 1983). The perception of light either directly from

the sun or reflected from objects is mediated by the photoreceptor cells in the retina of the compound eye and in dorsal ocelli (Warrant and Nilsson, 2006). Although the general structures of the compound eyes are similar, many species specific differences and variations exist. These differences concern the optical construction and the spectral sensitivity of the photoreceptor pigments. Especially the neural processing and response system of light stimuli differs between species (Briscoe and Chittka, 2001). In general the compound eyes consist of numerous ommatidia which represent independent optical units. The number of ommatidia determines the spatial resolution of the eye. Quite simple herbivorous insects of limited mobility like the greenhouse whitefly have only around 84 ommatidia per eye (Mellor et al., 1997), while the eye of a predatory and highly mobile dragonfly contains the maximum number of up to 3000 ommatidia (Cronin et al., 2014). Each ommatidium has its own dioptric apparatus with a biconvex corneal lens and a crystalline cone which directs the light to the underlying photoreceptor cells. An ommatidium contains six to nine elongated rotationally symmetrically arranged photoreceptor cells exhibiting a large surface area achieved by microvilli which form a light guiding rhabdomere. The membranes of the microvilli contain the visual pigments finally responsible for the uptake of photons (Warrant and Nilsson, 2006).

The dorsal ocelli are two or three very small organs whose basic building blocks are similar to the ommatidia but of much smaller size. They form a retina of one or more layers but are covered by only one thick biconvex lens. The ocelli do not provide spatial resolution, but are highly sensitive to UV and visible light and have a high signal transmission speed. As a separate channel their function is the detection of light to provide general information for navigation and orientation during the flight (Lazzari et al., 2011).

The basic structure of photoreceptor pigments is common to all animals and contains a UV-sensitive chromophore that is bound to an opsin protein which shifts the absorption range towards the longer wavelengths. Three different types of chromophores were found in invertebrates which are all derivatives of vitamin A. The most common chromophore is retinal, the aldehyde derivative of vitamin A1. Most insects use only one and rarely two chromophores (Briscoe and Chittka, 2001). Pigments based on the same chromophore have similar shaped spectral sensitivity curve which can be described by template formulas (Stavenga et al., 1993; Govardovskii et al., 2000). Differences in spectral sensitivities result from different amino acid sequences in the opsin protein. Different photoreceptor cells are arranged in one ommatidium, each containing usually only one type of visual pigment. Further modifications of the spectral sensitivity are achieved by filter and screening pigments, special arrangement of receptors or by coloured corneal lenses or crystalline cones. The presence of different photoreceptor pigments with different absorption spectrums is the prerequisite for the ability to discriminate colours. During the perception of light, photons cause a change in the conformation of the opsin protein and trigger the visual signal transduction cascade. The signal is then transmitted

via the axons of the photoreceptor cells to the higher-order neurons where it undergoes further processing (Cronin et al., 2014; Warrant and Nilsson, 2006).

1.4 Visual processing and behaviour

The presence of different photoreceptors provide only the basis for the perception of colours. According to the principle of univariance, a single receptor is colour blind because it acts like a photon counter and cannot distinguish between different wavelengths. The sensitivity curve of the photoreceptor determines only the ability to count photons and a bright light far away from the peak sensitivity can cause the same signal like a dim light at the peak sensitivity (Naka and Rushton, 1966; Skorupski and Chittka, 2011; Döring and Chittka, 2007). At a simple level, a colour blind behaviour results from the stimulation of one receptor and the corresponding channel is referred to as achromatic. If more receptors are directly involved to discriminate colour stimuli, the behaviour is not colour blind any more, even when receptors do not interact. Furthermore, receptor signals can interact on a subsequent neural stage which facilitates the extraction of chromatic signals. Inhibitory interactions of visual neurons enable the comparison of receptor outputs because they rely on ratios or differences which facilitates the extraction of constant chromatic signals independent from intensity. They also result in a shift of the behavioural action spectrum as compared to the underlying photoreceptor sensitivity and a more specific and narrow separation of relevant wavelength ranges. Achromatic mechanisms rely only on the summation of receptor signals (Kelber et al., 2003; Skorupski and Chittka, 2011).

Based on these mechanisms, different levels of the complexity of colour vision exist in insects, representing an evolutionary continuum. The simplest form is colour guided photokinesis and phototaxis which lacks any spatial vision. The second level are innate colour sensitive responses often referred to as ‘wavelength-specific behaviours’ which involve spatial resolution and are used for object detection. They cannot be modified by learning or experience and are common behaviours in particular for rather simple herbivorous insects. The third level involves colour learning and cognition and adds flexibility to the object detection. It is used by more complex insects such as flower visitors and enables to relate colours to certain food rewards or olfactory cues which are relevant only in specific environments or times (Kelber and Osorio, 2010).

If the spectral sensitivity of a photoreceptor is known, either from physiological investigations or approximated by template formulas, the photon catch from a stimulus light of known spectral distribution can be calculated. For reflecting objects, the wavelength of the stimulus is a result of the illumination and the reflection spectrum (Kelber et al., 2003; Döring, 2014). Based on knowledge from visual neuroscience, photon catches can be calculated to photoreceptor

excitations by rather simple non-linear functions (Naka and Rushton, 1966). In combination with behavioural data from colour choice experiments, the photoreceptor excitations can be used for more or less complex modelling of potential chromatic interactions of photoreceptors with regard to certain behaviours (Chittka, 1996; Kelber, 2001; Döring et al., 2009).

1.5 Target insects and knowledge gaps

This work deals with two important greenhouse pests, the greenhouse whitefly (*Trialeurodes vaporariorum*) and the black fungus gnat (*Bradysia difformis*), with a clear focus on the former.

T. vaporariorum is a worldwide distributed polyphagous phloem sucking pest which causes severe damage due to the withdrawal of assimilates, honeydew excretion and virus transmission. While the larval stages are sessile, the adults are mobile and the orientation is guided visually to a large extend (Byrne, 1991). Whiteflies are commonly monitored with yellow sticky traps in greenhouses worldwide and it is known that LEDs can improve the attractiveness of such traps considerably (Pinto-Zevallos and Vänninen, 2013; Chen et al., 2004; Stukenberg et al., 2015). Nevertheless, a detailed screening of LED wavelengths and intensities regarding an optimal fitting with the visual system is not available. Basic research on the visual system was neglected for a long time, thus existing behavioural and physiological studies to which one could refer are relatively old. It is known that green-yellow light stimulates settling behaviour while ultraviolet (UV) radiation is in involved in migratory behaviour, both as a result of wavelength-specific behaviours (Coombe, 1981; 1982). Physiological measurements of the photoreceptors' spectral efficiency showed peaks in the green and the UV range and the visual system is said to be dichromatic (Mellor et al., 1997). Some studies give indications that *T. vaporariorum* exhibits a blue-green opponent mechanism and possesses a trichromatic receptor setup similar to aphids (Stukenberg et al., 2015; Kirchner et al., 2005), but a clear proof and detailed characterisation is still missing.

B. difformis represents a common fungus gnat species in Europe. While the adults do not cause any damage and live only a few days, their mobility within the crop is responsible for the distribution of eggs in the substrate. The larvae which are hatching in the soil, finally can cause severe direct and indirect damages to the roots of different horticultural plants, especially in ornamentals (Cloyd, 2015). Furthermore, they are severe pests in edible mushroom cultivation (Cloyd, 2010; Shin et al., 2012). Monitoring as well as direct control is commonly performed with yellow traps and promising studies with LED and light enhanced traps have been conducted as well (Cloyd et al., 2007; Chen et al., 2004; Sonoda et al., 2014). Nevertheless, a detailed screening for attractive LED wavelengths and intensities is still missing and their visual behaviour and physiology is largely unknown.

1.6 Objectives

The overall objective of this thesis in order to improve visual traps for whiteflies and fungus gnats is to screen for attractive LEDs regarding colour (wavelengths) and intensity. Which LED colour (wavelength) is needed at which intensity to achieve a sufficient improvement of the trap efficiency as compared to conventional yellow sticky card traps?

The objective of the first chapter is a detailed investigation of the colour choice behaviour and visual processing of the greenhouse whitefly, *Trialeurodes vaporariorum*, on the basis of LED choice experiments and subsequent modelling.

The objective of the second chapter is to explore for the first time the visual behaviour of the black fungus gnat *Bradysia difformis* on the basis of LED choice experiments.

The third chapter aims at the development of an LED enhanced yellow trap which is equipped with cameras and an automatic image acquisition system for the identification of whiteflies and fungus gnats. The efficiency of these traps should be compared with conventional yellow traps in small-scale tomato crop stands.

2 Blue-green opponency and trichromatic vision in the greenhouse whitefly (*Trialeurodes vaporariorum*)

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2.1 Abstract

Visual orientation in the greenhouse whitefly (*Trialeurodes vaporariorum* Westwood, Hemiptera: Aleyrodidae) is the result of 'wavelength-specific behaviours'. Green-yellow elicits 'settling behaviour' while ultraviolet (UV) radiation initiates 'migratory behaviour'. Electroretinograms of the photoreceptors' spectral efficiency showed peaks in the green and the UV range and whitefly vision was said to be dichromatic.

In order to study the visual behaviour of *T. vaporariorum*, nineteen narrow-bandwidth LEDs covering the UV-A and visible range were used in combination with light scattering acrylic glass screens in a small-scale choice arena under greenhouse conditions. Multiple-choice and dual-choice assays were performed, resulting in LED-based behavioural action spectra of settling (green) and migratory behaviour (UV). A potential inhibitory blue-green chromatic mechanism was studied by combining yellow with different blueish LEDs. Intensity dependencies were illustrated by changing LED intensities.

Regarding the 'settling response', highest attraction was achieved by a green LED with a centroid wavelength of 550 nm, while a blue LED with 469 nm proved to be most inhibitory. Behaviour was distinctly intensity dependent. 'Migratory behaviour' was elicited the most by the UV LED with the shortest available wavelength of 373 nm. The results clearly prove the presence of a green and a yet undescribed blue sensitive photoreceptor and a blue-green opponent mechanism. Furthermore, empirical colour choice models were built and receptor peaks were estimated around 510 - 520 nm (green), 480 - 490 nm (blue) and 340 - 370 nm (UV). Consequently, *Trialeurodes vaporariorum* possesses a trichromatic receptor setup.

Key words: wavelength-specific behaviour, visual behaviour, opponent chromatic mechanism, colour vision, colour choice model, LEDs

2.2 Introduction

Visual orientation is crucial for initial host plant detection and migration in the greenhouse whitefly (*Trialeurodes vaporariorum* Westwood, Hemiptera: Aleyrodidae), a worldwide occurring horticultural pest in greenhouses (Byrne, 1991). Two different behavioural patterns, so called 'wavelength-specific behaviours', were identified in *T. vaporariorum*. Orientation to host plants is guided by a 'settling' behaviour which is elicited by green-yellow light while ultraviolet (UV) radiation is responsible for a pattern which can be broadly defined as 'migratory behaviour' (Coombe, 1981; 1982).

Those 'wavelength-specific behaviours' are generally defined as innate colour-sensitive behavioural responses to different wavelength bands which cannot be modified by experience or learning. On a basic level they enable insects to find and discriminate targets by their specific patterns of reflected light (Kelber and Osorio, 2010). In herbivorous insects the green-yellow range is commonly used for host plant detection (Prokopy and Owens, 1983). UV radiation is generally known to be involved in spatial orientation, flight activity, and dispersal in a variety of insects (Briscoe and Chittka, 2001).

The physiological basis for the visual perception of light are the photoreceptor cells in the insects' compound eyes containing the visual pigments. The absorption spectrum of visual pigments can be expressed by its sensitivity function which can be described using template formulas (Govardovskii et al., 2000; Kelber et al., 2003). According to the principle of univariance, a single photoreceptor is colour-blind because wavelength and intensity-dependent stimulation are confounded. The receptor screens a certain wavelength range but the same signal can be elicited by low intensity light at the sensitivity peak wavelength or by high intensity light further away from peak sensitivity (Skorupski and Chittka, 2011; Naka and Rushton, 1966).

'Wavelength-specific behaviour' can be based on the output of a single photoreceptor and achromatic, i.e. brightness-related, processing. Furthermore, it can be the result of colour opponency which is a chromatic mechanism in which the outputs of several photoreceptors are compared by antagonistic neuronal processing. Colour opponency is a prerequisite of colour vision defined as the ability to detect spectral variations in the light independent of their intensity (Skorupski and Chittka, 2011; Kemp et al., 2015; Kelber and Osorio, 2010; Kelber et al., 2003).

Many studies indicate that for herbivorous insects such as aphids, the 'settling' behaviour is controlled by such an inhibitory interaction of two overlapping photoreceptors sensitive for blue and green light. In this so called 'opponent mechanism' or 'blue-green opponency' the signal from the blue receptor inhibits the signal from the green receptor eliciting 'settling' (Döring and Chittka, 2007; Döring, 2014; Döring and Röhrig, 2016; Döring et al., 2009). This mechanism

facilitates to extract a constant chromatic signal that detects reflected long-wavelength light (green-yellow) associated with host plants and discriminates it from short- or broad-wavelength light independent from illumination intensity. It also results in a shift of the behavioural action spectrum to the longer wavelength range as compared to the underlying photoreceptor sensitivity and a more specific and narrow tuning in to the relevant green wavelength range. An apparent shortcoming of this dichromatic mechanism is the common preference of many herbivorous insects for yellow instead of green which can be explained by higher reflection in the relevant green range resulting in higher relative input to the green receptor. Therefore, this simple chromatic mechanism, which should be independent of light intensity, is influenced by brightness in terms of changing blue and green photoreceptor excitation ratios. Thereby, it may be that the whole mechanism lies on a mixed achromatic and chromatic axis (Döring and Chittka, 2007; Kelber and Osorio, 2010; Skorupski and Chittka, 2011).

Similar to aphids and other herbivorous insects, *Trialeurodes vaporariorum* shows a clear preference for yellow-reflecting objects. At an early stage, Moericke et al. (1966) identified a 'fall reflex' consistently elicited above yellow surfaces independent of the intensity of the reflected colour and suggested some form of 'wavelength-specific behaviour' or colour vision. This preference for yellow was later confirmed in behavioural studies with coloured surfaces, and bright yellow with little to no reflectance in the violet-blue spectrum was identified as being most attractive compared to darker or less saturated yellow. Violet-blue proved to be not attractive and it even inhibits the attraction towards yellow. Moreover, it was shown that highly reflected intensities in the green-yellow range contribute positively to their attractiveness (Vaishampayan et al., 1975; Affeldt et al., 1983; Webb et al., 1985). All these results with coloured surfaces have contributed to the development and use of yellow sticky traps for monitoring and control of whiteflies in horticultural greenhouse crops (Böckmann et al., 2015; Gillespie and Quiring, 1987).

In a behavioural study with monochromatic light of controlled intensities MacDowall (1972) determined the spectral efficiency function for a wavelength pattern from blue to red. The revealed action spectrum peaked at 550 nm and corresponded with the reflection spectrum of a tobacco leaf. Coombe (1981) extensively investigated the visual behaviour using monochromatic light in a 'settling' paradigm and a 'phototactic' paradigm. An action spectrum for the 'settling response' was generated based on spectral sensitivity which peaked at 550 nm and had a second peak in the UV range at 350 nm. Based on intensity response functions and different methods for the determination of 'settling' it was concluded that *T. vaporariorum* exhibits 'wavelength-specific behaviour'. In the phototactic paradigm it could be shown that two different antagonistic behavioural patterns are elicited by 400 nm (UV) and 550 nm (green) which do not interact with each other. In a follow-up study (Coombe, 1982), it was further revealed that UV elicits a variety of responses associated with migratory behaviour, such as

take-off behaviour and maintenance of flight. For example, increased walking activity and take-off rates were observed under 400 nm light and UV was preferred over green light but only during flight activity. In accordance with that, it is reported from many applied studies that whiteflies show less flight activity in UV-deficient environments leading to a general avoidance of such conditions (Gulidov and Poehling, 2013; Kumar and Poehling, 2006; Antignus et al., 2001).

For aphids, clear physiological evidence of a trichromatic receptor setup involving UV-sensitive photoreceptors exists (Kirchner et al., 2005). In contrast, trichromacy has not been confirmed in *Trialeurodes vaporariorum*. Mellor et al. (1997) investigated the physiological properties of the compound eye of *T. vaporariorum* and determined its spectral efficiency using the electroretinogram (ERG) technique. Efficiency peaks were identified in the green-yellow region (520 nm) and in the UV region (340 nm). Furthermore, the eye is divided in a dorsal part with 54-55 ommatidia and a ventral part containing 29-31 ommatidia. The dorsal region was thereby more sensitive to UV. Based on these results the visual system was concluded to be dichromatic.

New insights could be achieved by Stukenberg et al. (2015) using choice experiments with narrow bandwidth light emitting diodes (LEDs). Green LED traps were preferred over yellow sticky traps but this attraction was supressed when simultaneously combined with blue LEDs. This is the first clear indication that a yet undetected blue photoreceptor close to a green receptor and an inhibitory chromatic interaction between both might be present in the greenhouse whitefly. A moderate attractiveness towards UV could also be shown and it seemed to have an enhancing or synergistic effect on the attractiveness of green light as the combination of UV and green LEDs was more attractive than green alone, especially under night-time conditions. In a recent study, yellow rollertraps with reduced translucency were more attractive than those with common translucency. The authors determined the spectral properties of the traps and explained the results on the basis of the potential blue-green opponency. The brighter reflection in the green-yellow range and the low transmission of blue light had a greater influence on the opponent mechanism, resulting in higher attraction (Sampson et al., 2018).

Considering the referred studies it is quite likely that *T. vaporariorum* exhibits blue-green opponency and possesses a trichromatic receptor setup. Nevertheless, a clear proof and a detailed characterisation of the mechanism which connects behavioural data with potential photoreceptor sensitivities is still missing. LEDs are a very useful tool to study insects' visual behaviour since wavelengths and intensities can be individually adjusted and combined (Tokushima et al., 2016; Booth et al., 2004). In this study, we explored the visual behaviour and wavelength discrimination ability of *T. vaporariorum* using a fine-tuned selection of LEDs

ranging from UV to red. Behavioural action spectra were generated under semi-natural greenhouse conditions, thereby taking changing ambient light conditions into account. We further investigated and characterized in detail the potential blue photoreceptor and the blue-green chromatic mechanism by LED mixing experiments. From the data, we built simple empirical colour choice models which explain the choice behaviour and enable approximate estimation of the spectral location of photoreceptors.

2.3 Material and Methods

2.3.1 Experimental LED trap screens

In order to study the visual behaviour of *T. vaporariorum*, nineteen individual high-power (HP) light emitting diodes (LEDs) covering the UV-A and visible spectra were selected (Table 1, Fig.1). LEDs underlie limitations concerning wavelength availability and homogeneity of bandwidths and intensities and show variations among equally coloured LEDs. Criteria for the selection were the fine-tuned fitting to the spectral regions of interest, narrow bandwidths, and sufficient spectral distances and intensities. In the selection process, spectra of various HP LEDs were recorded with the spectrometer Avaspec 2048-2 (Avantes, Apeldoorn, The Netherlands).

LEDs of each colour were attached to aluminium-panels (100 x 100 x 1 mm). To obtain sufficient intensities for yellow LEDs, two or four LEDs had to be used. Most HP LEDs were common single chip emitters but for chartreuse green and yellow specific multichip emitters had to be used (Table 1). They required additional cooling by heat sinks (Fischer Elektronik GmbH & Co. KG, Lüdenscheid, Germany) or even active cooling with a fan (LED cooling module, LA001-011A9DDN, Sunonwealth Electric Machine Industry Co., Ltd, Kaohsiung City, Taiwan).

As LED traps, boxes (0.1 x 0.1 x 0.13 m) were constructed out of grey PVC (4 mm) to insert the LED panels on the backside via grooves in the side walls. The front side of the box was closed by transparent a opal acrylic glass plate (100 x 100 x 3 mm, PLEXIGLAS® LED 0M200 SC, Evonik Industries AG, Essen, Germany) which served as scatter screens (Fig. 2A). In addition, mirror film (PEARL GmbH, Buggingen, Germany) was used to laminate the insides of the boxes. For whitefly trapping, the screen was covered with transparent plastic film (PET) coated with insect glue (Temmen GmbH, Hattersheim, Germany), which was shown in preliminary tests to not influence the emitted spectra.

For the operation and adjustment of intensities of each LED panel, a device with 16 LED drivers (Mini Jolly, TCI, Saronno, Italy) was constructed. The 16 separate channels could be dimmed (0-100%) by external control signals (0-10 V) which were provided by two USB analogue output

modules (ME RedLab 3104, Meilhaus Electronic GmbH, Alling, Germany) in combination with a notebook and the software ProfiLab-Epert 4.0 (ABACOM, Ganderkesee, Germany).

Photon flux densities (µmol m^{-2} s^{-1}) of LEDs from the long-wave UV-A to red (UV3 - R, Table 1, Fig. 1) were measured and adjusted using the LI-250 A Light Meter with LI 190 Quantum Sensor (LI-COR Biosciences, Lincoln, NE, USA). As the sensor is only suitable to measure broadband photosynthetic active radiation (PAR, 400 – 700 nm), the sensor sensitivity data provided by LI-COR (starting at 385 nm) was included in the measurement of UV and violet LEDs (UV3 – V3, Table 1, Fig. 1). Extrapolation of the non-measurable parts of LED spectra below 385 nm had to be conducted. For the other two UV-A LEDs (UV1, UV2, Table 1, Fig. 1), the Almemo® 2390-5 datalogger (Ahlborn Mess- und Regelungstechnik GmbH, Holzkirchen, Germany) in combination with a UV-A sensor (Type 2.5, Indium Sensor GmbH, Neuenhagen, Germany) were used. The intensities were indicated in W m^{-2} and were converted to µmol m^{-2} s^{-1} using the LED spectra, Planck's constant, and Avogadro's number. The sensitivity data of the sensor was included as the sensor is matched for UV-A measurement in broadband sunlight. All measurements were conducted in darkness by placing the sensor directly on the centre of the LED screen surface.

Table 1. Specifications of high-power LEDs and constructed LED panels used in the experiments.

LED colour	Abbreviation	Peak- / Centroid wavelength / Full-width-half-max (nm)	Manufacturer	Type (Design*)	LEDs/Panel (cooling**)
Ultraviolet	UV1	371 / 373 / 10	Roithner	H2A1-H365-E (sc)	1 (nc)
Ultraviolet	UV2	376 / 378 / 11	Roithner	H2A1-H375-E (sc)	1 (nc)
Ultraviolet	UV3	382 / 385 / 11	Roithner	H2A1-H385 (sc)	1 (nc)
Ultraviolet	UV4	398 / 400 / 14	Roithner	H2A1-H395 (sc)	1 (nc)
Violet	V1	408 / 410 / 15	Roithner	H2A1-H405 (sc)	1 (nc)
Violet	V2	414 / 415 / 14	Roithner	H2A1-H410 (sc)	1 (nc)
Violet	V3	432 / 435 / 18	Roithner	H2A1-435 (sc)	1 (nc)
Blue	B1	444 / 447 / 17	Osram	Oslon SSL LD CQ7P (sc)	1 (nc)
Blue	B2	467 / 469 / 21	Osram	Oslon SSL LB CP7P (sc)	1 (nc)
Cyan	C	499 / 500 / 42	Roithner	H2A1-505 (sc)	1 (nc)
Bluegreen	BG	511 / 512 / 41	Roithner	H2A1-515 (sc)	1 (nc)
Green	G1	521 / 524 / 30	Roithner	H2A3-520 (sc)	1 (nc)
Green	G2	524 / 528 / 31	Luxeon	Rebel LXML-PM01 (sc)	1 (nc)
Green	G3	530 / 533 / 33	Osram	Oslon SSL LT CP7P (sc)	1 (nc)
Green	G4	546 / 550 / 38	Roithner	LED550-66-60 (mc)	1 (pc)
Yellow	Y1	578 / 574 / 15	Roithner	LED570-66-60 (mc)	4 (ac)
Yellow	Y2	592 / 590 / 15	Osram	Oslon SSL LY CP7P (sc)	2 (nc)
Amber	A	619 / 614 / 14	Osram	Oslon SSL LA CP7P (sc)	1 (nc)
Red	R	634 / 630 / 16	Osram	Oslon SSL LR CP7P (sc)	1 (nc)

* sc = single chip emitter, mc = multi chip emitter
** nc = no additional cooling, pc = passive cooling with heat sink, ac = active cooling with fan

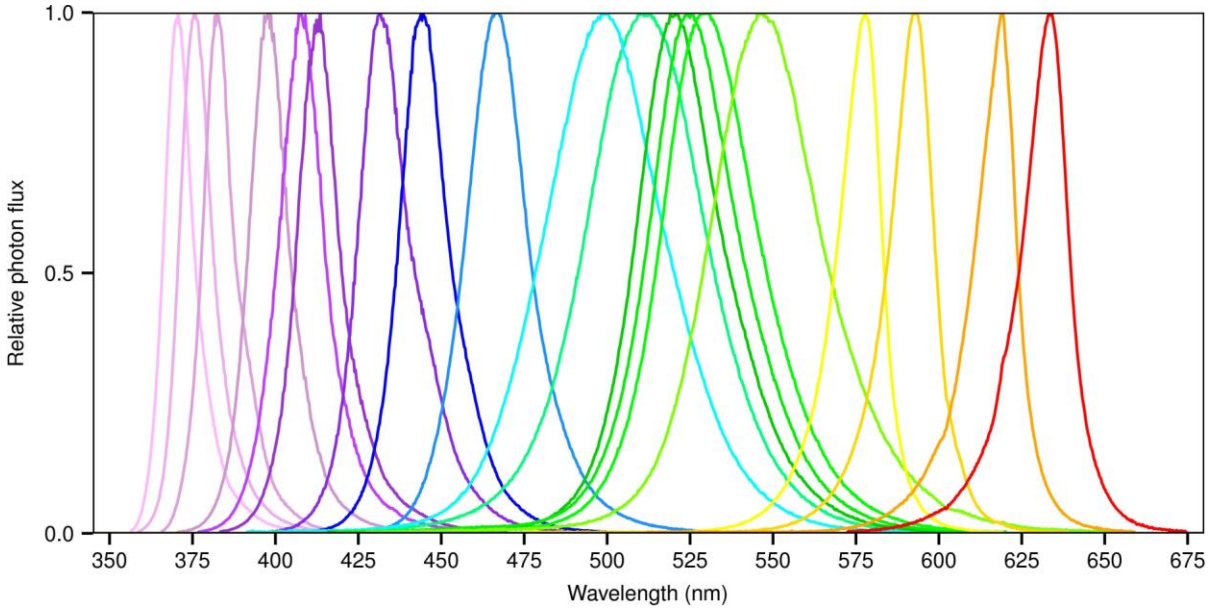

Fig. 1. Spectra of high-power LEDs used. Data refer to LED specifications given in Table 1, in spectral order.

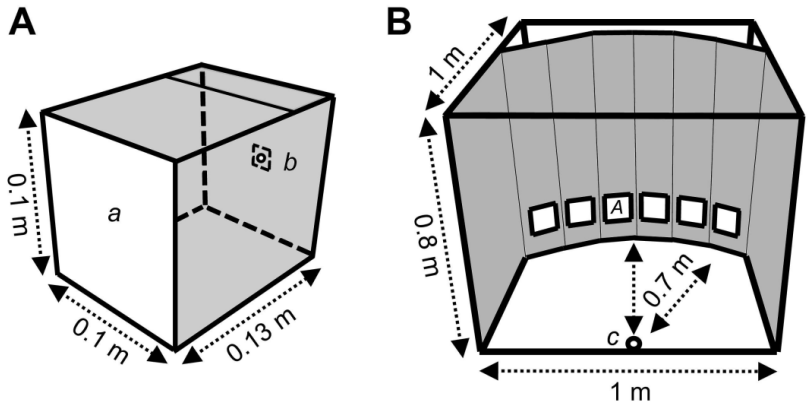

Fig. 2. Schemes of LED trap screen and choice arena. (A) LED trap screen with acrylic glass screen front side (*a*) and LED panel backside (*b*). The inner side of the box was laminated with mirror film. (B) Choice arena with whitefly release point (*c*) and position of LED traps (*A*). The background was black and the bottom was black-brown.

2.3.2 LED choice arena

Choice experiments were conducted close to the whitefly rearing in the same greenhouse compartment. A gauze-covered flight cage (1 x 1 x 0.8 m, Fig. 2B) with a waterproof black-brown plywood bottom was placed on stands at a height of one meter. The foldable front side faced in northern direction and was equipped with an additional lockable circular opening (0.25 m diameter) enabling the releasing of whiteflies. A semicircular background made of carton sprayed with matt black acrylic paint (Dupli Color, Motip Dupli GmbH, Hassmersheim, Germany) was inserted into the cage at a distance of 0.7 m to the release point. The

background was equipped with six square holes of 0.1 x 0.1 m at a height of 0.1 m and a distance of 0.05 m to each other. The LED trap screens could be optionally inserted from the backside by placing them on 0.1 m high wooden blocks (Fig. 2A,B). The cage backside was covered with gauze and black-silver reflective mulch film (Sunup Reflective Films, Oceanside, CA, USA). The cables for each LED panel were connected from the cage backside to the LED control placed under the cage.

The ambient solar radiation during the experiments was measured using a sensor for visible light (FLA 623 PS, Ahlborn Mess- und Regelungstechnik GmbH, Holzkirchen, Germany) and a UV-A sensor (300 – 400 nm, Type 2.5, Indium Sensor GmbH, Neuenhagen, Germany) placed next to the whitefly release point. Measurements were recorded at 20 second intervals with the Almemo® 2590-4AS datalogger (Ahlborn Mess- und Regelungstechnik GmbH, Holzkirchen, Germany) which was also placed under the cage. Temperature was recorded with a Tinytag Plus 2 TGP-4500 datalogger (Gemini Data Loggers Ltd., Chichester, UK).

2.3.3 Whiteflies

Greenhouse whiteflies (*Trialeurodes vaporariorum*) were reared on tobacco plants (*Nicotiana tabacum* L. cv. 'Xanthi') in two gauze cages (0.75 x 0.5 x 0.8 m) at the Leibniz-Universität, Hannover, Institute of Horticultural Production Systems, Section Phytomedicine in Germany at 23 ± 3 °C. For each experimental trial, vital individuals were carefully collected with an aspirator from the underside of the top leaves into a snap-on lid glass vial (h x d = 50 x 30 mm) and immediately released into the experimental choice arena.

2.3.4 Experimental overview and classification

According to literature, the behavioural response to the green-yellow range corresponds with 'settling' while the response to the UV-violet range is presumably related to 'migratory behaviour' (Coombe, 1981; 1982). The conducted experiments can be classified into wavelength dependence experiments characterized by the predominant main colours (green, blue, UV) and intensity experiments in the green-yellow range, resulting in four experimental blocks (Table 2). Wavelength dependence experiments on the 'settling response' are referred to as 'Green response experiments' (Block 1). Subsequently, 'intensity dependencies' in the green-yellow range were determined (Block 2). An inhibitory blue-green chromatic mechanism in the 'settling response' was studied by combining yellow LEDs of the same wavelength with blueish LEDs of different wavelengths, referred to as 'Blue inhibition experiments' (Block 3). Wavelength dependence experiments of the 'migratory behaviour' are referred to as 'UV response experiments' (Block 4).

Wavelength-dependent responses were initially investigated in multiple-choice experiments and subsequently relevant LEDs were selected and tested in dual-choice experiments to

determine standardized spectral efficiencies. All multiple-choice and dual-choice experiments were performed in 2015. The experiments took place in the described choice arena and replicates were conducted in consecutive trials on different daytimes and days. Trials were conducted between 10:00 and 17:00 h. Experiments regarding the ‘settling response’ were conducted from February to May. With increasing day length and brighter ambient light conditions in the greenhouse, whiteflies orientated more readily to the traps, hence trial durations could be reduced and number of trials per day could be increased within this time. UV response experiments were conducted from September to November but suffered from weaker responses and low recapture rates and trial durations were adjusted accordingly (Table 2).

In multiple-choice experiments, the LED trap screens in question (six or five) were placed in the holes of the choice arena background and the order was changed randomly for each replication. 150 or 200 whiteflies were released per replication and the number of trapped individuals on each trap were counted after a given period (0:30 – 1:30 h). Afterwards the cage was cleaned carefully from remaining whiteflies with a handheld vacuum cleaner before starting the next trial. The procedure for dual-choice experiments was similar, but four holes for trap screens were covered with black plastic film and only the two inner holes were equipped with the two LED traps. Again, trap positions were changed randomly for each replication. The measurement of ambient light conditions were averaged over each experimental trial and considered in the dataset as co-variable.

Table 2. Experimental overview and design

Experimental			LEDs **	Intensity on trap screen (µmol m^{-2} s^{-1})	Number of				Trial Duration (h) ***	Dates
Block	No.	Design *			Replicates	Days	Trials / day	Whiteflies / trial		
(1) Green response	1	m-c	B2, C, BG, G1, G2, G3	28	20	5	4	150	01:15	12-17/02/2015
	2	m-c	G3, G4, Y1, Y2, A, R	28	20	5	4	150	01:15	06-11/02/2015
	3	m-c	G1, G2, G3, G4, Y1, Y2	28	20	5	4	200	01:15	20-25/02/2015
	4	m-c	B1, B2, C, BG, A, R	28	10	2	5	150	01:15	09-10/04/2015
	5	d-c	G4 vs. BG, G1, G2, G3, Y1, Y2, A	28	10	10	7	150	00:40	02-16/03/2015
(2) Intensity dependence	6	d-c	G4 at 50% intensity vs. G1, G3, Y1, Y2 at 100%	14 vs. 28	10	5	8	150	00:40	23-30/03/2015
	7	m-c	Y2 at different intensities (100, 83, 66, 50, 33, 16%)	54, 45, 36, 27, 18, 9	20	5	4	200	00:40	24-27/11/2015
	8	m-c	Y2 at equal intensities	50	10	2	5	200	00:40	15-16/12/2015
(3) Blue inhibition	9	m-c	Y2 only & Y2 mixed with +V2, +V3, +B1, +B2, +C	50 + 5	10	1	10	150	00:30	08/05/2015
	10	m-c	Y2 mixed with +V2, +V3, +B1, +B2, +C	50 + 2.5	21	3	7	150	00:30	09-12/05/2015
	11	d-c	+B2 vs. +V2, +V3, +B1, +C	50 + 2.5	10	5	8	150	00:30	19-23/05/2015
(4) UV response	12	m-c	UV(1, 2, 3, 4), V(1, 2)	40	20	4	5	150	01:30	22-28/09/2015
	13	m-c	UV(1, 3, 4), V(2, 3), B1	40	20	4	5	150	01:30	06-10/10/2015
	14	d-c	UV1 vs. UV3, UV4, V2, V3	40	10	10	4	150	01:30	12/10-02/11/2015

* m-c = Multiple-choice experiment, d-c = Dual-choice experiment / ** LED colour abbreviations according to Table 1 / *** Trial duration varied by ± 0:10 h at most

2.3.5 Block 1: Green response experiments (Exp. 1-5)

The wavelength dependence of the ‘settling response’ in the green-yellow range was studied using 12 LED colours at equal photon fluxes including the adjacent blue and red ranges. Four multiple-choice experiments were conducted comparing six LEDs simultaneously in one experiment. Experiment 1 compared LEDs ranging from blue to green and exp. 2 those ranging from green to red. These experiments were interlinked by one green LED (G3) presented in both experiments. Then the most targeted LEDs from these two experiments were selected and compared in exp. 3. Here, the sex ratio of the trapped whiteflies on each LED colour was also determined in five of the 20 replicates (last trial of each day). Finally, the previously less preferred blue and red LEDs were compared separately in exp. 4.

The most attractive chartreuse green LED (G4 - 550 nm centroid wavelength) from the multiple-choice experiments was selected as a reference to determine standardized spectral efficiencies of seven LED colours (test lights) from blue-green to amber (BG, G1-3, Y1-2, A) and successively tested against the green reference LED in dual-choice assays (exp. 5). The responses were the relative choice frequencies on the test lights which were graphically displayed relative to the reference light which was set to maximum response. The spectral efficiencies of the tested LED colours were normalized to obtain a standardized LED based action spectrum of the ‘settling response’ under daylight conditions. The experiment was conducted with one replicate per colour per day and a randomized order of the colours per day.

2.3.6 Block 2: Intensity dependencies (Exp. 6-8)

Following the determination of the spectral efficiency in the ‘settling response’ (see exp. 5) the intensity dependence of the choice behaviour was determined in the same dual-choice setup (exp. 6). The intensity of the chartreuse green reference light (G4) was reduced by 50% and tested against four spectrally adjacent green and yellow LEDs (G1, G3, Y1, Y2). The data of this experiment were merged with the initial data of these colours (exp. 5, LEDs at equal intensity) to illustrate the intensity-dependent changes in the spectral efficiencies.

The influence of different intensities of the same colour on the preference in a multiple-choice setup was looked at in another experiment with six yellow (Y2) LED traps at different intensities (exp. 7). One trap was set to maximum intensity and intensities of the others were reduced evenly.

In a final multiple-choice experiment, the same yellow LED traps were tested at equal intensities with randomized order to evaluate the bias regarding their positions in the choice arena (exp. 8).

2.3.7 Block 3: Blue inhibition experiments (Exp. 9-11)

A potential inhibitory blue-green chromatic mechanism was studied combining five panels with yellow LEDs (Y2 - 590 nm centroid wavelength) with two violet LEDs (V2 - 415, V3 - 435 nm), two blue LEDs (B1 - 447, B2 - 469 nm), and one cyan LED (C - 500 nm), respectively. Yellow LEDs were used here because we assume that they stimulate mostly the green receptor on the long wavelength side to ensure that inhibitory interaction effects can be attributed to the mixture with blueish LEDs. One additional panel remained with only yellow LEDs and the intensity of all six yellow LED panels was set to 50 µmol m^{-2} s^{-1} on the trap screen. A small amount of 5 µmol m^{-2} s^{-1} (= 9.1% relative intensity) of the respective blueish LED light was added.

In a first multiple-choice experiment, the five LED trap screens with yellow-blueish mixture and the pure yellow LED trap were compared (exp. 9). The pure yellow LED trap consequently had a 9.1% lower total intensity due to the lack of additional blueish light. In a second multiple-choice experiment (exp. 10), the pure yellow LED trap was excluded from the setup and the intensities of blueish LEDs were further reduced to 2.5 µmol m^{-2} s^{-1} (= 4.8% relative intensity).

The most unattractive yellow-blue combination (Y2+B2) was selected as reference to determine standardized spectral efficiencies of the other four yellow-blue combinations (test lights) in successive dual-choice assays (exp. 11). Here, the responses were the relative choice frequencies on the reference light, representing a measure of inhibition. A standardized LED based action spectrum of ‘settling inhibition’ was constructed according to the procedure in the green response experiments. The experiment was conducted with two replicates per colour per day and randomized order of the colours within the day.

2.3.8 Block 4: UV response experiments (Exp. 12-14)

The wavelength dependence of the ‘migratory behaviour’ in the UV range was studied using eight LEDs from UV to blue at equal photon fluxes. The first multiple-choice experiment compared LEDs from the narrow UV to violet range (exp. 12). In the second multiple-choice experiment, the spectral range was extended to blue with larger spectral steps between the LED colours (exp. 13).

The most attractive UV LED (UV1 - 373 nm centroid wavelength) was selected as reference to determine the standardized spectral efficiencies of four LED colours (test lights) from UV to violet (UV3, UV4, V2, V3) in dual-choice assays (exp. 14). A standardized LED based action spectrum of the UV response was constructed according to the procedure in the green response experiments (see Block 1). The experiment was conducted with two replicates per colour per day and randomized order of the colours within the day.

2.3.9 Colour choice models

An empirical colour choice model was built to describe the wavelength preference in the 'settling response' based on opponent chromatic interaction of a green and a yet undescribed blue photoreceptor. Modelling of the UV response was performed assuming achromatic processing based only on the UV receptor. As no reliable data of photoreceptor sensitivities are available for whiteflies, the peak sensitivities were approximated by this method.

Photoreceptor sensitivity templates (Govardovskii et al., 2000) were fitted for different photoreceptor peak sensitivities of a putative UV, blue, and green photoreceptor, respectively. The peak sensitivities of the green and the blue receptor were altered in 5 nm steps in the range of 500 - 545 nm (green) and 470 - 495 nm (blue) resulting in 60 potential combinations. The peak sensitivities of the UV receptor was changed in 10 nm steps in the range of 340 - 370 nm.

The photon catch P of a photoreceptor can be calculated with the photoreceptor sensitivity function $S(\lambda)$ and the spectrum of the (LED) stimulus light $I(\lambda)$ (Kelber et al., 2003):

$$P = \int I(\lambda)S(\lambda)\mathrm{d}\lambda \tag{1}$$

The photon catches of each LED colour (and its combinations) were calculated for each potential photoreceptor position. Photoreceptor excitations E were calculated from photon catch values using a nonlinear transformation (Chittka, 1996):

$$E = P/(P+1) \tag{2}$$

This resulted in excitation values for each LED and each photoreceptor (E_{UV}, E_{B}, E_{G}) at varying positions. The excitations of the colour opponent mechanism E_{opp} were calculated as difference between green and blue photoreceptor excitations:

$$E_{\mathrm{opp}} = E_{\mathrm{G}} - E_{\mathrm{B}} \tag{3}$$

These values were connected to the LED choice datasets of the 'green response', the 'blue inhibition' and the 'UV response', resulting in three separate models.

For the 'green response model' the mean relative choice frequencies from the 'green response experiments' (exp. 1-3, 5) were combined and plotted against E_{opp} values of each receptor configuration. The data from multiple-choice experiments were thereby normalized to the most attractive chartreuse green LED (G4). The first dataset was built based on the outcome of exp. 1 and 2 which were connected via the linking green LED (G3) used in both experiments. Exp. 3 was taken as second dataset and the normalized spectral efficiencies from exp. 5 as third dataset.

A preference restriction was implemented which considers that the highest excitation value should correspond with the most attractive chartreuse green LED (G4):

$$E_{\mathrm{opp}}(\mathrm{LED\ G4}) > E_{\mathrm{opp}}(\mathrm{LED\ G1\text{-}3, Y1,2}) \quad (4)$$

For the 'blue inhibition model' the data from the 'blue response experiments' with mixed yellow and blue LEDs (exp. 10, 11) were plotted against E_{opp} values. Here, the indirect response was the inhibition of the attraction and the highest response was referred to the most inhibiting blue LED. Therefore, the mean relative choice frequencies from the multiple-choice experiment (exp. 10) were inverted and normalized to the most unattractive yellow-blue combination (Y2+B2). The normalized spectral efficiencies of inhibition from exp. 11 were taken as second dataset. Here, the lowest excitation value should correspond with the blue LED (B2) inhibiting the attraction towards yellow LEDs the most:

$$E_{\mathrm{opp}}(\mathrm{LED\ Y2{+}B2}) < E_{\mathrm{opp}}(\mathrm{LED\ Y2{+}V2,3, B3, C}) \quad (5)$$

For the 'UV response model', achromatic processing based solely on the UV receptor was assumed. Therefore, the excitation values E_{UV} were directly plotted against the normalized relative response data from the multiple-choice experiments (exp. 12, 13) and the dual-choice spectral efficiency experiment (exp. 14). The restriction that the highest excitation value should correspond with the most attractive UV LED is described by:

$$E_{\mathrm{UV}}(\mathrm{LED\ UV1}) > E_{\mathrm{UV}}(\mathrm{LED\ UV2\text{-}4, V1\text{-}3, B1}) \quad (6)$$

All models' significant linear regressions (α = 0.05) fulfilling the preference restrictions were fitted and the models were assessed based on R^2 values. All analyses and graphical display related to the colour choice models were performed in Microsoft Excel 2016.

2.3.10 Statistical analysis

The statistical analyses were performed in R (Version 3.2.1; R Core Team, 2015).

The multiple-choice experiments (exp. 1-4, 7-8, 9-10, 12-13) were analysed with linear models using the lm() function. The response variables were the ln(x + 1) transformed numbers of trapped whiteflies on each LED trap. In colour choice experiments, the explanatory variable was the LED colour. The ambient light intensity (visible light or UV radiation) measured throughout the experiments was included as co-variable for the experiments of the green and UV response. Initial Block factors (day, daytime) of the consecutive experiments were excluded after model selection using Akaike's Information Criterion (Burnham and Anderson, 2010). Interactions between the colour and the ambient light intensity were included in the analyses of the green response experiments 2 and 3. Separate linear models were fitted to analyse the total numbers of trapped whiteflies in the given time dependent on the ambient light intensity. In the analyses of the multiple-choice experiments with different LED intensities

(exp. 7), LED trap intensity and its interaction with ambient light intensity were explanatory variables. In the analyses of the multiple-choice experiment with equal LED intensities (exp. 8), the individual LED trap number and the position in the choice arena were the explanatory variables. ANOVA was used to determine influences of explanatory variables and interactions in the linear models. Tukey-type pairwise comparisons regarding LED colours and intensities were performed at α=0.05 using the lsmeans package (Lenth, 2015).

The sex ratio in the multiple-choice experiment 3 was analysed with a generalized linear model using the glm() function with binomial distribution and logit link. The response variable was the odds ratio between males and females on each trap and the explanatory variable was the colour. The dual choice experiments (exp. 5, 6, 11, 14) were analysed with generalized linear models (quasibinomial, logit link). The response variable was the odds ratio between the number of trapped individuals on test and reference LED traps. Explanatory variable was the respective colour of the test LED. The ambient light intensity was included as co-variable for the spectral efficiency experiments on green and UV response. An interaction between colour and ambient light was further included in the green response analysis. Deviance analyses were performed to determine influences of explanatory variables and interactions in the generalized linear models. In the intensity dependence dual choice experiment (exp. 6), pairwise comparisons were performed between intensity levels (α=0.05, lsmeans package). User-defined interaction contrasts were created to compare intensity-dependent changes of choice frequencies between colours using the package statint (Kitsche and Schaarschmidt, 2015). Tukey-type comparisons on interaction contrasts were performed using the multcomp package (Hothorn et al., 2008). Graphs were created using the ggplot2 and gridExtra package (Wickham, 2016; Auguie, 2012).

2.4 Results

2.4.1 Block 1: Green response experiments (Exp. 1-5)

Experiment 1: The results showed hardly any response of whiteflies to the blue (B2 - 469 nm), cyan (C - 500 nm), and blue-green (BG - 512 nm) LED, and a steep significant increase in the preference among green LEDs (G1-3) with only slightly different centroid wavelengths of 524, 528, and 533 nm (Fig. 3A).

No significant influence of the ambient light or the interaction with colours were observed in the fitted linear model (Fig. 4A). This indicates that whiteflies discriminated green LEDs over the whole ambient light intensity range. The overall recapture rate was 69.0 ± 6.6% (Mean ± s.d.) within 1:15 ± 0:10 h. A separately fitted linear model shows no significant increase of the total recaptures with rising ambient light intensity.

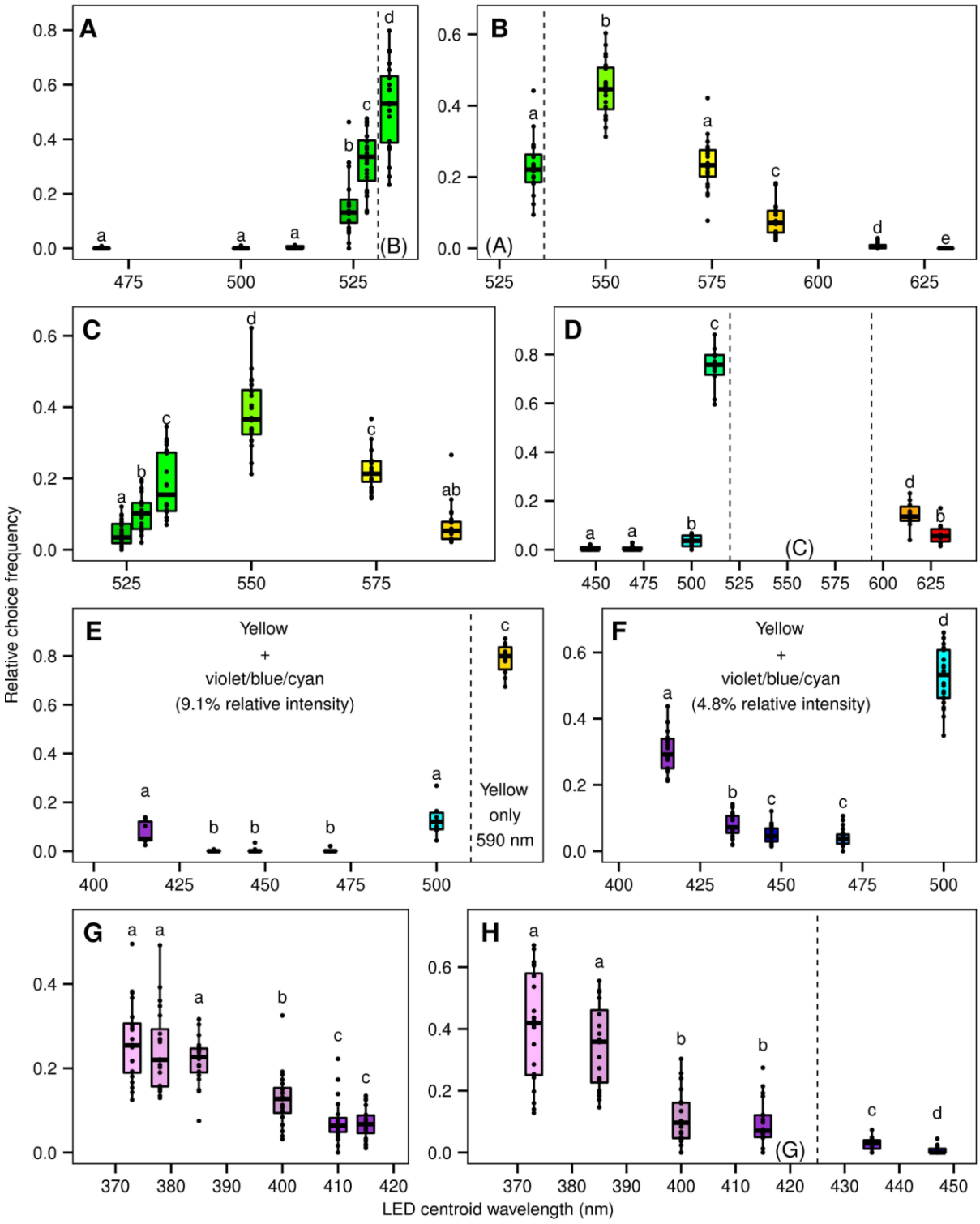

Fig. 3. Wavelength preferences of *Trialeurodes vaporariorum* in LED multiple-choice experiments. (A) blue - green (exp. 1), (B) green - red (exp. 2), (C) green - yellow (exp. 3), (D) blue & red (exp. 4), (E) yellow + violet - cyan & pure yellow (exp. 9), (F) yellow + violet - cyan (exp. 10), (G) UV - violet (exp. 12), (H) UV - blue (exp. 13). See Table 2 for experimental details. Dashed vertical lines and panel letters in brackets on the bottom indicate spectral overlapping of the experiments. Dots show original data points. Boxes indicate interquartile ranges (IQR) with median (thick line). Whiskers comprise values within 1.5 × IQR. Different letters indicate significant differences of the factor LED colour within each experiment (Linear Model: ln (x+1) ~ LED colour × ambient light intensity, Tukey post-hoc tests, P=0.05).

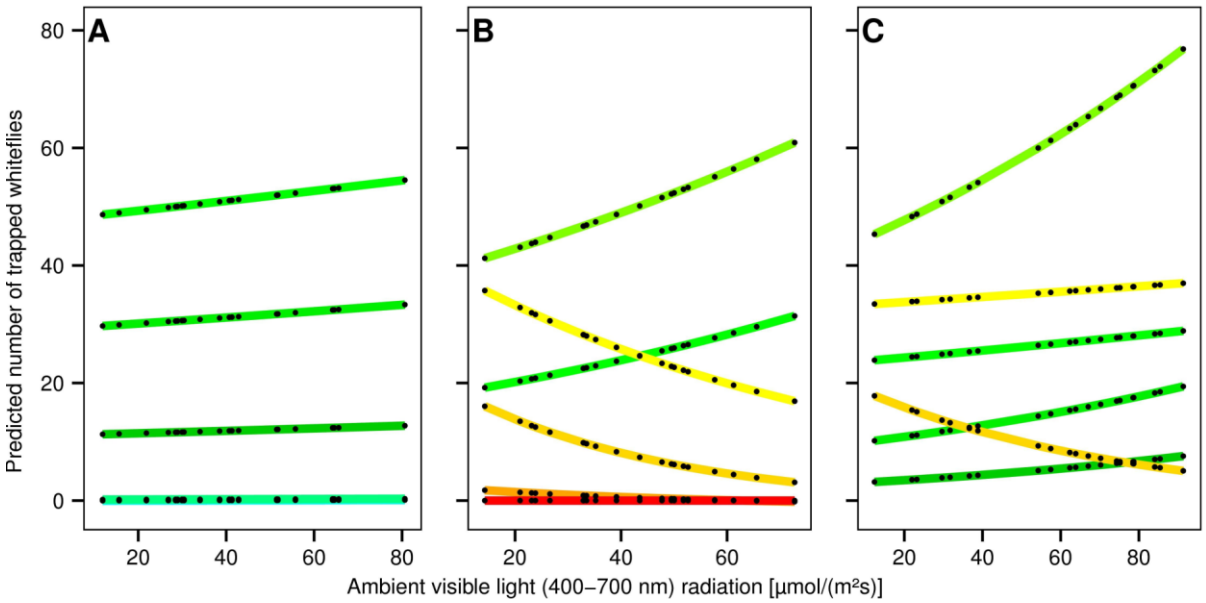

Fig. 4. Interactions of wavelength preferences in *Trialeurodes vaporariorum* with ambient light intensity in LED multiple-choice experiments based on the fitted models. Black dots connected by coloured lines show backtransformed predictions from the linear models (ln(x+1) ~ LED colour × ambient light intensity) fitted to the data of (A) exp. 1, (B) exp. 2, (C) exp. 3. See Fig. 3A, B, C for original data and associated line colours (lines at zero partly overlay each other).

Experiment 2: In the green range, the preference further increased revealing chartreuse green (G4) with 550 nm centroid wavelength as the most attractive LED (Fig. 3B). Towards the yellow spectrum with the two yellow LEDs (Y1 - 574, Y2 - 590 nm), the preference declined and only a weak response to amber (A - 614 nm) and no response to the red (R - 630 nm) LED were noticed.

In contrast to exp. 1, a significant influence of ambient light and the interaction with colour were observed (both $P<0.001$). At darker conditions, the response to yellow was relatively stronger while the corresponding response to green was weaker (Fig. 4B). With increasing ambient light intensity, the response to green LEDs (G3, G4) increased while the response to yellow LEDs (Y1, Y2) decreased correspondingly. This resulted in a cross-over interaction between the second most attractive green (G3) and yellow (Y1) LEDs which are on average of similar attractiveness but with increasing ambient light intensity G3 became more attractive. The overall recapture rate was 75.6 ± 10.9% (mean ± s.d.) within 1:15 ± 0:10 h and total recaptures were not influenced by ambient light.

Experiment 3: When the selected attractive green and yellow LEDs (G1-4, Y1-2) were compared, the results show that the relative preferences resemble an action spectrum (Fig. 3C).

No significant influence of the ambient light but a significant interaction with the colour could be determined ($P=0.019$). At darker conditions, the preferences were more evenly distributed

across all colours and with rising ambient light intensity the preference was pointed more towards the most attractive chartreuse green LED (G4) while the preference towards the second most attractive yellow (Y2) decreased (Fig. 4C). The overall recapture rate was 82.8 ± 10.0% (mean ± s.d.) within 1:15 ± 0:10 h. The totally recaptured numbers increased significantly with rising ambient light (P=0.003), primarily due to the strongly increasing preference for the most attractive chartreuse green (G4).

The ratio of females on the LED colours were 68% on G1, 72% on G2, 72% on G3, 72% on G4, 81% on Y1, and 81% on Y2; the overall ratio was 74.5%. The ratio of females was slightly higher on the yellow LEDs but statistically no significant effect of LED colours on the sex ratio was observed (GLM, Analysis of Deviance, P=0.16).

Experiment 4: When the previously attractive range was excluded, whiteflies significantly preferred the blue-green (BG - 512 nm) LED and only few landings were recorded on cyan (C - 500 nm), amber, and red LED traps (Fig. 3D). The overall recapture rate was 41.9 ± 10.6% (mean ± s.d.) within 1:15 ± 0:10 h.

Experiment 5: In the spectral efficiency dual-choice experiment the response declined steeply over the three green LEDs to very little relative response towards the blue-green LED. On the long wavelength side, the response declined a bit wider over the two yellow LEDs to almost zero response on the amber LED. The obtained action spectrum was similar to the action spectra derived from previous multiple-choice experiments (Fig. 5). No significant influence of the ambient light but a significant interaction with colour could be determined (GLM, Analysis of Deviance, P=0.005). The recapture rate was 82.0 ± 13.5% (mean ± s.d.) within 0:40 ± 0:10 h.

2.4.2 Block 2: Intensity dependencies (Exp. 6-8)

Experiment 6: When the intensity of the green reference light was reduced following the determination of the spectral efficiency (exp. 5), the choice frequencies on the respective green and yellow LEDs increased significantly (G1, G3, Y1: P<0.001; Y2: P=0.003; Fig. 6A). The increase was strongest on G1, thereby almost reaching equal response (choice frequency=0.5, Logit=0, indicated as dashed line in Fig. 6A) as on the reference LED (G4). The strength of increase was slightly lower on G3 and Y1 but the choice frequencies reached an even higher level than on the reference LED. The increase in attractiveness was significantly lower on Y2 compared to the other LEDs (Y2 vs. G1, G3: P<0.001; Y1 vs. Y2: P=0.015), and the response remained below the corresponding response to the reference LED.

Experiment 7: Different intensities of the same yellow (Y2) in a multiple-choice experiment showed the strongest response on the brightest LED and a constant decrease of attractiveness towards the lowest intensity (Fig. 6B).

A significant influence of ambient light intensity on the trapped numbers on each colour was observed (P=0.048, Fig. 6C). The interaction between ambient light and LED intensity was an explanatory factor according to model selection by AICs (P=0.079).

Experiment 8: When the yellow LEDs from the previous experiment were compared at equal intensities, the LED position had a significant influence on the numbers trapped (P=0.018, data not shown). More whiteflies were trapped on the outer side positions compared to the inner positions. But due to randomization and repetitions this effect could be neutralised resulting in no significant effect on the trapped numbers on respective LED traps (P=0.28).

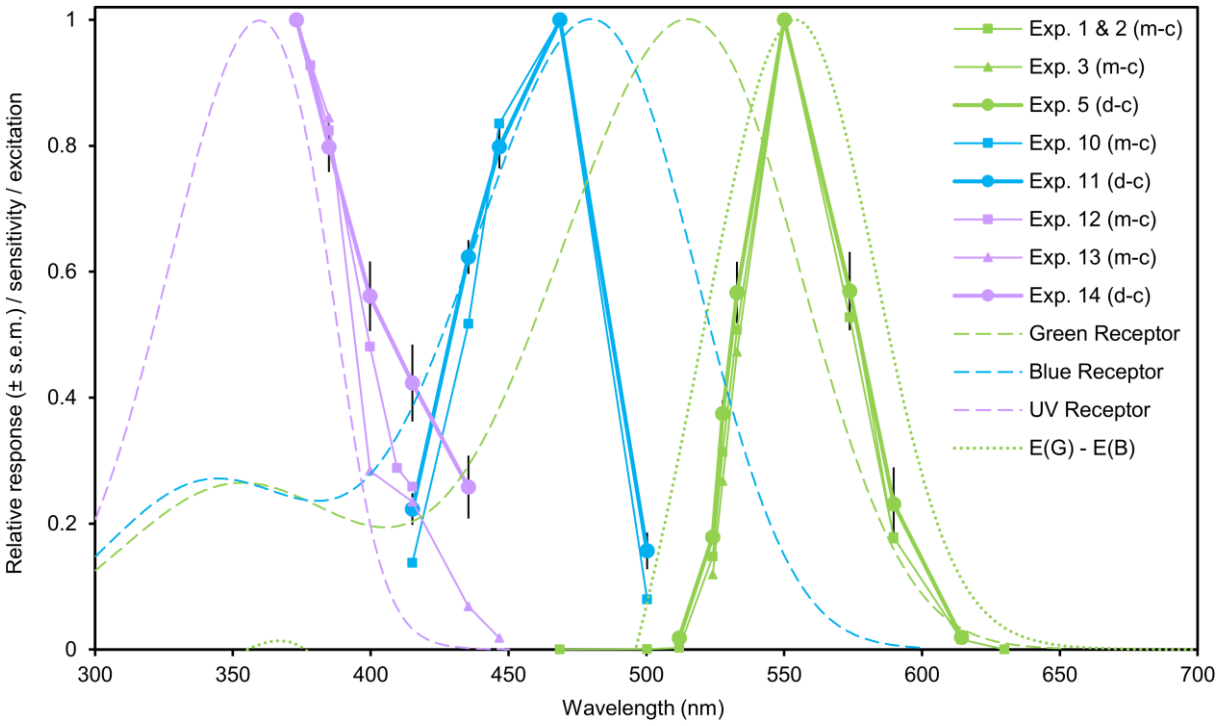

Fig. 5. Spectral efficiencies of *Trialeurodes vaporariorum* derived from LED choice experiments and modelled putative photoreceptor sensitivities with resulting theoretical action spectrum of the 'settling response' based on blue-green opponency. Coloured symbols connected by solid lines show relative responses in the respective spectral range dependent on LED centroid wavelengths (see Table 1 and Fig. 1 for LED centroid wavelengths and spectra). Green data points refer to the green response ('settling') and violet data points indicate the UV response. Blue data points are derived from mixing experiments with equal yellow and different blueish LEDs indicating the 'settling inhibition' as an inverse response to blue. Thin solid lines with squares or triangles show normalized mean relative choice frequencies from multiple-choice (m-c) experiments. Thick solid lines with circles (± s.e.m) show normalized mean relative choice frequencies from dual-choice (d-c) spectral efficiency experiments. See Table 2 for experimental overview and Fig. 3 for original data in multiple-choice experiments. Dashed coloured lines show photoreceptor sensitivity templates (Govardovskii et al., 2000) with peak sensitivities at 360 nm (UV), 480 nm (blue), and 515 nm (green) estimated in colour choice models. The dotted green line describes the modelled blue-green opponency as difference of photoreceptor excitations and represents the theoretical action spectrum of the 'settling response'.

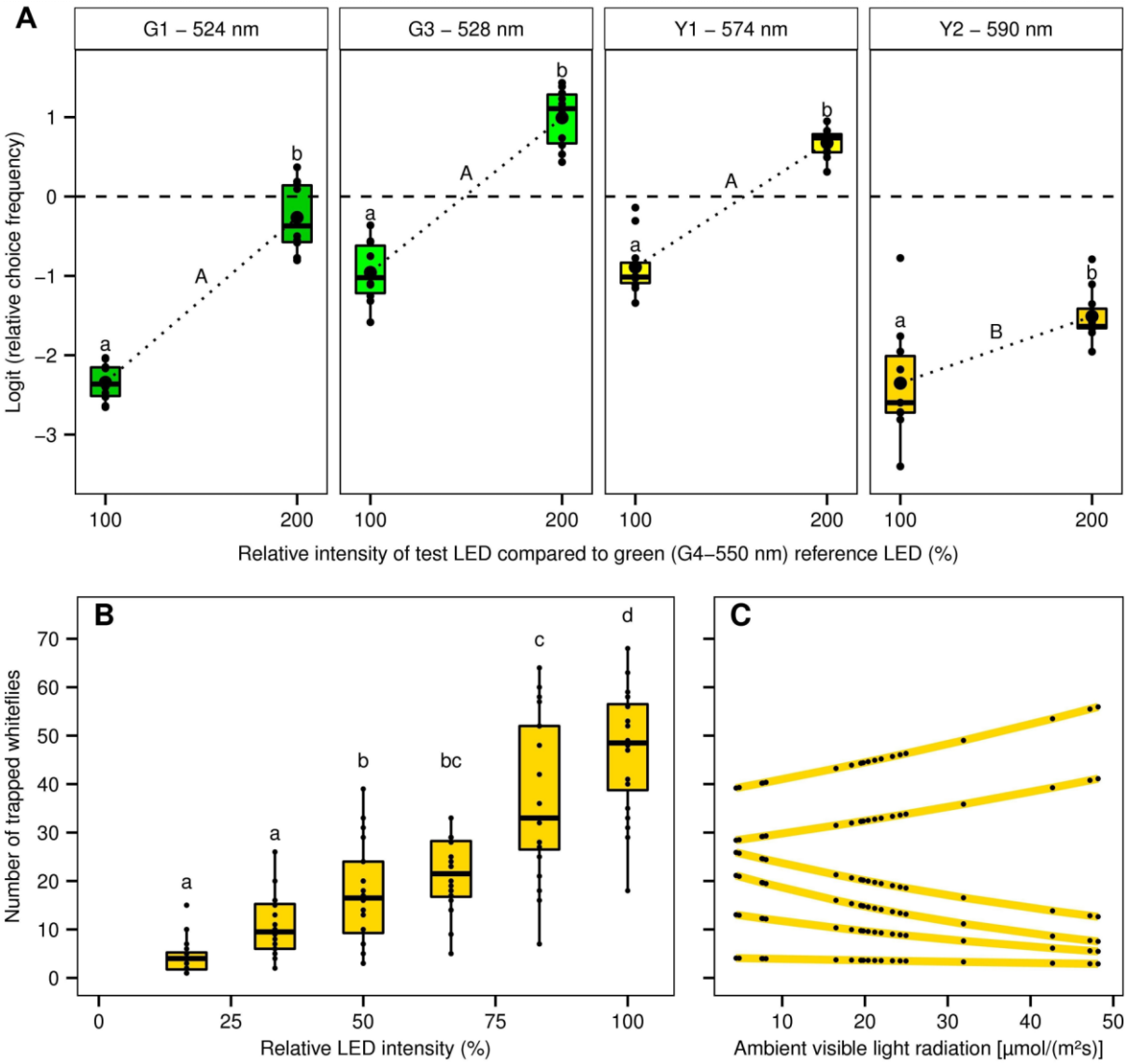

Fig. 6. Intensity dependencies in the colour choice behaviour of *Trialeurodes vaporariorum*. (A) Intensity dependences between spectral efficiencies. Panels show Logit-transformed choice frequencies in dual-choice experiments with four LED colours at equal (=100%) and double (=200%) relative intensity of the reference LED (G4 - 550 nm). Relative intensity changes were created by reducing the reference LED intensity by 50%. The dashed horizontal line indicates equal choice frequencies (=0.5, Logit=0) of test and reference LED. The dotted line connects mean choice frequencies of intensity levels. Different small letters indicate significant differences between intensity levels within each colour (GLM, pairwise comparisons, P=0.05). Capital letters indicate significant differences of intensity-dependent changes of choice frequencies between colours (GLM, user defined interaction contrasts, P=0.05). (B) Intensity Preference for equal yellow LEDs with 590 nm centroid wavelength at different relative intensities. Different letters indicate significant differences of the factor LED intensity (Linear Model, Tukey post hoc test, P=0.05). (C) Corresponding interaction with ambient light intensity based on the fitted model. Black dots connected by yellow line show predicted values from the linear model (Linear Model: ln (x+1) ~ LED intensity × ambient light intensity).

2.4.3 Block 3: Blue inhibition experiments (Exp. 9-11)

Experiment 9: Most of the whiteflies were trapped on the LED trap with pure yellow (Y2 - 590 nm). Little response was obtained when yellow was additively combined with small intensities of the shortest wavelength violet (V2 - 415 nm) or the longest wavelength cyan (C - 500 nm) LED. Almost no trappings were recorded on the combinations with the intermediate violet (V3 - 435 nm) and blue (B1 - 447, B2 - 469 nm) LEDs. The results clearly indicate that the "settling response" was inhibited by blueish light (Fig. 3E). The overall recapture rate was 92.8 ± 4.9% (mean ± s.d.) within 0:30 ± 0:10 h.

Experiment 10: When the pure yellow light was excluded from the setup and the intensity of blueish light was further reduced, the preferences exhibited in the previous experiment were clearly emphasized. Highest trap catches were recorded on the yellow-cyan combination and lowest catches on the yellow-blue combinations (B1 - 447, B2 - 469 nm). The preference increased again for the adjacent violet (V3 - 435 nm) and for the shortest wavelength violet (V2 - 415 nm) LED in particular. The data resemble an inverse action spectrum of inhibition of the 'settling response' (Fig. 3F). The overall recapture rate was 89.7 ± 10.5% (mean ± s.d.) within 0:30 ± 0:10 h.

Experiment 11: On the short wavelength side, the inhibition declined successively from UV to blue (B1) and violet (V2, V3) LEDs. On the long wavelength side, the inhibition strongly decreased in one big step to the cyan (C) LED. Again, the obtained action spectrum was quite congruent with the one derived from the multiple-choice approach (Fig. 5). The recapture rate was 75.4 ± 13.0% (mean ± s.d.) within 0:30 ± 0:10 h.

2.4.4 Block 4: UV response experiments (Exp. 12-14)

Experiment 12: The highest responses were recorded on the first three UV-A LEDs (UV 1-3) with closely related centroid wavelengths of 373, 378, and 385 nm but these preferences did not differ among each other. The preference declined over 400 nm (UV4) to the violet (V1 - 410, V2 - 415 nm) LEDs which showed the lowest but still detectable response (Fig. 3G).

A significant influence of the ambient UV radiation on the trapped numbers on the colours was observed in the fitted linear model (p=0.003). The overall recapture rate was 46.8 ± 10.7% (mean ± s.d.) within 1:30 ± 0:10 h. A separately fitted linear model showed that the totally recaptured numbers decreased with rising UV radiation intensities (P=0.006).

Experiment 13: When the tested spectral range was extended to blue, the preference further declined on the long wavelength violet (V3 - 435 nm) and very low responses were still detected on the short wavelength blue (B1 - 447 nm) LED (Fig. 3H).

UV radiation had a significant influence on the trapped numbers on the colours (P=0.046). The overall recapture rate was 46.8 ± 10.7% (mean ± s.d.) within 1:30 ± 0:10 h and total numbers were not significantly influenced by ambient UV radiation.

Experiment 14: The response declined successively over the tested UV and violet colours but was still quite prominent on the long wavelength violet (V3). The obtained half-sided action spectrum was wider and not entirely congruent with the ones derived from the previous multiple-choice experiments (Fig. 5). The recapture rate was 23.6 ± 10.0% (mean ± s.d.) within 1:30 ± 0:10 h.

2.4.5 Colour choice models

In the 'green response model' and the 'blue inhibition model', several combinations of blue and green photoreceptor peak combinations led to significant linear regressions which fulfil the preference restrictions (Table 3).

For the 'green response model', regressions with good fits ($R^2 \geq 0.8$) were found for receptor peak combinations from 470 & 525 nm at widest distance to 495 & 500 nm at lowest distance from each other. In the blue inhibition model, good fits ($R^2 \geq 0.9$) were found for combinations from 470 & 545 nm at widest distance to 495 & 500 nm at lowest distance. Well-fitting regressions which fulfil the restrictions in both models overlap at receptor combinations of 480 & 515 nm, 485 & 510 nm, 490 & 505 and 495 & 500 nm (Tab. 3). Selected regressions for potential blue and green receptor peaks at 480 and 515 nm are shown in Fig. 7A,B. The modelled potential photoreceptors based on template formulas (Govardovskii et al., 2000) and the resulting theoretical relative action spectrum of the 'settling response' based on blue-green opponency are shown in Fig. 5.

Fig. 7C shows the best fitting linear regression of the 'UV response model' with a photoreceptor peak at 360 nm ($R^2 = 0.93$) and the modelled receptor is also shown in Fig. 5. The restriction that the highest excitation value corresponds with the most attractive UV LED is also fulfilled for adjacent receptor peaks at 340, 350, and 370 with R^2 values of 0.77, 0.90, and 0.86, respectively.

Table 3. R^2 values from linear regressions of colour choice models for different photoreceptor combinations based on blue-green opponency.

		Colour choice model											
		Green Resp.	Blue Inh.	Green Resp.	Blue Inh.	Green Resp.	Blue Inh.	Green Resp.	Blue Inh.	Green Resp.	Blue Inh.	Green Resp.	Blue Inh.
		Blue receptor peak wavelength (nm)											
		470		475		480		485		490		495	
Green receptor peak wavelength (nm)	500			0.32		0.48		0.68		0.82		0.81	0.93
	505	0.30		0.47		0.67		0.83		0.82	0.93		0.98
	510	0.45		0.66		0.83		0.83	0.92		0.98		0.95
	515	0.64		0.83		0.84	0.90		0.97		0.96		0.85
	520	0.82		0.86			0.96		0.98		0.89		0.72
	525	0.87			0.93		0.98		0.93		0.77		0.58
	530				0.97		0.96		0.84		0.65		0.46
	535		0.93		0.98		0.91		0.75		0.55		
	540		0.96		0.96		0.85		0.66		0.46		
	545		0.98		0.93		0.78		0.58				

Only R^2 values from significant (α=0.05) regressions are shown which fulfill the restriction $E_{\mathrm{opp}}(\mathrm{G4}) > E_{\mathrm{opp}}(\mathrm{Y1, G3})$ for the green response model and $E_{\mathrm{opp}}(\mathrm{Y2{+}B2}) < E_{\mathrm{opp}}(\mathrm{Y2{+}B3})$ for the blue inhibition model. Fields framed with dotted line indicate overlapping of appropriate regressions from both models.

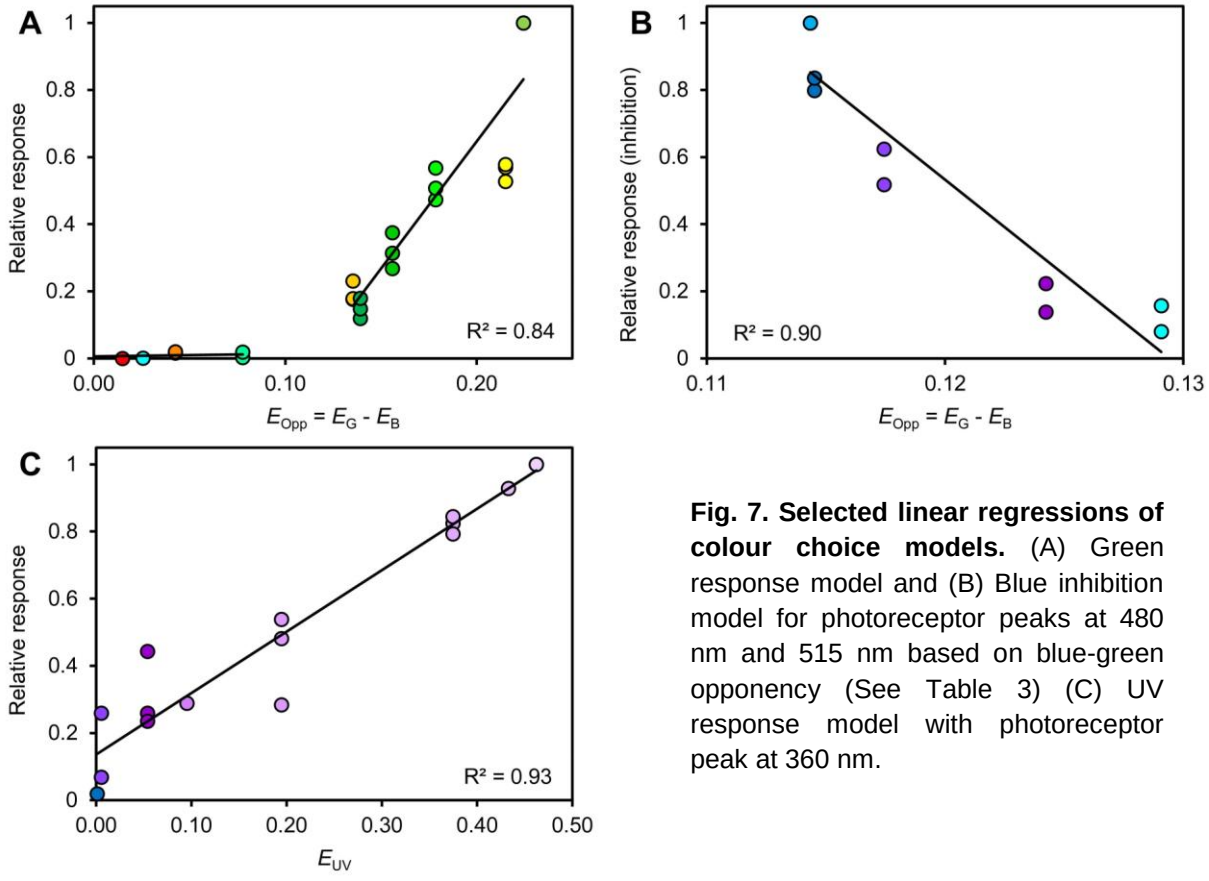

Fig. 7. Selected linear regressions of colour choice models. (A) Green response model and (B) Blue inhibition model for photoreceptor peaks at 480 nm and 515 nm based on blue-green opponency (See Table 3) (C) UV response model with photoreceptor peak at 360 nm.

2.5 Discussion

2.5.1 Main findings

This study reveals that *Trialeurodes vaporariorum* possesses a yet undescribed photoreceptor sensitive towards blue light and an inhibitory blue-green chromatic mechanism which controls a 'wavelength-specific behaviour' referred to as 'settling response' (Coombe, 1981). Besides this chromatic processing, the behavioural control is distinctly intensity-dependent. The known response to UV radiation based on a UV sensitive photoreceptor related to migratory behaviour could also be confirmed in our study (Coombe, 1981; 1982). As a consequence, we could conclude that *T. vaporariorum* possesses a trichromatic visual system.

2.5.2 Wavelength dependence of the 'settling response' and ambient light interaction

The chartreuse green LED with 550 nm centroid wavelength proved to be most attractive (Fig. 3B,C) and consequently constitutes the peak of the LED based action spectrum of the 'settling response' (Fig. 5). This meets our expectations as it is in line with earlier studies from MacDowall (1972) and Coombe (1981) also showing action spectra peaking at 550 nm. As only this LED was available in the region between 533 and 574 nm, it is possible that the actual peak slightly differs which is also possible for both reported studies which used monochromatic light in wide steps of 10 and 50 nm. When only one receptor controls the behaviour, the action spectrum should roughly exhibit the shape of the underlying receptor (Skorupski and Chittka, 2011). But our action spectrum as well as the reported data are more narrowly tuned to the green-yellow range and shifted to the longer wavelength range compared to the spectral efficiency peak at 520 nm which was determined by ERG recordings by Mellor et al. (1997). This discrepancy suggests the involvement of opponent processing and the extraction of chromatic signals (Skorupski and Chittka, 2011). Nevertheless, from an evolutionary perspective it seems natural that these action spectra peak around 550 nm which corresponds quite accurately with the peak reflectance and transmittance of green leaves, corroborating the fact that the visual systems of herbivores are adapted to host plant detection (MacDowall, 1972; Döring et al., 2009; Prokopy and Owens, 1983; Kelber and Osorio, 2010).

An important observation with regard to potential chromatic processing was that green LEDs with similar spectra of only 4-5 nm difference could be differentiated by *T. vaporariorum* as shown by the multiple-choice experiments (Fig. 3A,C). Moreover, the discrimination was exhibited consistently over the whole range of ambient light intensity, whereas yellow LEDs were to some extent confused with green ones at darker conditions (Fig. 4). Compared to naturally reflecting objects, the constant intensity of LED light is uncoupled from illuminating light intensity and should theoretically appear as brighter or darker in relation to changing ambient light intensity. Colour vision is defined as the ability to detect spectral variations in the

light independent of the intensity (Kelber et al., 2003). Photoreceptors adapt to the intensity of perceived light versus the background light by adjusting their responses through various mechanisms (Laughlin and Hardie, 1978; Arshavsky, 2003; Warrant and Nilsson, 2006). This avoids saturation of the photoreceptors and is a mechanism to maintain colour constancy (Foster, 2011; Kemp et al., 2015). Our results therefore suggest that green LEDs are discriminated based on opponent processing. In the longer wavelength range above 550 nm, yellow LEDs are presumably discriminated mainly by different stimulation of the green receptor with only low inhibitory input from a blue receptor. At darker conditions and relatively bright LED light, the green receptor might have been saturated resulting in similar signals for different wavelengths. Constant wavelength discrimination should then be possible only in the green region with distinctly overlapping receptor sensitivities resulting in different inhibitory input from a non-saturated blue receptor.

2.5.3 Blue-green chromatic mechanism

The results from blue-yellow mixing experiments provide the strongest evidence for blue-green opponency (Fig. 3E,F). Small amounts of blue light decreased the preference for yellow LEDs, and thus inhibited the elicited 'settling response' to some extent. This reveals the presence of a blue photoreceptor with inhibitory input to an adjacent green receptor. The inverse response resembles an action spectrum of opponent inhibition and enables a first approximate estimation of the spectral location of the blue receptor (Fig. 5). These results expand the study of Stukenberg et al. (2015) which already showed that the attractiveness of green LEDs is suppressed when simultaneously combined with blue LEDs. Similarly, a blue-green chromatic mechanism was identified in the mate finding behaviour of the glow-worm *Lampyris noctiluca* also using the technique of mixing green and blue LEDs (Booth et al., 2004).

Descriptive evidence for the blue-green chromatic mechanism comes from the empirical colour choice models built from the green response and the blue inhibition experiments (Tab. 3, Fig. 7A,B). Both models explain the observed colour choice behaviour and fit well into the theory of opponent processing based on the difference of concurrent excitations of the green and blue photoreceptors. Similar models have already been shown for aphids or the pollen beetle (Döring et al., 2009; Döring et al., 2012; Döring and Röhrig, 2016). In contrast to the reported studies which were based on physiological and behavioural data, reliable physiological data on photoreceptor sensitivities were not available for *T. vaporariorum*. Therefore our flexible approach does not enable us to estimate exact positions of the photoreceptors since linear modelling based on excitation differences of several combinations of blue and green photoreceptor peak sensitivities led to well-fitting linear regressions (Tab. 3). The preference restriction that the highest receptor excitation should correspond with the LED of highest response is thereby fulfilled either in one or the other model. The position of the green receptor

is limited to longer wavelengths by the preference restriction in the ‘green response model’ while the ‘blue inhibition model’ sets a limit towards shorter wavelengths. Both models follow a slightly different pattern with receptor peaks either far away from each other or close together but have a converging area in the range where receptors are close together and the restrictions are fulfilled in both models. These four combinations are 480 & 515 nm, 485 & 510 nm, 490 & 505 nm, and 495 & 500 nm (Tab. 3) which all lead to similarly shaped theoretical action spectra peaking at 554 - 556 nm (Fig. 5). While the very close combinations appear quite unlikely with regards to a reliable signal from the opponent mechanism, the more distant combinations (480 & 515, 485 & 510 nm) appear relatively realistic (Fig. 5). In comparison, the known photoreceptor sensitivities of aphids, which are also phloem-sucking herbivores show similar configurations. Receptor peaks for the green peach aphid *Myzus persicae* were determined around 490 and 530 nm and for the pea aphid *Acyrthosiphon pisum* at 518 nm, respectively (Kirchner et al., 2005; Döring et al., 2011). However, the exact positions and sensitivities of photoreceptors in the greenhouse whitefly still remain uncertain from this study, but only within a small range: The blue photoreceptor should be present with a peak around 480 - 490 nm, while a green receptor exists between 510 - 520 nm. The presence of a green receptor around 520 nm is also supported by the former ERG recording by Mellor et al. (1997). Obviously, this ERG investigation did not detect the blue receptor and measured a mixed peak of the green and blue receptor. It is unclear why the green peak was so prominent in ERG recordings but the blue photoreceptor cells may be underrepresented and contribute only a low electrophysiological input which is then strongly weighted in the nervous system.

The possible reasons for the incongruence of both models and the inaccuracies of their outcomes are diverse because they rely on simple assumptions and incalculable factors. The sensitivity functions of photoreceptors based on template formulas could slightly differ from real sensitivities for various reasons like self-screening properties or filter and screening pigments. Moreover, the calculations from photon catches to excitation values by the nonlinear transformation might not explain the reality completely. Furthermore, the relative contributions of the inputs from blue and green photoreceptors most likely differ from the assumed one-to-one ratio. Possible reasons for this could be different amounts of blue and green-sensitive photoreceptor cells in the compound eye or different weighting of the signals in the nervous system (Warrant and Nilsson, 2006; Cronin et al., 2014).

2.5.4 Intensity dependence in the ‘settling response’

It could be shown that the ‘settling response’ exhibits a clear intensity dependence (Fig. 6) which is in line with findings in whiteflies and other insects (Coombe, 1981; Scherer and Kolb, 1987; Booth et al., 2004). Normally, colour vision is characterized to be independent of intensity and most studies implicate that behaviours are processed either purely chromatic or

achromatic and it often remains unclear if both aspects are involved (Kelber and Osorio, 2010). But our results demonstrate that the suggested dichromatic mechanism shows both chromatic and achromatic properties, hence both colour (wavelength) and intensity are crucial in the 'settling' behaviour. This is an aspect which has already been implied by the colour choice model (see above) since excitation values as outcome of the opponent mechanism can theoretically be increased at the same wavelength by increasing their intensity. Our results show that within the green-yellow range of the action spectrum higher intensities can compensate for not optimally attractive wavelengths, thus colour constancy is not completely achieved. Furthermore, the sensitivity to relative intensity changes was higher in case of green LEDs compared to yellow LEDs (Fig. 6A). This represents a further clue that an interaction between receptors takes place, as these intensity dependencies would be parallel if they are based only on one receptor, following the principal of univariance (Naka and Rushton, 1966). Obviously, the intensity dependence is more distinct and stable in the green region in which the action spectrum is mainly shaped by opponent processing as compared to the yellow region where it should be primarily formed by the sensitivity of the green receptor.

Also, amongst equally coloured yellow LEDs preferences follow a brightness gradient which further demonstrates the influence of intensity on the choice behaviour in a multiple-choice setup (Fig. 6B). The interaction between the relative preferences and the ambient light intensity may be explained with photoreceptor adaptation (Fig. 6C), as has already been discussed for the wavelength choice experiments. Under bright background light conditions, the relative receptor sensitivity might be lower resulting in higher relative attractiveness of the two brightest LEDs. Under darker conditions, the relative sensitivity was probably higher resulting in a more even attractiveness of the traps.

2.5.5 UV response

The moderate attraction of the greenhouse whitefly to UV radiation supposed to be a wavelength-specific behaviour involved in flight initiation, migration, and dispersal could be confirmed in our study. Apparent differences in the choice behaviour compared to the experiments in the green-yellow range corroborate that another antagonistic behaviour aside from 'settling' is most likely the reason for the attraction (Coombe, 1981; 1982; Stukenberg et al., 2015). One important indication was the low speed of orientation and generally low recapture rates resulting in long trial durations to achieve sufficient numbers of trapped individuals. Moreover, it could be visually observed that the orientation was not as target-oriented as the response to green since individuals tended to rest somewhere in the upper part of the cage before the traps were approached.

A relatively ambiguous wavelength dependence was determined with no significantly distinguished LEDs in the UV range below 400 nm and a high variance of the choice data (Fig.

3G). The attractiveness decreased at 400 nm but was still present in the blue range (Fig. 3H), indicating a relatively wide sensitivity. Nevertheless, the half-sided action spectrum can most likely be attributed to a uniform behaviour (Fig. 5). The observed peak of the action spectrum at 373 nm allows no final conclusion about the most attractive UV LED because we could not test high power LEDs with smaller wavelengths as they are not yet available. According to these results, it could be assumed that the observed behaviour is based only on one receptor in the UV range because no indication for a chromatic interaction with an adjacent receptor could be found. This is also supported by the colour choice model which explains the data best with a receptor peak sensitivity at 360 nm (Fig. 5, 7C). Therefore, it can be concluded that the position and peak sensitivity of the UV receptor lies between 340 and 370 nm supporting the existing study by Mellor et al. (1997) showing a UV peak around 340-350 nm in ERG recordings.

However, the conclusion that the UV receptor does not at all interact with another receptor in a behavioural context might be misleading and has to be scrutinized carefully. In a natural environment, significant intensities of UV radiation are always associated with skylight which contains all wavelengths while the light reflected from natural objects usually contains relatively small amounts of UV. It is suggested that insects could generally use a threshold-based UV-green contrast to detect skyline features and to perform landmark navigation tasks (Möller, 2002). This opponent interaction with inhibitory input from a distant green receptor would enable insects to discriminate the sky from terrestrial objects in most cases. A UV-green contrast allows a better discrimination in this context than an assumed UV-blue contrast. Therefore, it might not be the total intensity but rather the UV-green ratio in the perceived light which determines the classification into sky and object. Light with a UV ratio above a certain threshold might be classified as sky while objects with a lower UV ratio should theoretically appear as a dark silhouette.

We can assume that the UV radiation emitted by the traps in our setup competes with the UV radiation naturally entering the cage, thus skylight and trap should appear similar in this behavioural context. Theoretically, the UV traps in our setup could be perceived by the whiteflies as additional entry points for skylight which elicit an ‘open-space reaction’ as described for butterflies (Scherer and Kolb, 1987). Similarly, UV patches could be used by *T. vaporariorum* in the natural environment to find a way out of a plant canopy in order to conduct dispersal flights. But it is important to note that the solitary UV radiation emitted from LEDs in our setup is highly artificial as compared to the green-yellow LEDs which basically imitate host plants. Although not much UV radiation is transmitted through the greenhouse glass, the UV intensity measured at the release point was frequently higher than received from the traps. Only with closer distance to the traps the UV intensity became higher compared to skylight. The reason why the traps with comparably low UV intensities under such daylight conditions

were attractive for whiteflies could be the mentioned UV-green ratio (Möller, 2002) which should be high due to the lack of any green light. The possibility of a UV-green contrast coincides with the antagonistic character of the behavioural pattern towards UV as compared to green (Coombe, 1981; 1982). The rationale of such a UV-green contrast mechanism to discriminate sky and object represents a convincing explanation but needs further investigations in the future.

2.5.6 Conclusion and Outlook

Translated into the natural environment, *T. vaporariorum* uses the discussed chromatic mechanism to extract a colour signal to decide if the perceived object is a host plant or not. All objects with significant reflection in the green-yellow range (500-600 nm) and low reflection in the violet-blue range (400-500 nm) are potentially seen as host plants and elicit settling. Within the green-yellow range of the action spectrum the whitefly selects the brightest stimulus with the highest excitation of the opponent mechanism. The intensity dependence may be used to detect young leaves of brighter green or top leaves exposed to the sun and showing higher reflectance or transmittance than shaded ones.

This study represents the first detailed LED-based investigation on whitefly visual behaviour resulting in LED-based action spectra under natural sunlight conditions. This has profound relevance for the basic understanding of the visual mechanism of *Trialeurodes vaporariorum* and provides the basis for the improvement of visual trapping methods for monitoring and control in greenhouses. A recent study already showed the effect of modifying the reflective properties of yellow card traps (Sampson et al., 2018). This can be further specified according to the determined wavelength and intensity dependence, the photoreceptor sensitivity estimations, and the colour choice model from our study. Moreover, LEDs which enhance the attractiveness of visual traps can be more appropriately selected according to our results (Stukenberg et al., 2015). The developed method generally provides great possibilities for future studies on the visual ecology of insects. An important notion is that we obtained comparable results regarding spectral efficiencies with multiple-choice and dual-choice experiments, thus for rapid wavelength screenings time consuming dual-choice experiments could be neglected in the future. The method could also be extended to more detailed LED mixing experiments under controlled ambient light conditions to provide a better basis for more precise modelling of photoreceptor sensitivities and interactions. Future studies should especially focus on the behaviour related to UV radiation and the underlying mechanisms.

3 Visual orientation of the black fungus gnat, *Bradysia difformis*, explored using LEDs

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3.1 Abstract

Fungus gnats occur worldwide with more than 1 700 described species. They can cause serious damages on ornamentals, crop plants, and edible mushrooms, and are considered to be a serious pest in the last years. *Bradysia difformis* Frey (Diptera: Sciaridae) represents a common species in Europe. Usually, yellow sticky traps are used for monitoring and control in greenhouses and fluorescent tube-based light traps are additionally applied for control in mushroom cultivation. The importance of such visual trapping measures for efficient monitoring or alternative control increases in biological and integrated plant protection. However, detailed color preferences of fungus gnats are mostly unknown.

We studied the visual orientation of *B. difformis* with light-emitting diodes (LEDs) in a broad range of peak wavelengths from 371 nm (ultraviolet, UV) to 619 nm (amber). We determined attractive wavelengths in consecutive choice experiments in daylight and darkness.

Highest numbers of adult *B. difformis* were attracted to UV radiation (382 nm) followed by green-yellow light (532-592 nm). The responses to UV and the green-yellow range were relatively unspecific and mostly independent from intensity. Combination of UV and yellow LEDs improved trapping efficacy compared to a single UV or yellow LED trap, as well as compared to a common yellow sticky trap. When both wavelengths were compared to a black surface to increase contrasts, the black surface was preferred over yellow, but was less attractive than UV. Thus, *B. difformis* displays two, probably wavelength-specific, behaviors to UV radiation and green-yellow light, with UV being the most attractive stimulus. These behaviors might be directly related to underlying photoreceptors, suggesting dichromatic vision in *B. difformis*.

Key words: sciarid fly, Diptera, Sciaridae, color preference, wavelength-specific behavior, greenhouse pest, light-emitting diode, LED trap, monitoring, biological control

3.2 Introduction

Bradysia difformis Frey (Diptera: Sciaridae) is an important greenhouse pest in Europe. It causes heavy damages in several crops and commercial mushrooms but has been neglected for many years (Rinker et al., 2010; Menzel et al., 2003; Shin et al., 2012). The most damaging stage in the fungus gnats' life cycle is the larval stage in the growing medium. The larvae are extremely polyphagous and feed on roots and root hairs of many crops like *Capsicum* spp. and *Geranium* spp. and on mycelium of mushrooms (Cloyd, 2010; Shin et al., 2012). Feeding on the roots interferes directly with the plants' ability to take up water and nutrients (Cloyd, 2010; 2015). Indirect damages are caused by soil borne pathogens, which can infect the plants through wounds created by larval feeding (Ferguson et al., 2006). Adults can carry spores of pathogens (e.g., *Fusarium* spp.) on their body and spread them to other greenhouse areas, but do not cause direct damages (Ferguson et al., 2006; Cloyd, 2010; Bethke and Dreistadt, 2013). Adult fungus gnats feed on water and nectar and thereby can pollinate flowers (Bealmer, 2010; Duque Buitrago et al., 2014).

Biological control is possible with entomopathogenic nematodes (*Steinernema* spp., *Heterorhabditis* spp.) and/or predatory mites such as *Hypoaspis miles* Berlese, but efficacy of these antagonists is dependent on time of application (Gouge and Hague, 1995; Jagdale et al., 2007; Scheepmaker et al., 1997; Cloyd, 2010; Cloyd and Sadof C. S.). Therefore, a key factor for successful biological control is efficient and reliable detection of fungus gnats with an appropriate monitoring technology. Vision-based mass trapping can be an appropriate strategy in small crops like potted ornamental plants. Mass trapping and monitoring fungus gnats in greenhouses involves yellow sticky traps (Cloyd, 2010) but also studies with visual traps equipped with narrow-bandwidth light emitting diodes (LEDs) have been performed (Chen et al., 2004; Sonoda et al., 2014). In contrast, light traps emitting a broad wavelength spectrum with peaks in the UV-violet range, or fluorescent black lamps emitting UV radiation and visible blue light, have been proven suitable tools for trapping sciarids in mushroom cultivation (Ishitani et al., 1997; Jess and Bingham, 1999). Light traps have also been used in mushroom cultivation to monitor flight activity of fungus gnats for the determination of pesticide application thresholds (Jess and Kilpatrick, 2000). The attractiveness of colored sticky or water pan traps is based on reflection of incident radiation by the color pigments. The reflection pattern is often relatively broad and not exactly adapted to the peak sensitivity of the target insect's photoreceptors. Moreover, the reflection pattern and in particular its intensity depends on environmental conditions like position of the sun, clouds, and shades (Johansen et al., 2011). For instance attractiveness of yellow traps for fungus gnats is related to brightness of illumination (Cloyd et al., 2007).

To overcome these drawbacks and to improve the efficiency of conventional visual traps, Chen et al. (2004) combined yellow sticky traps with green LEDs (525 nm) and Sonoda et al. (2014) equipped pan traps with UV (365 nm) and green (530 nm) LEDs. Both attempts increased the capture of fungus gnats compared to non-LED-supplemented traps. Apart from the known yellow and UV sensitivity, undetermined sciarid species are attracted by cyan (502 nm) and blue (471 nm) light in dark caves (Stringer and Meyer-Rochow, 1994).

In general, the use of LEDs seems to be a promising approach for improvement of conventional reflective traps. Reasons are the bright narrow-band emission and the possibility to select the emitted wavelength specifically with regard to color preferences of target insects (Burkett et al., 1998; Chen et al., 2004; Cohnstaedt et al., 2008; Johansen et al., 2011; Sonoda et al., 2014). Moreover, the narrow-bandwidth LED radiation offers the possibility to study combinations of wavelengths which could act synergistically or inhibitory as, for example, shown with whiteflies. This could lead to better understanding of potential interactions of the photoreceptors (Stukenberg et al., 2015). Contrasts, which are crucial for object detection in insects, might also play an important role (Prokopy and Owens, 1983).

So far, only the general attractiveness of UV, green LEDs, and yellow sticky traps is known for fungus gnats. Detailed differentiation of the preferences in narrow wavelength ranges or combinations of wavelengths with controlled intensities is still missing. This study aims to determine the color preferences of *B. difformis* basically by using 13 LEDs with peak wavelengths from UV (371 nm) to amber (619 nm) in choice tests. Moreover, we evaluated combinations of wavelengths as well as the influence of contrasts on the choice behavior. We expect basic new findings about the visual system of *B. difformis* with regard to potential underlying photoreceptors and interactions between them. This information may help develop selective and sensitive monitoring tools, to control this pest by visual trapping technology and to improve integrated pest management (IPM) and biological control strategies.

3.3 Material and methods

3.3.1 LED-traps

High-power (HP) LEDs (Table 1) were attached to aluminum panels (10 × 10 × 0.1 cm) at the rear of grey PVC (4 mm thick) boxes (10 × 10 × 13 cm) (Figure 1A). The front of the boxes was enclosed by transparent opal acrylic glass plates (10 × 10 × 0.3 cm; Plexiglas LED 0M200 SC; Evonik Industries, Essen, Germany) which served as scatter screens for the punctual radiation of the LEDs. In addition, the inner walls of the boxes were coated with mirror foil (Pearl, Buggingen, Germany). The screen was covered with transparent household plastic wrap (polyethylene terephthalate; REWE Handelsgruppe, Cologne, Germany) coated with

insect glue (Temmen, Hattersheim, Germany) to capture the adult fungus gnats after contact to the screen. To investigate the influence of contrasts on the visually controlled choice behavior of *B. difformis*, the transparent foils were substituted for a piece of black plastic Sunup Reflective film (10 × 10 cm; Star Metallizing, Oceanside, CA, USA). Similarly, a same sized yellow sticky trap (10 × 10 cm; Neudorff, Emmerthal, Germany) was used for two-choice comparisons with LEDs. The LEDs operated with 24 V (PLC 100-24; Mean Well, New Taipei City, Taiwan) and HP LED drivers (LED-Slave V4 PWM; PCB-Components, Hildesheim, Germany) which enabled adjustment of individual intensities with universal 10 kΩ rotary potentiometers. The LED drivers and potentiometers were installed in an aluminum box. For most of the tested wavelengths, one HP LED was inserted per trap. For 579 and 592 nm, four and two HP LEDs, respectively, were installed because due to technical limitations, the required intensity could not be achieved with single HP LED of these wavelengths. The 579 nm peak-wavelength HP LED (multichip emitter) had to be cooled additionally by a heat sink with a fan (LED cooling module LA001-011A9DDN; Sunonwealth Electric Machine Industry, Kaohsiung City, Taiwan) and the green HP LED (547 nm peak-wavelength, multichip emitter) was cooled by a passive heat sink (Fischer Elektronik, Lüdenscheid, Germany).

Table 1 Specifications of High-Power (HP) LEDs used in experiments

LED color and codes		Peak wavelength (nm)	Manufacturer	Type
UV	UV1	371	Roithner	H2A1-H365-E
	UV2	375	Roithner	H2A1-H375-E
	UV3	382	Roithner	H2A1-H385
	UV4	397	Roithner	H2A1-H395
Violet	V1	408	Roithner	H2A1-H405
	V2	414	Roithner	H2A1-H410
Blue	B	446	Osram	Oslon SSL 80 LD CQ7P
Cyan	C	501	Roithner	H2A1-H490
Green	G1	532	Osram	Oslon SSL 80 LT CP7P
	G2	547	Roithner	LED550-66-60
Yellow	Y1	579	Roithner	LED570-66-60
	Y2	592	Osram	Oslon SSL 80 LA CP7P
Amber	A	619	Osram	Oslon SSL 80 LA CP7P

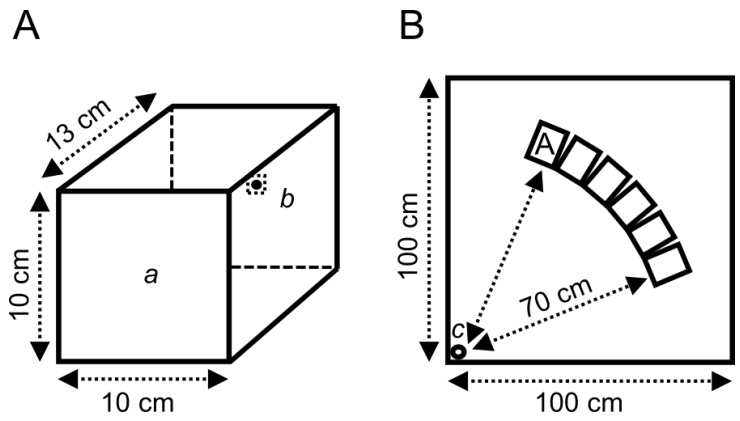

Figure 1 (A) Scheme of experimental LED trap screens, with an acrylic glass screen at the front (a) and the LED on an aluminium panel in the back (b). (B) Cage setup used for the fungus gnat LED choice experiments, indicating a range of trap screens (A), all at 70 cm from the release point (c).

3.3.2 Spectral and intensity measurement

Emission spectra of the HP LEDs were measured in a dark room with a spectrometer (AvaSpec-2048-2 and AvaSoft v.7.0.3 Basic; Avantes, Apeldoorn, The Netherlands). The reflection spectrum of the yellow sticky trap was measured with the spectrometer Lambda 900 UV/VIS/NIR (PerkinElmer, Waltham, MA, USA) containing a 30-cm integrating sphere and a tungsten-halogen and deuterium lamp (Figure 2). To measure and equalize intensities (photon flux, µmol m^{-2} s^{-1}) of HP LEDs with peak-wavelengths >385 nm, the LI-250 A Light Meter with LI 190 Quantum Sensor (LI-COR Biosciences, Lincoln, NE, USA) was used. For peak-wavelengths <385 nm the datalogger Almemo 2390-5 (Ahlborn Mess- und Regelungstechnik, Holzkirchen, Germany) in combination with a UV-A sensor (Type 2.5; Indium Sensor, Neuenhagen, Germany) was used. The data for peak-wavelengths <385 nm were indicated in W m^{-2} and were converted to µmol m^{-2} s^{-1} using the LED spectra, Planck's constant, and Avogadro's number. The intensity measurements were also performed in darkness.

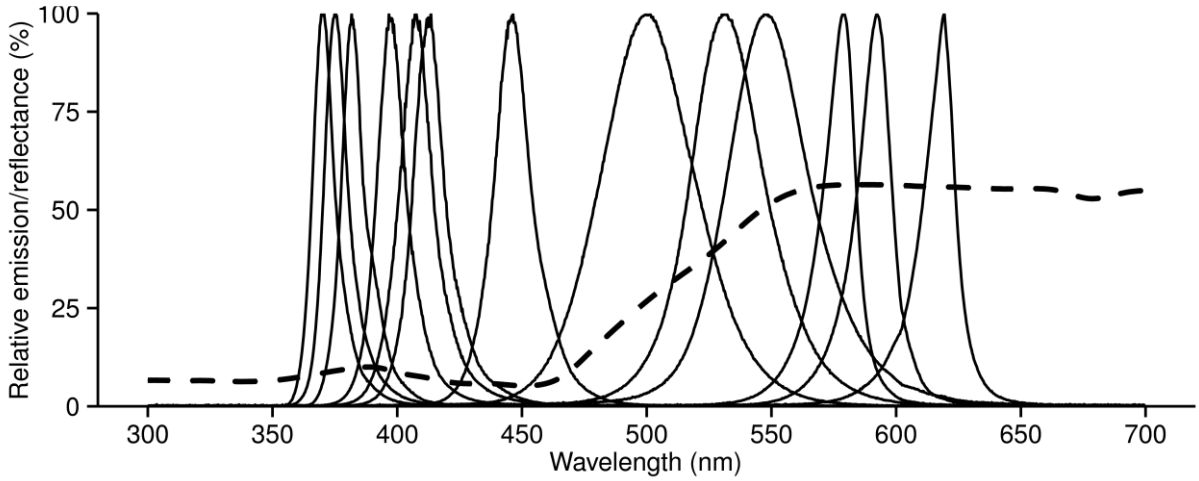

Figure 2 Spectra of LEDs (solid lines) and the yellow trap (dashed line) used in the experiments.

3.3.3 Fungus gnat rearing and handling

Bradysia difformis larvae were initially obtained from the Julius Kühn-Institut (Federal Research Center for Cultivated Plants, Braunschweig, Germany). The insects were maintained in gauze cages in an air-conditioned room of the Section of Phytomedicine (Institute of Horticultural Production Systems, Leibniz Universität Hannover, Germany) at an average temperature of 22 °C and 16 h light per day. The basis for rearing was coir terrarium substrate (635 g, Plantation Soil 8.8 L; Exo Terra, Montreal, Canada) in a plastic tray (40 × 60 cm), which was initially mixed with 8 l water. Weekly, 1.5 l water was added and additionally 100 g oat flakes were strewed on the surface and moistened twice a week with approximately 0.2 l water. For each experimental trial 3- to 5-day-old fungus gnats of undetermined sex were carefully collected with an aspirator into a snap-on lid glass vial (10 cm high, 3 cm diameter) and immediately released in the experimental gauze cage.

3.3.4 Experimental setup

Experiments were conducted in the central greenhouse compartment of the Institute of Horticultural Production Systems, Section Phytomedicine (Leibniz Universität Hannover) at 20-35 °C. Inside a gauze-covered flight cage (1 × 1 × 0.8 m; Figure 1B) the adult fungus gnats were released in the left corner opposite the trap row by means of snap-on lid glass vials. In multiple-choice experiments, the LED-traps were placed adjacent to each other in a curved line 70 cm in front of the release point, so that each trap had the same distance to it (Figure 1B). In two-choice experiments two traps were placed in similar distance to the release point, but with a distance of 10 cm between each other. All experiments were conducted during daytime under greenhouse conditions. Experiments in ‘darkness’ were conducted in the same time but the gauze cage was completely covered with a black plastic film (Sunup Reflective Films; Star Metallizing, Oceanside, USA). Therefore, experimental light conditions refer to either daylight (uncovered cage) or darkness (covered cage).

3.3.5 Experiments

Overview: A series of experiments was conducted to investigate the color preferences and choice behavior of *B. difformis*. At first, visible light of five peak-wavelengths with (experiments 1 and 2) and without (experiments 3 and 4) UV radiation were tested in daylight (1 and 3) and in darkness (2 and 4) to get basic data about color preferences. Second, the UV-violet and the green-yellow range were investigated in darkness more in detail with six peak-wavelengths, respectively (experiments 5 and 6). Afterwards, peak-wavelengths with highest capture rates of fungus gnats from experiments 5 and 6 were selected and compared to each other at various intensities (experiment 7). UV and yellow were combined in one trap and compared to each of both wavelengths alone and to a yellow sticky trap (Neudorff) in daylight (experiment 8). Finally, the choice behavior of *B. difformis* between a black surface (Sunup Reflective Films;

Star Metallizing, Oceanside, USA) and selected color targets (UV and yellow LED-trap) was compared (experiment 9) to check for possible influences of light/dark contrast on the choice behavior.

General experimental design and procedure: All experiments were conducted in the same cage with consecutive replications over time. Depending on the availability of sufficient numbers of adult fungus gnats, consecutive replications were performed from 08:00 to 16:00 hours. Experiments 1-6 consisted of 20 replications with a duration of 2 h each. Experiments 7-9 were performed with 10 replications of 0.5 h. The intensity of each HP LED was adjusted to 0.25 (experiments 1-6) or 0.4 µmol m^{-2} s^{-1} (experiment 8) at 70 cm distance from the light source. In each trial 40 adult fungus gnats were released and after the given duration the trapped individuals were counted per trap. The total number of released individuals was corrected by subtracting the number of dead fungus gnats remaining in the release vial. The flight cage was carefully cleaned with a small vacuum and the position of the traps was randomly rearranged before each replication.

Details of multiple-choice and two-choice experiments: The details of multiple-choice experiments 1-6 regarding compared LEDs, light conditions, and the timescales of replications are given in Table 2.

Table 2 Details of multiple-choice experiments 1-6. LED codes refer to Table 1

Experiment		Compared LEDs						Ambient light	Timescale
1	Wide	UV1	V2	B	C	G1	Y2	Daylight	15 September - 2 October 2014
2	range							Darkness	20 November 2014 - 9 January 2015
3			V2	B	C	G1	Y2	Daylight	20 February - 4 March 2015
4								Darkness	12 January - 3 February 2015
5	Narrow	UV1	UV2	UV3	UV4	V1	V2	Darkness	23 March - 7 April 2015
6	range	C	G1	G2	Y1	Y2	A	Darkness	27 April - 5 May 2015

Experiment 7: Five intensities of yellow (Y2, 592 nm) were compared in two consecutive multiple-choice experiments (daylight and darkness). Two intensities of UV LED-traps (UV3, 382 nm) were compared in a two-choice experiment in darkness. To obtain five intensities of the yellow LED-trap, the maximum intensity was reduced in 20% tiers. Relative intensities of 100, 80, 60, 40, and 20% corresponded with 0.41, 0.33, 0.25, 0.16, and 0.08 µmol m^{-2} s^{-1} absolute intensity at 70 cm distance. For UV, the maximum intensity was reduced by half (100 and 50%, i.e., 0.93 and 0.47 µmol m^{-2} s^{-1} at 70 cm distance). Replicates were conducted on 19 May 2015 (yellow, daylight), 13-18 May 2015 (yellow, darkness), and on 12-15 June 2015 (UV, darkness).

Experiment 8: Yellow (Y2) and UV (UV3) LEDs were combined in one trap by adjacent adjustment on one aluminum panel. The combination was tested against either yellow and UV alone or a yellow sticky trap in three consecutive two-choice experiments. The intensity of the single wavelength LED-traps (Y2 or UV3) was set to 0.4 µmol m^{-2} s^{-1} at 70 cm distance, whereas UV and yellow LEDs in combination were set to 0.2 µmol m^{-2} s^{-1} each to achieve an equal and comparable overall intensity. Replicates were conducted on 28 May 2015 (UV), 28-29 May 2015 (yellow), and on 1-2 June 2015 (yellow sticky trap).

Experiment 9: The LED-traps from the previous experiment (UV and yellow, same intensity) were compared with a black surface trap in two consecutive two-choice experiments. Replicates were conducted on 23-25 June 2015 (yellow LED) and 21-23 July 2015 (UV LED).

3.3.6 Statistical analysis

Analyses were performed in R v.3.2.1 (R Core Team, 2015). All experiments were analyzed with generalized linear models (glm) with quasi-Poisson distribution and log link function. The response variable was the number of captured adult fungus gnats on each trap as randomized treatment effects. Depending on the experiment, the explanatory treatment variables were the LED colors, intensities, or trap types. Trials of an experiment, which were consecutively performed in the same cage, were included as block factors. Deviance analysis was applied to determine whether there is a main effect of LED colors, intensities, or trap types on the number of trapped fungus gnat adults. Tukey-type multiple comparisons (R-package multcomp; $\alpha = 5\%$) were performed to determine differences between explanatory variables (McCullagh and Nelder, 1989; Hothorn et al., 2008).

3.4 Results

3.4.1 Choice experiments

Experiments 1-4: The wide-range spectral screening at daylight clearly indicated high attractiveness of the UV LED-trap to fungus gnats, which was preferred over the other wavelengths (experiment 1; $P<0.001$; Figure 3A). This attractiveness was slightly extended to the violet LED ($\lambda \approx 410$ nm), which trapped higher numbers of adult fungus gnats than cyan ($\lambda = 501$ nm; $P = 0.043$). Although of minor distinctness, a second attractive domain came up around the green LED ($\lambda \approx 540$ nm) which trapped higher numbers than blue ($\lambda = 446$ nm; $P = 0.025$) and cyan ($P = 0.012$) (Figure 3A).

In darkness (experiment 2), the attractiveness of the UV domain was more distinct and extended to violet and blue, thereby reducing the relative attractiveness of green (Figure 3B). UV was equally attractive ($P<0.001$) as in daylight (experiment 1) but comparatively more

fungus gnats were trapped on violet, which differed from blue (P = 0.030), cyan (P<0.001), and yellow (λ ≈ 585 nm; P = 0.0099).

When the UV LED was excluded, the number of trapped fungus gnats was higher on violet, green, and yellow LED-traps than on blue and cyan LED-traps in the daytime (experiment 3; P<0.001; Figure 3C). Therefore, violet and green-yellow appeared as two attractive spectral ranges. In darkness, violet alone was more attractive than the other wavelengths (experiment 4; P<0.001; Figure 3D). This resembles the relative shift in attractiveness to the shorter wavelengths as found in experiment 2.

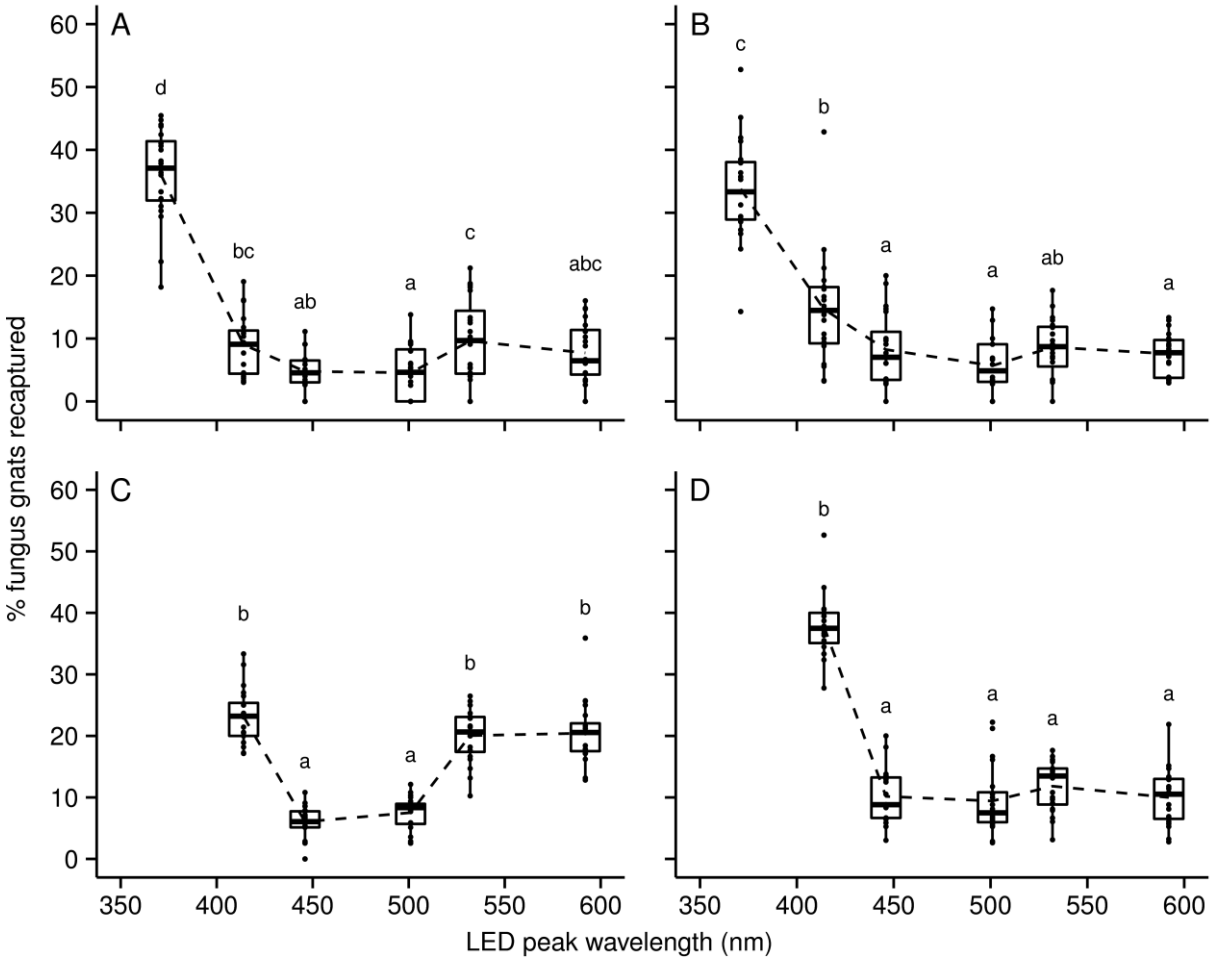

Figure 3 Trapped fungus gnats (%) in multiple-choice experiments with various LED colors (V2, B, C, G1, Y2; see Table 2) in combination with UV in (A) daylight (experiment 1) and (B) darkness (exp. 2), and without UV in (C) daylight (exp. 3) and (D) darkness (exp. 4). Mean (± SD) total recapture rate = 71.7 ± 4.0% (exp. 1), 78.6 ± 4.1% (exp. 2), 77.2 ± 4.3% (exp. 3), and 79.2 ± 3.3% (exp. 4). Dots represent original data points (n = 20). The boxes represent interquartile ranges (IQR) showing the first quartile (bottom), median (thick line) and third quartile (top). Lower and upper whiskers indicate highest and lowest value within 1.5 × IQR respectively. Single points (outliers) are outside 1.5 × IQR. The dashed line connects mean values. Means within a panel capped with different letters are significantly different (GLM, Tukey test: P<0.05).

Experiments 5-6: In the narrow-range UV-violet experiment (experiment 5) more adult fungus gnats were trapped by the 382-nm UV LED than by any of the others (P<0.014; Figure 4A). All UV LEDs were more attractive than violet LEDs with 408 or 414 nm (P<0.0015).

In the narrow-range green-yellow experiment (experiment 6), the highest number of fungus gnats was trapped on the yellow LED trap (592 nm) with a significant difference only for cyan with 501 nm peak wavelength (P = 0.049; Figure 4B).

Experiment 7: When different intensities of the same yellow LED wavelength were compared, differences in the number of trapped fungus gnats were only found with the LEDs in darkness (Figure 5B). Here, the maximum intensity was preferred over the lowest intensity (P = 0.029) whereas in daylight (Figure 5A) no differences were recorded. For UV, the trap catches did not differ between the two intensities tested (P = 0.16) (Figure 5C).

Experiment 8: The combination of UV and yellow LEDs (382 and 592 nm) was significantly more attractive to fungus gnat adults than yellow LEDs alone or the yellow sticky trap (P<0.001), but not than UV alone (Figure 6).

Experiment 9: The black surface was more attractive to fungus gnat adults than the yellow LED-trap (592 nm), but less attractive than the UV LED-trap (382 nm) (P<0.001; Figure 7).

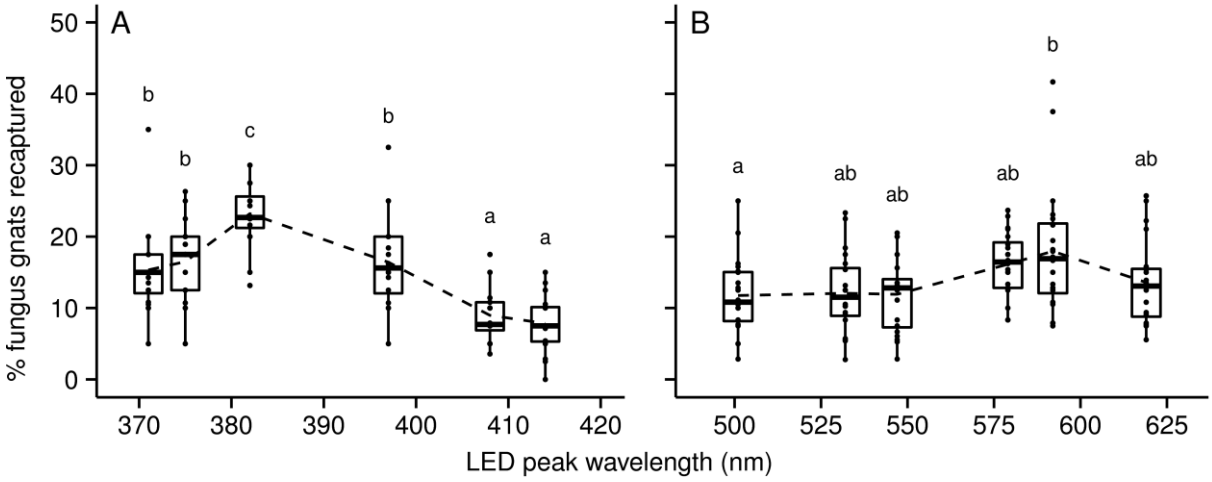

Figure 4 Trapped fungus gnats (%) in multiple-choice experiments with various LED colors in the (A) UV-violet (experiment 5; UV1-4, V1, V2; see Table 2) and (B) green-yellow (exp. 6; C, G1, G2, Y1, Y2, A; see Table 2) spectral range. Mean (± SD) total recapture rate = 88.3 ± 5.3% (exp. 5) and 83.4 ± 3.8% (exp. 6). Dots represent original data points (n = 20). The boxes represent interquartile ranges (IQR) showing the first quartile (bottom), median (thick line) and third quartile (top). Lower and upper whiskers indicate highest and lowest value within 1.5 × IQR respectively. Single points (outliers) are outside 1.5 × IQR. The dashed line connects mean values. Means within a panel capped with different letters are significantly different (GLM, Tukey test: P<0.05).

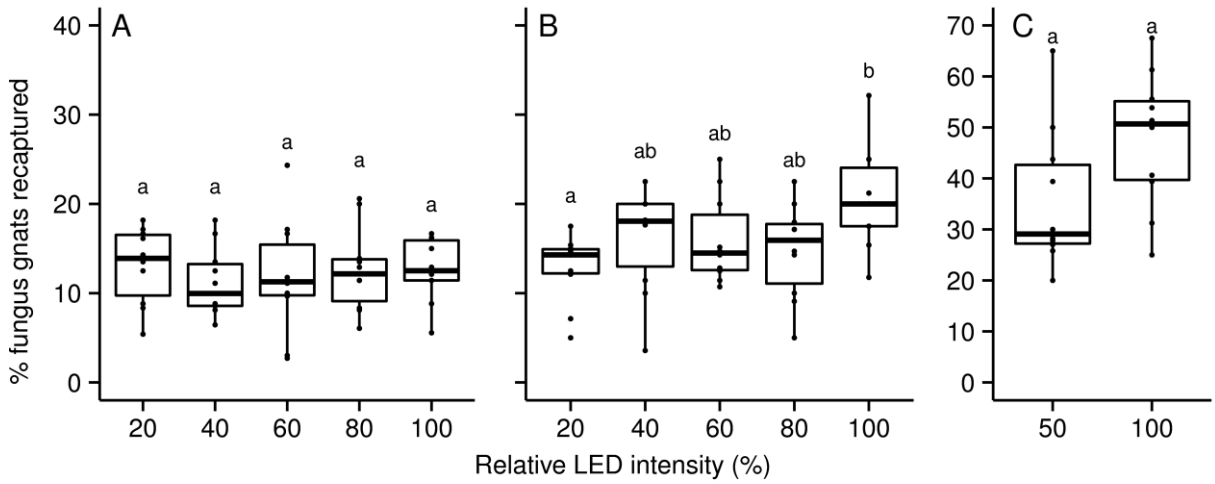

Figure 5 Trapped fungus gnats (%) in multiple-choice experiments with various intensities of yellow LEDs in (A) daylight and (B) darkness, and (C) in a two-choice experiment with different intensities of UV LEDs (experiment 7). Mean (± SD) total recapture rate = 61.4 ± 4.9% (A), 80.2 ± 7.5% (B), and 83.3 ± 3.9% (C). Dots represent original data points (n = 10). The boxes represent interquartile ranges (IQR) showing the first quartile (bottom), median (thick line) and third quartile (top). Lower and upper whiskers indicate highest and lowest value within 1.5 × IQR respectively. Single points (outliers) are outside 1.5 × IQR. Means within a panel capped with different letters are significantly different (GLM, Tukey test: P<0.05).

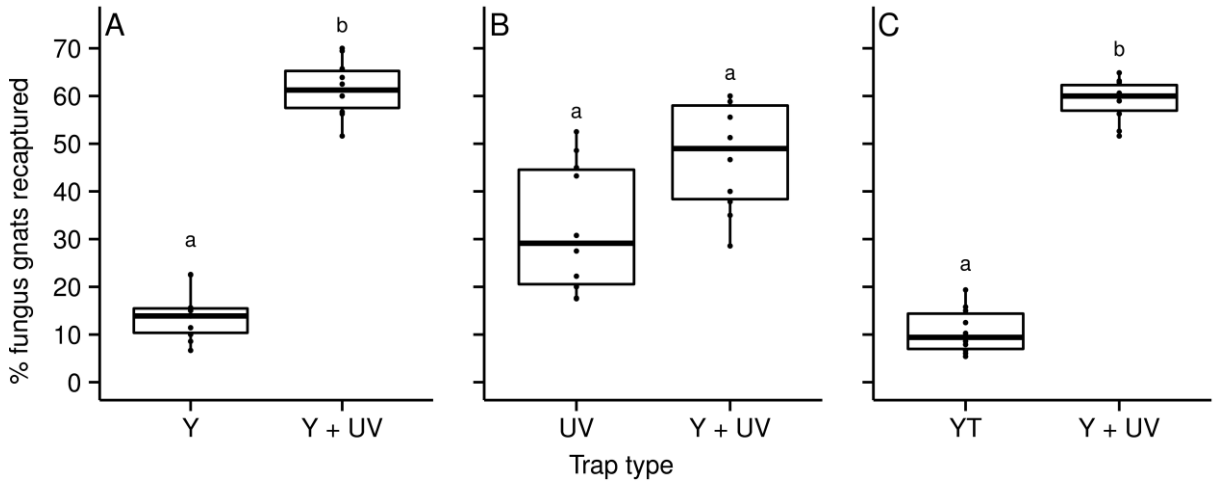

Figure 6 Trapped fungus gnats (%) in two-choice experiments with (A) yellow LED (Y), (B) UV LED (UV), and (C) yellow traps (YT) compared to a combination of yellow and UV LED (Y + UV). Mean (± SD) total recapture rate = 75.6 ± 5.1% (A), 79.9 ± 6.2% (B), and 69.8 ± 2.5% (C). Dots represent original data points (n = 10). The boxes represent interquartile ranges (IQR) showing the first quartile (bottom), median (thick line) and third quartile (top). Lower and upper whiskers indicate highest and lowest value within 1.5 × IQR respectively. Single points (outliers) are outside 1.5 × IQR. Means within a panel capped with different letters are significantly different (GLM, Tukey test: P<0.05).

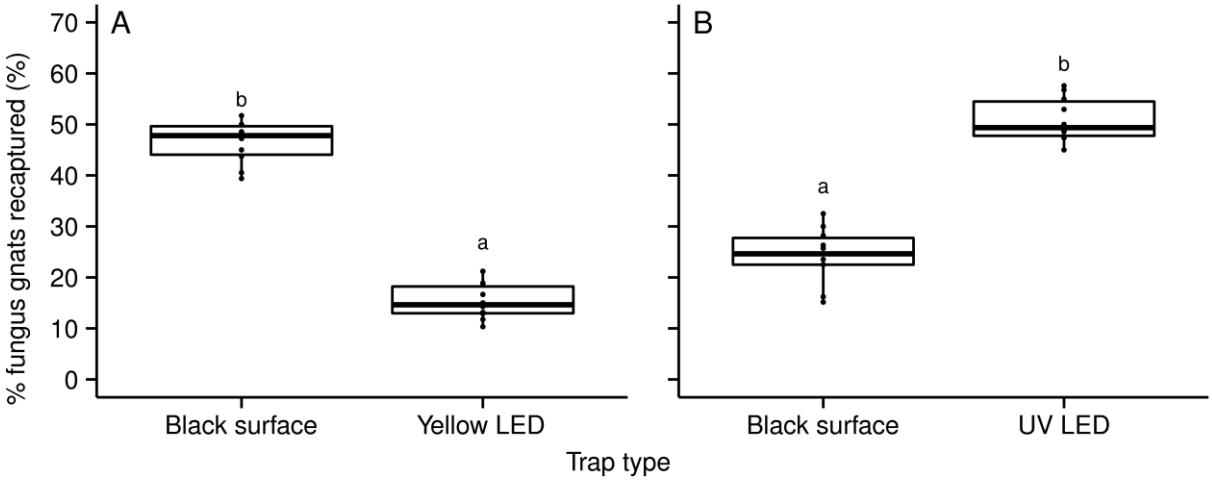

Figure 7 Trapped fungus gnats (%) in two-choice experiments with (A) yellow LED and (B) UV LED compared to a black surface. Mean (± SD) total recapture rate = 61.7 ± 1.4% (A) and 75.2 ± 2.4% (B). Dots represent original data points (n = 10). The boxes represent interquartile ranges (IQR) showing the first quartile (bottom), median (thick line) and third quartile (top). Lower and upper whiskers indicate highest and lowest value within 1.5 × IQR respectively. Single points (outliers) are outside 1.5 × IQR. Means within a panel capped with different letters are significantly different (GLM, Tukey test: P<0.05).

3.4.2 Recapture rates and mortality

The recapture rates of live individuals of the various experiments cannot be directly compared statistically, as they were obtained in independent experiments on different dates under different conditions (temperature, humidity, solar radiation) and with different cohorts of fungus gnats. Nevertheless, the recapture rates were relatively consistent ranging from 61.4 ± 4.9% (mean ± SD; Figure 5A) to 88.3 ± 5.3% (Figure 4A) with an overall mean recapture rate of 77.2%. Recapture rates were always higher when obtained in darkness and when UV LED-traps were involved. Mortality in the experiments ranged from (mean ± SD =) 3.1 ± 7.2 to 24.5 ± 13.3% and is unlikely to have affected the results much, as ample vital individuals were present in each trial.

3.5 Discussion

UV radiation proved to be the strongest attractive stimulus for fungus gnats in daylight and in darkness. The green-yellow spectral range was second most attractive. The attraction of *B. differmis* to UV LEDs was highest around 382 nm peak wavelength and might directly correspond with an underlying UV receptor. However, this peak was not very distinct and all UV LEDs displayed a comparably high attractiveness. A decline was obvious in the violet-blue spectral range, which was not as attractive as UV or green-yellow. This clear gap shows that *B. differmis* can clearly distinguish visually between UV radiation and long wavelength visible light. Consequently, there should exist at least one further photoreceptor for longer wavelengths from 532-592 nm, presumably more in the yellow spectral range, indicating a

dichromatic vision for *B. difformis* (Briscoe and Chittka, 2001; Döring and Chittka, 2007). Distinct spectral sensitivities, however, could not be shown in the longer wavelength range, assuming a receptor with broad sensitivity.

Differences were obtained in the spectral reactions in daylight or darkness. Green and yellow were as attractive as violet in daylight but not in darkness. With presence of the UV LED-trap the attraction to the green LED was stronger under daylight conditions, resulting in a lower reaction to violet compared to darkness. This could be caused by an overlap of UV radiation from the LED-traps with the UV radiation from ambient light, causing a lower response to the UV-trap and a stronger response to visible light traps. It is also known that photoreceptors adapt to the background light intensity by adjusting their responses (Laughlin and Hardie, 1978). Therefore, under daylight conditions where small intensities of UV radiation were present in the greenhouse, the response to UV might have been decreased. Most probably, under daylight conditions, violet stimulates also partly the UV-related response but the attraction to green and yellow is stronger in the absence of the UV stimulus. An explanation could be that the attraction to colored LEDs in the visible range is bound to simultaneous background perception of daylight because colored objects naturally appear only by reflection of sunlight. Whiteflies, for example, responded only weakly to green LEDs at nighttime, and were most sensitive in daylight (Stukenberg et al., 2015).

UV radiation triggers orientation of insects and controls take off and flight behavior (Scherer and Kolb, 1987; Costa et al., 2002; Chyzik et al., 2003). It is well known that several insects display less flight activity and avoid immigration in UV-deficient environments (Kumar and Poehling, 2006). The attraction of fungus gnats to UV radiation in daylight and darkness should therefore be an achromatic phototactic response generally controlling orientation and flight activity. Interestingly, the response to UV did not differ much among UV intensities in our short-range setup, which would indicate a chromatic and intensity independent mechanism (Kelber et al., 2003; Kelber and Osorio, 2010). But most likely this could be explained by the high and broad sensitivity for UV radiation, resulting in a poor distinction of intensity gradients, especially when the UV targets are close together like in our experiments. Probably, the existence of UV radiation, widely independent of intensity or wavelength, is sufficient for stimulation of the observed behavior in *B. difformis*.

We did not observe significant differences in the attraction to green-yellow with regard to wavelength or intensity. Apart from the blue range, fungus gnats seem to sense the green-yellow range in a broad manner and mostly independent of its intensity, indicating again a chromatic mechanism (Kelber et al., 2003; Kelber and Osorio, 2010). A slight preference for the highest intensity in darkness only, indicated a slight intensity dependence under such special backlight conditions. The broad spectral sensitivity in the visible and in the UV range,

as well as the missing intensity dependence, could be explained by the putative 'self-screening' properties of the underlying photoreceptors. Self-screening is the result of absorbance over a long photoreceptor with a multi-layer arrangement of photoreceptor pigments, which broadens the spectral sensitivity due to increased absorptance. High absorptance is conducive to photon capture for vision in twilight, but it is a disadvantage for color vision in daylight (Cronin et al., 2014).

Broad spectral sensitivity of the UV and green receptor may explain why *B. differmis* showed only slightly different responses within the UV range and within the green-yellow range in our experiments. The photoreceptors may be matched for photon capture and vision in twilight or for coverage of the whole visible spectrum, which would also explain why the response was mostly intensity independent and elicited already by low intensities. We assume that *B. differmis* has no need to distinctly discriminate spectral domains in the visible range, because they do not have to locate and settle on plants like other herbivorous insects. Their eyes should be optimized to sense reflective surfaces by high photon captures over a broad spectral range.

The combination of UV radiation (382 nm) – the most attractive stimulus – and yellow light (592 nm) – the inferior stimulus – was far more attractive than yellow light alone. The UV stimulus most likely was the key factor for the preference and might just have overruled the yellow stimulus. When the combination was compared with UV radiation alone, attractiveness was slightly (but not significantly) higher. We assume, that the attraction to both wavelengths (UV and yellow) is most likely based on two different 'wavelength-specific behaviors' of *B. differmis*, as suggested for example for whiteflies (Coombe, 1981; Stukenberg et al., 2015). Hypothetically, skylight is associated with UV radiation and yellow light represents reflecting objects. However, daylight also contains yellow and therefore the response towards UV is maybe even slightly enhanced by yellow, as indicated by the observed preferential tendency. It remains unclear whether the behaviors interact additively or synergistically and further experiments should be conducted. Yellow sticky traps normally used for fungus gnat monitoring (Cloyd, 2010) were not as attractive as the trap with UV and yellow LEDs. Most likely, yellow traps lack UV reflection, whereas LED-traps emitted radiation constantly and most likely in higher intensities compared to reflective yellow traps.

The black surface was preferred by fungus gnats in comparison to yellow LEDs. The most likely reason is the high contrast between the black surface and the LED-trap, which is one of the key features for object perception of insects (Prokopy and Owens, 1983). A similar contrast may be found in nature between color-reflecting plant stems, leaves, and flowers, and the dark substrate. As adults of *B. differmis* are mainly searching for convenient egg substrate, it is reasonable that rather dark areas next to plants are preferred targets and that our black surface was associated with a substrate for egg laying. UV radiation, in contrast, was more attractive

than the black surface to adult fungus gnats. This may be due to the response generally involved in orientation and flight activity towards UV radiation, as discussed above (Scherer and Kolb, 1987; Kevan et al., 2001; Costa et al., 2002; Chyzik et al., 2003; Kumar and Poehling, 2006). However, the opposed reaction to UV radiation and yellow light is a further clue for two separate 'wavelength-specific behaviors' in *B. difformis*.

In summary, we demonstrated for the first time in detail that *B. difformis* has a visual system that senses UV radiation and green-yellow light, both with a broad spectral sensitivity. Thereby, UV radiation is the preferred stimulus, in daylight as well as in darkness. In daylight, black targets are approached when presented in contrast to yellow LED traps, which might agree with the needs of adult flies for detecting convenient egg laying habitats. The attraction to both spectral ranges is probably based on two separate 'wavelength-specific behaviors' and we assume dichromatic vision in *B. difformis*.

Regarding a practical use in IPM or biological control, especially UV, but also yellow or green LEDs, are promising tools to improve monitoring and trapping of fungus gnats in greenhouses. This could be achieved, for example, by equipping yellow traps with these LEDs, but also by constructing solely LED-based monitoring devices. However, it should be taken into account that also beneficial insects can be sensitive to light traps, hence LED traps with or without additional UV LEDs should be tested for selectivity (Mellor et al., 1997). Moreover, UV LEDs can be an innovative tool for mass trapping of fungus gnats in dark conditions in mushroom cultivation (Ishitani et al., 1997; Jess and Bingham, 1999). It may also be considered to use UV fluorescent tubes with an optimized spectrum according to the wavelength screening reported here. Furthermore, fluorescent tube based traps may be equipped with additional yellow or green high-power LEDs. This could further enhance their attractiveness by combining the attraction to UV and yellow.

4 LED enhanced yellow traps equipped with cameras for monitoring of whiteflies and fungus gnats

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4.1 Abstract

Yellow sticky card traps are used for monitoring and control of alate pests such as whiteflies and fungus gnats in greenhouses. The use of light emitting diodes (LEDs) has turned out as a promising approach to increase the efficiency and reliability of visual traps. The implementation of (semi-)automatic image acquisition and analysis methods is of great interest in order to improve and simplify the identification and counting of insects on traps.

LED enhanced yellow traps were constructed which combine a yellow card with specific edge lighting acrylic glass equipped with green high-power LEDs in a frame. In a next step, traps were equipped with cameras and white high-power LEDs were mounted in the frame and adjacent to the trapping surface to facilitate the acquisition of transmitted and incident light images at dark night-time conditions. The traps were compared with common yellow sticky traps in small-scale tomato crop stands in gauze cages with artificial whitefly infestation (*Trialeurodes vaporariorum*) and naturally occurring not further determined fungus gnats. A final experiment was conducted in a larger crop stand to evaluate the trap efficiency and the image acquisition method with subsequent pest counting on images.

The results show a significantly increased efficiency of the LED enhanced traps for whiteflies compared to yellow traps in experiments with high population densities and in choice situations with both trap types. A higher attractiveness for fungus gnats was observed throughout. The obtained images allowed reliable counting of both pests comparable with manual counting on traps.

Key words: greenhouse whitefly, *Trialeurodes vaporariorum*, *Bradysia difformis*, sciarid fly, visual trap, biological control

4.2 Introduction

Yellow sticky traps are a common tool for monitoring and control of alate pests such as whiteflies and fungus gnats in greenhouse crop production. As an essential part of integrated pest management they are mainly used for early detection of pest presence and identification of hot spots in the crop, but they can also provide information on the dynamics of pest densities and if economic thresholds are exceeded. This assist the decision making process for need-based biological or chemical plant protection measures in time and space (Böckmann et al., 2015; Pinto-Zevallos and Vänninen, 2013; Gillespie and Quiring, 1987; Cloyd et al., 2007). An important prerequisite for an efficient monitoring with yellow traps is the reliability, which is affected by several uncontrollable and controllable biotic and abiotic factors. Especially the trap efficiency is a controllable factor which can be well influenced in order to improve the reliability of sampling (Pinto-Zevallos and Vänninen, 2013). A reasonable definition of the trap efficiency is the percentage of trapped insects within the effective radius of the trap. The effective radius means the distance at which a trap becomes attractive (Hartstack et al., 1971). Traps which are generally referred to be more attractive than others could exhibit a higher trap efficiency and/or an increased effective radius. The efficiency of visual traps depends in particular on the fitting of wavelength distribution and intensity to the visual systems and behavioural patterns of the target insects.

The visual attraction of the greenhouse whitefly (*Trialeurodes vaporariorum*) is based on ‘wavelength-specific behaviours’ which are innate colour sensitive responses. Green-yellow targets or light elicits a ‘settling behaviour’ which guides host plant detection while ultraviolet (UV) radiation initiates ‘migratory behaviour’ (Coombe, 1981). The ‘settling behaviour’ is controlled by a chromatic interaction between two photoreceptors with an excitatory input from a green sensitive receptor and an inhibitory input from a blue sensitive receptor (Stukenberg and Poehling, 2018). This leads to the extraction of a chromatic signal resulting in an action spectrum which peaks around 550 nm and which perfectly matches with the reflectance spectrum of green leaves. Within the spectral range of the action spectrum, the behavioural response clearly depends on intensity which is the key for the manipulation by yellow traps as they represent a supernormal host plant stimulus for whiteflies and other herbivorous insects (Prokopy and Owens, 1983). A yellow trap shows a brighter reflection around 550 nm as compared to green leaves and therefore elicits a stronger response which explains the common yellow preference in whiteflies.

The described mechanism offers various possibilities for further improvement of the attractiveness of yellow traps resulting in an increased efficiency and reliability. The trap efficiency could be modified by reducing the translucency of yellow card and roller traps because less blue light is transmitted and more green-yellow light is reflected (Sampson et al.,

2018). Moreover, the implementation of black backgrounds and patterns increased the efficiency (Sampson et al., 2018; Kim and Lim, 2011), since contrasts play an important role for host finding in herbivorous insects and have the potential to increase the attractiveness (Prokopy and Owens, 1983; Döring and Röhrig, 2016). However, the most promising approach to increase the trap efficiency for whiteflies is the use optimal adopted narrow bandwidth light sources such as green LEDs. Alone or as supplementation to yellow traps, the intensity emitted from the trap at an appropriate target specific wavelength can be artificially increased. Yellow card traps and yellow cup traps equipped with single green LEDs increased catches of the silverleaf whitefly (*Bemisia tabaci*) in commercial greenhouse crop stands (Chen et al., 2004; Chu et al., 2003; Chu et al., 2004). Traps containing twelve green high-power (HP) LEDs (517 nm peak wavelength) were highly preferred over yellow traps in choice experiments in net cages (Stukenberg et al., 2015). UV LEDs showed only a moderate attractiveness in this study but had a synergistic effect in combination with green LEDs, especially in dark night-time conditions. In a detailed behavioural study which tested a wide range of green and yellow LEDs in small scale multiple-choice and dual-choice experiments it was shown that a light green LED with a centroid wavelength of 550 nm was most attractive for *T. vaporariorum* (Stukenberg and Poehling, 2018). As a result of the intensity dependence, the attractiveness of shorter wavelengths green LEDs (533, 524 nm) could be relatively increased by increasing its intensity. This indicates that not perfectly fitting wavelengths can be compensated by the intensity of emission.

Compared to whiteflies, the visual behaviour of fungus gnats (*Bradysia spp.*) is less well researched. It is known that yellow traps are highly attractive to them and therefore are widely used for monitoring and control in greenhouses (Cloyd, 2010). A lab study shows that yellow trap catches of the dark-winged fungus gnat *Bradysia coprophila* depend on the illumination intensity (Cloyd et al., 2007). Analog to whiteflies, the supplementation of yellow traps with green LEDs increases catches of adult fungus gnats (Chen et al., 2004). Also the supplementation of pan traps with green and especially UV LEDs increased trap catches of fungus gnats (Sonoda et al., 2014). In a detailed behavioural study (Stukenberg et al., 2018), it was shown recently that the black fungus gnat *Bradysia difformis* shows two different responses to UV radiation and green-yellow light, with UV being the most attractive stimulus. Both attractive ranges are targeted with a broad sensitivity and no distinct wavelength discrimination in the peak region. Furthermore, the results show the preference for yellow LEDs compared to conventional yellow traps in choice experiments.

In order to reduce the workload for monitoring and to improve its accuracy and the timing of plant protection measures the implementation of (semi-) automatic image acquisition and analysis methods for the assessment of yellow traps is of great interest. A commercial example is the Scoutbox® (SoilCares, The Netherlands), a semiautomatic device that allows

identification and counting of pests by inserting a yellow trap into a box equipped with camera and exposure technique. Another simple approach used a flatbed scanner in combination with an image processing system (Qiao et al., 2008). A more complex approach apart from classic yellow trap monitoring used a greenhouse robot with an automatic attraction system and an image analysis for on-site detection of pests on plants (Xia et al., 2012).

The aim of this study is to develop and evaluate an LED enhanced yellow trap and to compare the efficiency with common yellow sticky card traps in small-scale tomato crop stands, with the greenhouse whitefly as the main subject and fungus gnats as side study. Furthermore, a computer based automatic image acquisition method will be implemented which facilitates a simple and rapid identification and counting of whiteflies and fungus gnats on images.

4.3 Material and Methods

4.3.1 LED enhanced yellow trap

In order to construct a trap that combines a common yellow card with green high-power (HP) LEDs (Oslon SSL 80 LT CP7P, Osram Opto Semiconductors, Regensburg, Germany), we used specific transparent acrylic glass for LED edge lighting (PLEXIGLAS® LED 0E010 SM, Evonik Industries AG, Essen, Germany) which was placed in front of a non-sticky yellow card provided by W. Neudorff GmbH KG (Emmerthal, Germany). In a first version of the traps, six green HP LEDs were mounted in a frame made of folded aluminium sheets. The frame comprises a white PVC plate coated with a yellow card on the rear side, the edge lighting acrylic glass in the middle, and a standard transparent acrylic glass (PLEXIGLAS® XT clear 0A000 GT, Evonik Industries AG, Essen, Germany) cover plate on the front. In this way the reflective spectral properties of a yellow card (12 x 16 cm) were combined with the spectrum of an even LED light emitting surface (Fig. 1). The emission spectrum of the trap was measured in a dark room with a spectrometer (AvaSpec-2048-2 and AvaSoft v.7.0.3 Basic; Avantes, Apeldoorn, The Netherlands), and the reflection spectrum was measured with the spectrometer Lambda 900 UV/VIS/NIR (PerkinElmer, Waltham, MA, USA) containing a 30-cm integrating sphere and a tungsten-halogen and deuterium lamp. For insect trapping, the trap was covered with self-adhesive transparent plastic film (Rico Design GmbH & Co. KG, Brakel, Germany) and coated with insect glue (Temmen GmbH, Hattersheim, Germany). See Fig. 2 A-B for the general principle, but showing the modified version explained in the next paragraph. Four traps were produced in total and were used in the later presented experiments 1-4.

In the further modified final trap version, the number of green LEDs was increased to eight and they were mounted in a more robust frame made of aluminium profiles. Further aluminium profile pieces are attached on all sides for LED cooling. A frame of black cardboard with

aluminium foil on the backside has been inserted underneath the cover plate to define and increase the contrast to the trapping surface. In order to enable the acquisition of transmitted light images of high contrast for insect counting, the frame was additionally equipped with ten white HP LEDs (XB-D R4, Cree Inc., Durham, USA). For the acquisition of incident light images, profiles with three white HP LEDs each (a total of 6) are attached on two sides adjacent to the trap surface. USB mini cameras (uEye XS, IDS Imaging Development Systems GmbH, Obersulm, Germany) with 5 megapixel resolution were mounted adjustable on a rod 20 cm away from the trapping surface. These four camera equipped traps were used in the later presented experiment 5. See Fig. 2 A-C for detailed schemes and images of the illumination modes.

All LEDs in each trap operated with 700 mA, which were powered by four LED drivers (Jolly Slim, TCI, Saronno, Italy). The drivers were controlled by external control signals (0-10 V) which were provided by the analogue outputs of two USB control modules and a switching relay was used to change between illumination modes (LabJack U12 and ME-UBRE, Meilhaus Electronic GmbH, Alling, Germany). All control parts were placed in a control box which was connected to a PC together with the USB cameras. Operation and automation of illumination modes was realized using the software ProfiLab-Epert 4.0 (ABACOM, Ganderkesee, Germany) and the open source scripting language for desktop automation “AutoHotkey v.1.1.22.04” (AutoHotkey, 2015).

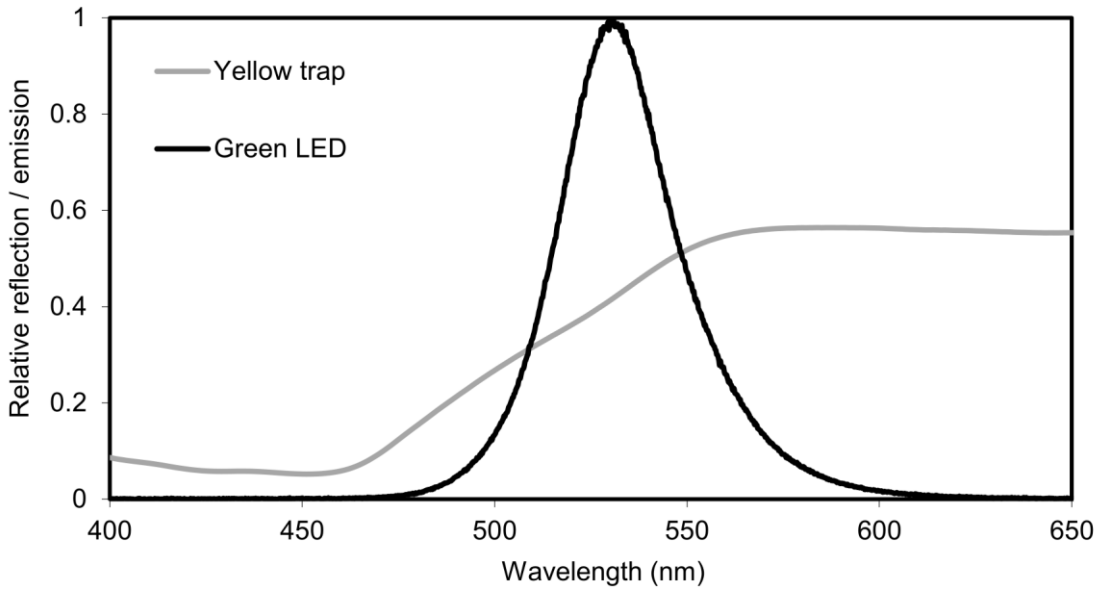

Fig. 1: Spectra of green LED and yellow trap which are combined in the LED enhanced yellow trap.

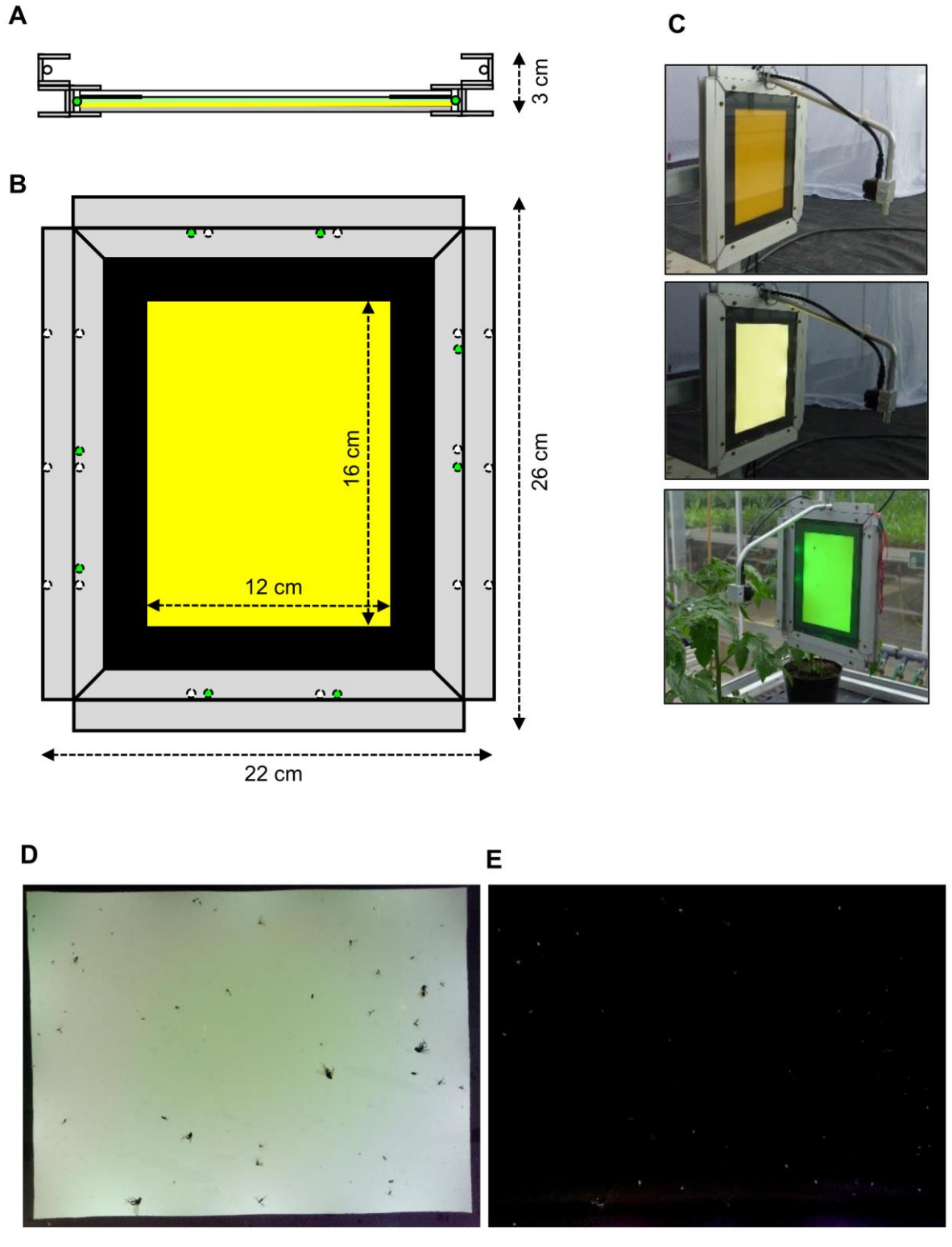

Fig. 2: LED enhanced yellow trap. (A) Cross-section scheme showing the aluminium profile frame which comprise LEDs, PVC plate coated with yellow card on the rear side, LED edge lighting acrylic glass in the middle and transparent acrylic glass cover plate. (B) Top view scheme showing trapping surface, black cardboard frame and layout of green and white LEDs in aluminium frame. (C) Pictures of the trap with camera without illumination (top), transmitted white light (middle) and transmitted green trapping light. (D) Example of generated transmitted light image with insects. (E) Generated incident light image at the same date showing whiteflies as small whitish dots.

4.3.2 Image acquisition and analysis

Two illumination modes are used for image acquisition at night in darkness: Transmitted light (white LEDs in profile) and incident light (white LEDs adjacent to trapping surface). The transmitted light images enable to visualize objects on the traps as dark contrasts, in particular larger objects such as fungus gnats and other insects (Fig. 2 D). Regarding the LED incident light, total reflection on the acrylic glass surface occurs due to small angle of incidence. In combination with the shielding of LEDs to the camera and a short exposure time, an almost black image is generated. Only the whitish whiteflies are visible, while the other insects are no longer visible (Fig. 2 E).

In experiment 5 the two illumination modes were consecutively turned on and two images per trap were successively taken at night around 03:00 (transmitted light and incident light) by accessing the firmware of the cameras (ueye cockpit, IDS Imaging Development Systems GmbH, Obersulm, Germany) via the “AutoHotkey” script (AutoHotkey, 2015). Subsequent to the experiment, rapid counting of fungus gnats (transmitted light images) and whiteflies (incident light images) on daily images was performed using the ‘point tool’ in the free software “ImageJ v.1.51” (Schneider et al., 2012).

4.3.3 Whiteflies and fungus gnats

Greenhouse whiteflies (*Trialeurodes vaporariorum* Westwood, Hemiptera: Aleyrodidae) were reared on small bush tomato plants (*Lycopersicon lycopersicum* cv. 'Miniboy') in gauze cages (1.4 x 0.7 x 1 m) in a greenhouse chamber of the Neudorff research nursery (Aerzen, Germany) at 22 ± 3 °C. For experiments, vital individuals were carefully collected with an aspirator from the underside of the top leaves into a snap-on lid glass vial (h x d = 50 x 30 mm).

Adult fungus gnats hatching from the substrate occurred naturally in the research greenhouse and in the experiments. Species of trapped fungus gnats were not further determined. Random samples of individuals in the greenhouse were identified as *Bradysia difformis* Frey (Diptera: Sciaridae), but it cannot be ruled out that other species were also present and trapped in the experiments.

4.3.4 Experimental overview

The experiments were carried out in the greenhouse of the Neudorff research nursery (Aerzen, Germany) at 22 ± 3 °C. Rollable greenhouse tables (1.6 x 1.5 x 0.8 m) with superimposed aluminium framed gauze cages (1.5 x 1.4 x 1.4 m) served as experimental units for experiments 1-4 (Fig 3 A, B). For experiment 5, the cages were arranged to a continuous area without cages (Fig. 3 C). Each table was connected to the ebb and flood irrigation system. About six week old bush tomato plants (*Lycopersicon lycopersicum* cv. 'Miniboy') were used as experimental plants. For experiments, eight plants were placed in two rows on the tables.

In each experiment, the four described LED enhanced yellow traps were compared with four common yellow sticky card traps (Neudorff, Emmerthal, Germany). They were cut to the same size (12 x 16 cm) as the trapping surface of the LED traps and only one side was used. The traps were installed about 45 - 50 cm above table level, approximately half below and half above top canopy level. With growing plants, trap height was adjusted accordingly during the experiments. In experiments 1-4 traps were placed on the northern side to ensure sun illumination from the south. In experiment 5, traps were placed on northern and southern side. The green trapping light was switched on each day from 06:00 to 22:00 in accordance with the supplementary light from sodium vapour lamps in the in the mornings and evenings greenhouse.

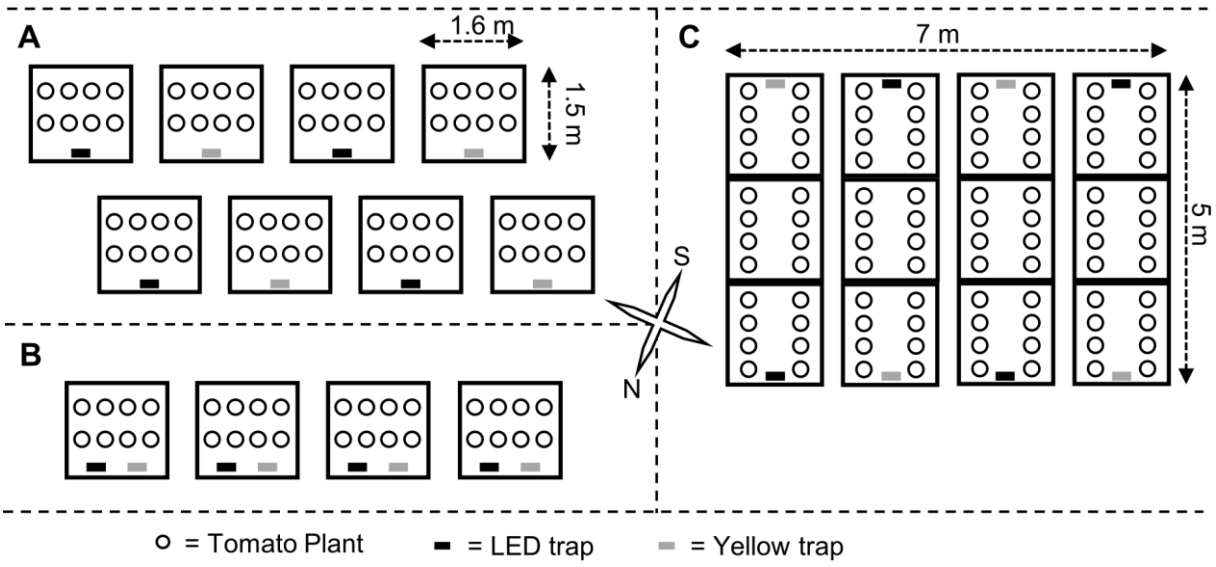

Fig. 3: Scheme of experimental setups. (A) Arrangement of rollable greenhouse tables with superimposed gauze cages containing plants and one trap type. (B) Arrangement with both trap types in one cage. (C) Arrangement of table without cages to a continuous area with plants and traps.

4.3.5 Experiment 1: Single trap comparison with few periodic releases of whiteflies

One LED trap or yellow trap was alternately placed in each of the eight cages together with eight plants per cage (Fig. 3 A). 200 whiteflies were released by means of snap-on lid glass vials in each cage two times, at the beginning and at day 3. The experiment lasted 14 days (28.10. - 11.11.2013). Whiteflies were counted on the traps daily on workdays in the morning between 09:00 and 10:00 and fungus gnats were counted on the traps at the end of the experiment.

4.3.6 Experiment 2: Double trap comparison with many periodic releases of whiteflies

One LED trap and a yellow trap were alternately placed in four cages together with eight plants per cage (Fig. 3 B). 200 whiteflies were released in each cage five times at the beginning and at day 3, 7, 10 and 14. The experiment lasted 18 days (9.12. - 27.12.2013). Whiteflies were counted on the traps daily on workdays in the morning between 09:00 and 10:00 and fungus gnats were counted on the traps at the end of the experiment.

4.3.7 Experiment 3: Single trap comparison with severe whitefly infection

Prior to the experiment, 64 two week old tomato plants were placed on two greenhouse tables with gauze cages. Each cage was infected with four heavily infested tomato leaves (approx. 400 whiteflies) from the rearing which were cut off and placed on the experimental plants. After 4 weeks with beginning of hatching, the plants occupied with eggs and larvae were placed in the experimental set-up. One LED trap or yellow trap was alternately placed in each of the eight cages together with eight plants per cage (Fig. 3 A). The experiment lasted 28 days (09.01. - 05.02.2014). Whiteflies were counted on the traps on Monday, Wednesday and Friday in the morning between 09:00 and 10:00.

4.3.8 Experiment 4: Single trap experiment with weak whitefly infection

One LED trap or yellow trap was alternatingly placed in each of the eight cages together with eight plants per cage (Fig. 3 A). Each cage was infected with 25 whiteflies. The experiment lasted 38 days (25.02. - 04.04.2014). Whiteflies were counted on the traps on Monday, Wednesday and Friday in the morning between 09:00 and 10:00.

4.3.9 Experiment 5: Trap comparison and image acquisition in a continuous crop stand

Twelve greenhouse tables (without gauze cages) were set up to a large continuous area. In between, narrow corridors were left free in one direction. 96 six week old bush tomato plants were placed on the tables in eight rows with eight plants per table. Initially, 15 whiteflies were released per table (a total of 180 on the area). The next day, four LED traps and four yellow traps were alternately placed on the northern and southern side of the area.

The experiment lasted 49 days (10.09. - 29.10.2015). Whiteflies, Fungus gnats and other insects were counted weekly on the traps. The daily image acquisition on LED traps at night at 03:00 was running throughout as described above. 49 transmitted light images and 49 incident light images were generated per trap, resulting in a total of 392 images for subsequent insect counting as described above.

4.3.10 Statistical analysis

Analyses were performed in R (Version 3.2.1; R Core Team, 2015). All experiments were analysed with generalized linear models with quasi-Poisson distribution and log link function. The response variable was the number of captured whiteflies or adult fungus gnats at each time point and the explanatory treatment variable was the trap type (LED or yellow trap). In experiment 1, 3, 4 the cage row and in experiment 5 the site of the trap (north, south) were included as block factors and an interaction with the trap type was assumed. Deviance analyses were performed to determine differences between trap types, influences of the blocks and interactions between trap types and blocks.

4.4 Results

4.4.1 Experiment 1: Single trap comparison with few periodic releases of whiteflies

Around 100 whiteflies totally were trapped on average on both LED and yellow trap, with no significant differences between them in the course of the 14-day experiment (Fig. 4). The trapped numbers per cage clearly increased after each of the two releases and remained almost constant from day 8 onwards, representing about 25 % of the totally released individuals (400).

The mean number of trapped fungus gnats was 72 on LED traps and 32 on yellow traps, but the data showed a high deviation and no significant differences could be calculated (Fig 6 A).

4.4.2 Experiment 2: Double trap comparison with many periodic releases of whiteflies

When both trap types were compared in one cage, the mean numbers of trapped whiteflies were significantly higher on yellow traps only on day 1 during the 18-day experiment. Afterwards accumulated numbers raised more on LED traps compared to yellow traps and differed significantly from day 10 onwards ($P<0.05$, Fig. 5). The numbers clearly increased after each release and reached finally 95 on LED traps and 39 on yellow traps. In this experiment more whiteflies were released (1000) and the experiment lasted longer as compared to the previous experiment, but only a smaller portion of released individuals (14 %) was trapped in total.

The mean number of trapped fungus gnats on LED and yellow traps (72 vs. 17) differed significantly ($P<0.001$, Fig.6 B).

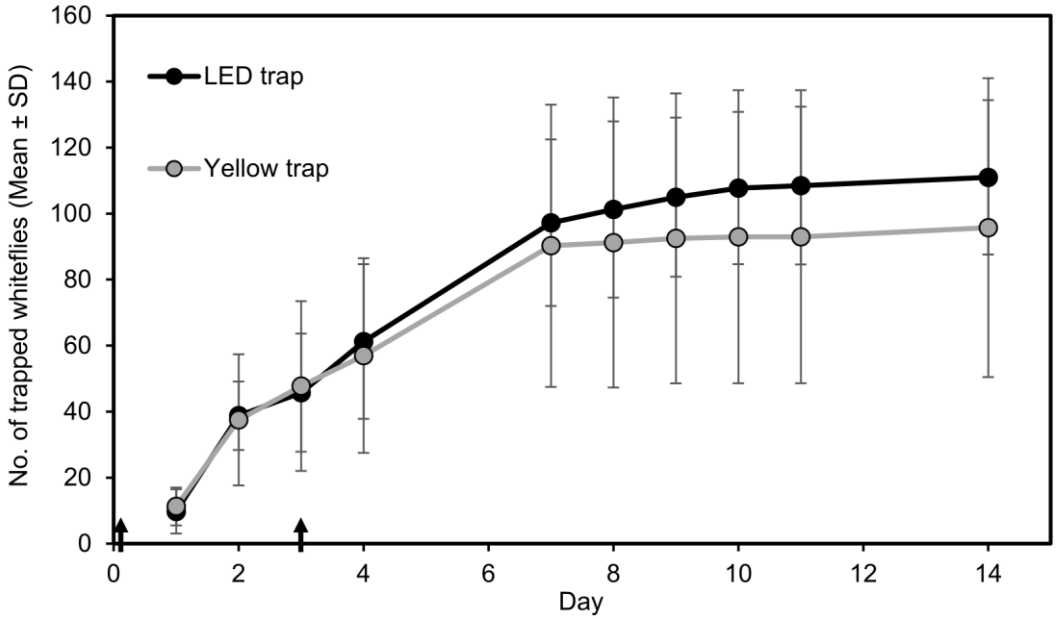

Fig. 4: Trapped whiteflies on LED and yellow trap in single trap comparisons in small-scale tomato crop stands. Cages with tomato plants were each equipped with one trap type (n = 4; experiment 1). Arrows on the x-axis indicate the release of 200 whiteflies per cage.

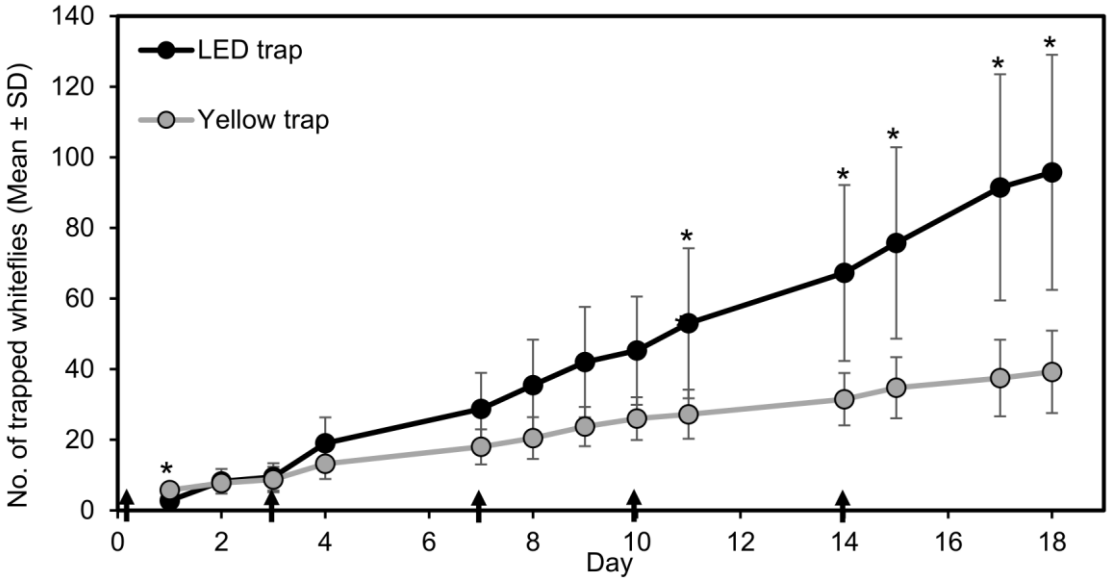

Fig. 5: Trapped whiteflies on LED and yellow trap in double trap comparisons in small-scale tomato crop stands. Cages with tomato plants were each equipped with both trap types (n=4; experiment 2). Arrows on the x-axis indicate the release of 200 whiteflies per cage. Asterisks indicate significant differences at each time point (P=0.05, GLM, Deviance Analysis).

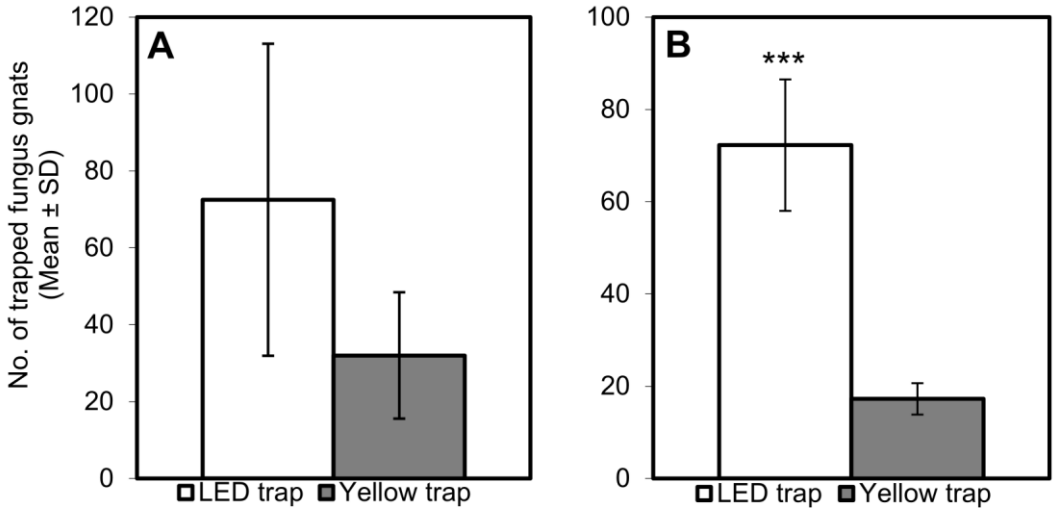

Fig. 6: Trapped fungus gnats on LED and yellow traps in single and double trap comparisons in small-scale tomato crop stands at the end of each experimental period. (A) Cages with tomato plants were each equipped with one trap type (n = 4; experiment 1). (B) Cages with tomato plants were each equipped with both trap types (n = 4; experiment 2). Asterisks indicate significant difference (P=0.001, GLM, Deviance Analysis).

4.4.3 Experiment 3: Single trap comparison with severe whitefly infection

The mean number of trapped whiteflies in the 28-day experiment was significantly higher on LED traps from day 23 onwards (P<0.05, Fig. 7). A significant block effect of the cage row was observed at day 7, 9, 12 and 14 (P<0.05). The heavy initial infection four weeks prior to the experiment lead to high numbers of trapped freshly emerged adults and reached an average of 1597 on LED traps and 517 on yellow traps.

4.4.4 Experiment 4: Single trap experiment with weak whitefly infection

The mean numbers of whiteflies on both LED and yellow traps steadily increased and reached around 68 on both trap types, with significant differences between them only on day 8 in the course of the 38-day experiment (P<0.05, Fig. 8). The trapped whiteflies in approximately the first 20 days can be attributed to the initially released individuals (25). Due to subsequent oviposition and development of a new generation, freshly emerged whiteflies should have contributed to the trapped numbers in the second half of the experiment.

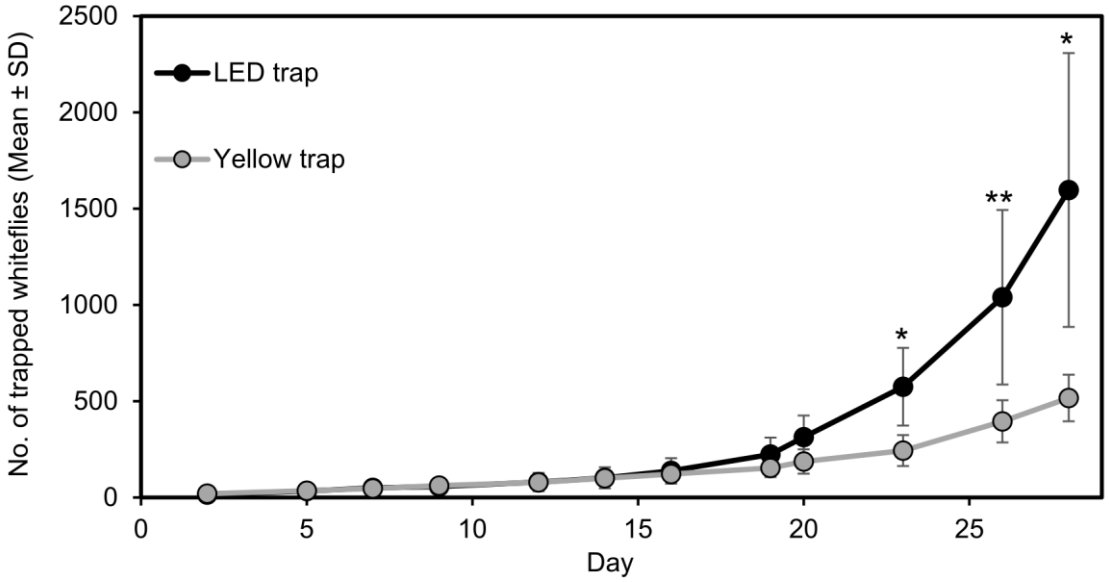

Fig. 7: Trapped whiteflies on LED and yellow trap in single trap comparisons in small-scale tomato crop stands. Cages with tomato plants were each equipped with one trap type (n = 4; experiment 3). Plants were heavily infected with whitefly larvae and eggs prior to experiment. Asterisks indicate significant differences at each time point (* P=0.05, ** P=0.01, GLM, Deviance Analysis).

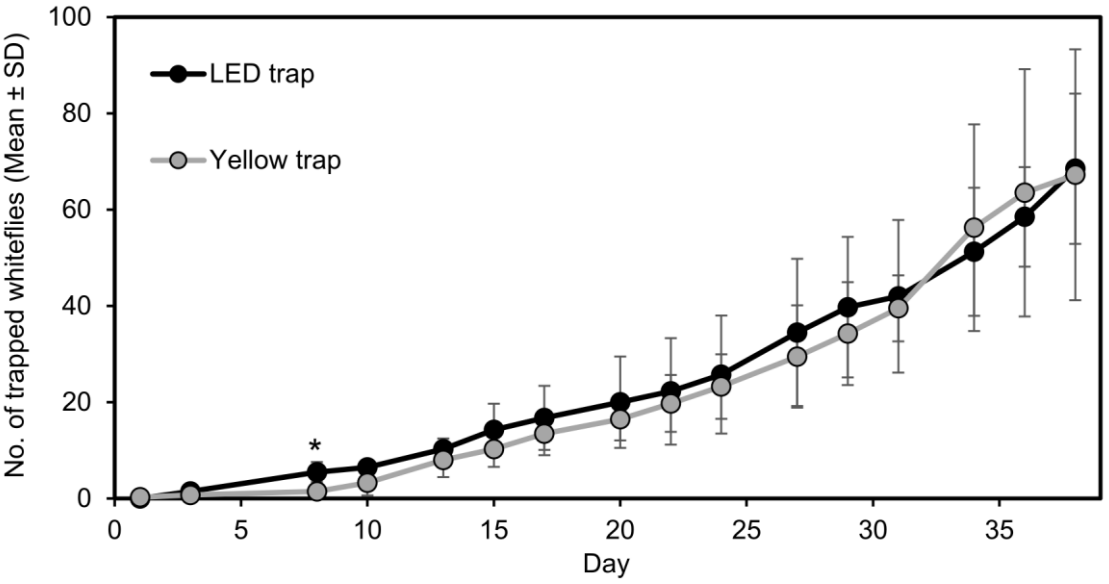

Fig. 8: Trapped whiteflies on LED and yellow trap in single trap comparisons in small-scale tomato crop stands. Cages with tomato plants were each equipped with one trap type (n = 4; experiment 4). Plants were infected with 25 whiteflies per cage at the beginning of the experiment. Asterisks indicate significant differences at each time point (P=0.05, GLM, Deviance Analysis).

4.4.5 Experiment 5: Trap comparison and image acquisition in a continuous crop stand

When both trap types were compared in a continuous crop stand, the mean numbers of trapped whiteflies on LED and yellow traps differed significantly throughout the whole 7-week experiment ($P<0.001$, Fig. 9). Significant effects of the trap site were observed in some weeks and are based on more whiteflies being caught on traps on the northern side in week 4 ($P<0.05$) and more trap catches on the southern side in week 6 ($P<0.05$) and 7 ($P<0.01$). Significant interaction effects were observed in weeks 2, 3, 4 with more catches only on yellow card traps on the northern side ($P<0.05$). A further significant interaction effect was determined in week 7 which is based on more catches only on LED enhanced traps on the southern side ($P<0.05$). A steep increase of the trapped numbers could be observed after 5 weeks due to freshly emerging adults subsequent to the initially released whiteflies which is also related to the described site and interaction effects.

The mean numbers of trapped fungus gnats on LED and yellow traps differed significantly throughout the whole 7-week experiment with a consistent rise from the first day onwards ($P<0.001$, Fig. 10).

The counting of whiteflies from images allowed daily determination and was quite comparable with manual counting on traps as long as the mean numbers remained below approximately 100 individuals up to week 6, but image countings underestimated real catches at high numbers in week 7 (Fig. 9). The counting of fungus gnats from images allowed daily determination and was generally comparable with manual countings on traps but showed higher relative variation compared to whitefly countings (Fig. 10).

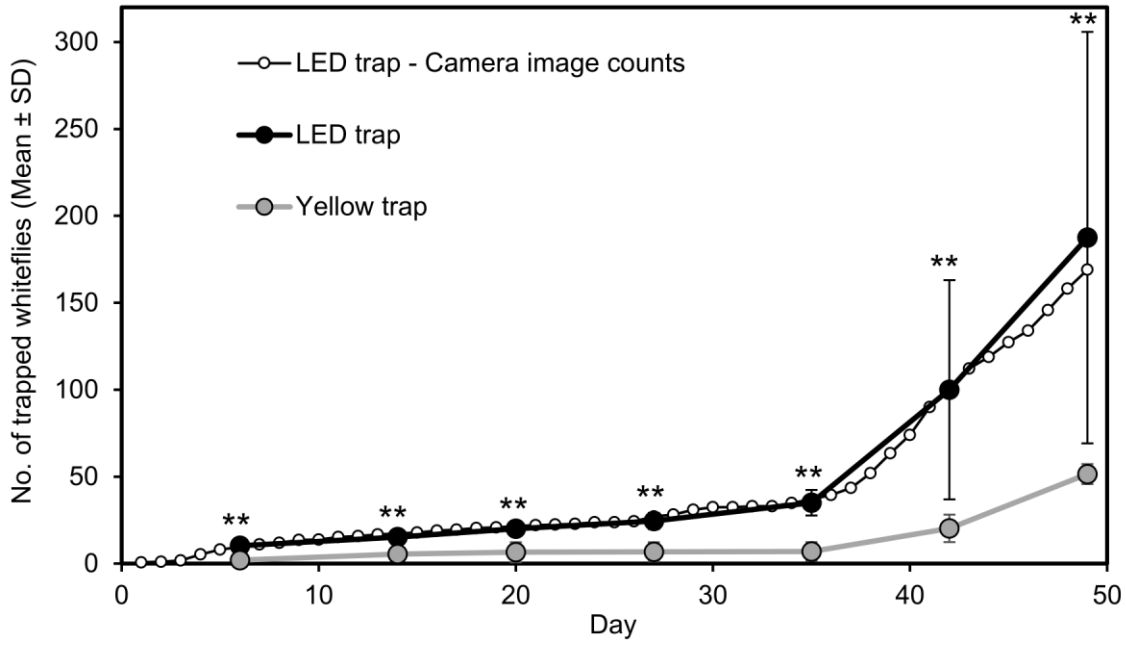

Fig. 9: Trapped whiteflies on LED and yellow trap in a small tomato crop stand. Both trap types (n = 4; experiment 5) were placed in a continuous crop stand. The area was evenly infected with 180 whiteflies at the beginning of the experiment. LED traps were equipped with cameras for daily image acquisition at night. Whiteflies were counted weekly on traps and additionally on images after the experiment. Asterisks indicate significant differences at each time point for manual counts on LED and yellow trap (* P=0.05, ** P=0.01, GLM, Deviance Analysis).

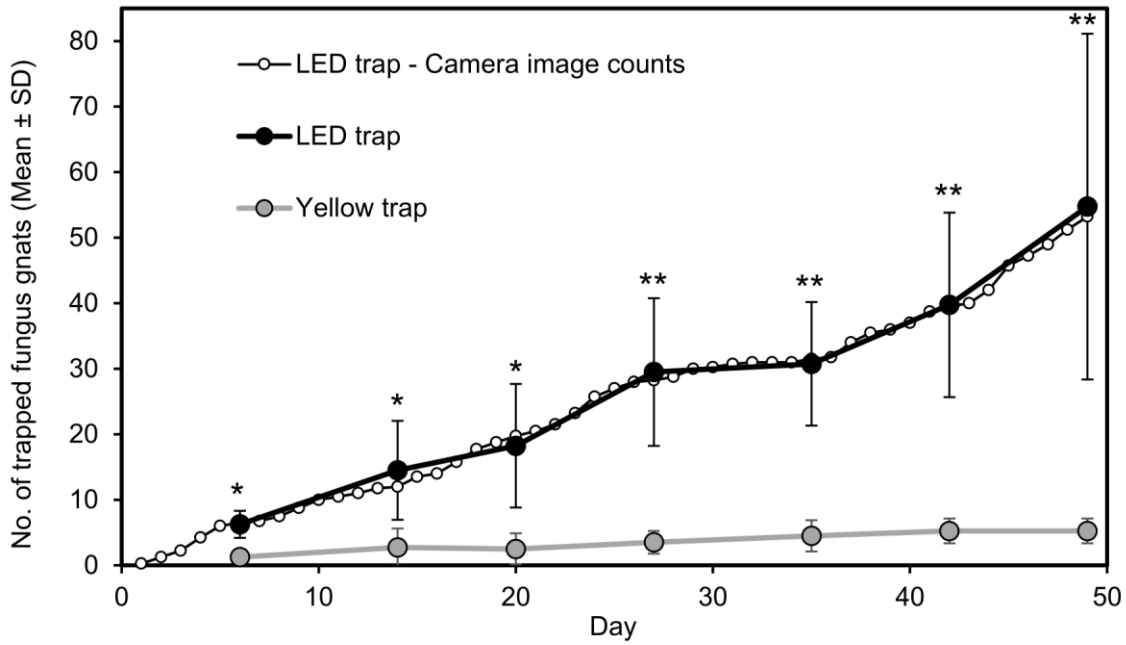

Fig. 10: Trapped fungus gnats on LED and yellow trap in a small tomato crop stand. Both trap types (n = 4; experiment 5) were placed in a continuous crop stand. LED traps were equipped with cameras for daily image acquisition at night. Fungus gnats were counted weekly on traps and additionally on images after the experiment. Asterisks indicate significant differences at each time point for manual counts on LED and yellow trap (* P=0.05, ** P=0.01, GLM, Deviance Analysis).

4.5 Discussion

The study generally shows the great potential of an increased trap efficiency of the LED enhanced yellow traps for both whiteflies and fungus gnats compared to conventional yellow traps, which corroborates other LED based studies. (Chen et al., 2004; Stukenberg and Poehling, 2018; Stukenberg et al., 2015; Stukenberg et al., 2018).

With regard to whitefly trapping, which is the main objective of this study, diverging results were obtained in different experimental set-ups but overall the superiority of LED enhanced traps could be shown. In the small-scale cage experiments clearly more whiteflies were trapped on LED enhanced traps when both trap types were presented in one cage in a kind of choice situation (Exp. 2, Fig. 5) and at heavy infection conditions with one trap type per cage (Exp. 3, Fig. 7). In contrast, no differences between trap types were observed in cage experiments with low numbers of released whiteflies (Exp. 1 & 4, Fig. 4 & 8). In particular in the last main experiment in the larger crop stand area with both trap types, the LED enhanced yellow traps exhibited their full potential with significantly higher captured numbers throughout the whole experimental period (Exp. 5, Fig. 9).

In experiment 1, 3 and 4 LED enhanced traps and yellow traps were each placed in one cage, representing a kind of no-choice situation in the sense that any whitefly which takes off from the plants could chose only one or the other trap type. These short-term experiments should evaluate whether the LED traps have the potential to attract higher overall numbers in the small scale system. In this experimental layout, a higher trap efficiency of LED traps could only be observed when plants were heavily infected prior to the experiment while with lower infestation conditions higher efficiencies were not observed. Two our understanding, these diverging results can be well explained with whitefly flight activity which is known to strongly affect yellow trap efficiency. The flight activity of whiteflies is influenced by several factors such as temperature, plant status, conspecific density, diurnal flight pattern and ambient UV radiation. Flight typology in greenhouse tomato crops between leaves and plants can be classified in short flights, long flights and dispersal flights (Bonsignore, 2015; Liu et al., 1994; Pinto-Zevallos and Vänninen, 2013). In experiment 1, the majority of the 400 released whiteflies settled on the host plants and the efficiency of the common yellow trap obviously was sufficient to trap the flying individuals subjected to trapping within their range. Similar results were obtained in experiment 4 with initially released and later on freshly emerged whiteflies present in the system. The potentially higher efficiency of the LED traps did not result in higher trap catches in these experiments, most likely because of limited whitefly flight activity. When tomato plants were heavily infected prior to experiment 3, huge numbers and far more whiteflies were overall trapped on the LED enhanced trap. Here uncontrolled numbers of adult whiteflies freshly emerged in the experiment which led to a high conspecific density most likely resulting in a

high flight activity and more long and dispersal flights. More whiteflies were directed to the LED enhanced yellow traps under such conditions, indicating a higher efficiency for whiteflies being in flight. In experiment 2, both trap types were placed in one cage in a choice situation to evaluate the general preference and not the overall efficiency. Again, the majority settled on the host plants but flying whiteflies subjected to trapping significantly chose the LED enhanced trap, which demonstrates the generally high attractiveness of the LED trap.

In line with the presented results, Stukenberg et al. (2015) showed that LED traps are highly preferred over yellow traps in recapture choice experiments but no differences are observed in no-choice experiments. Our results show that yellow traps already exploit sufficient attractiveness in small systems and that flight activity might be the limiting factor for trapping. Independent of how attractive the presented trap is, flight activity seems to be a prerequisite and cannot be influenced by LED traps. A potential shortcoming in the cage experiments with regard to the evaluation of the efficiency was the small size of the system. This did not allow longer flights and dispersal flights (Bonsignore, 2015), and might have underestimated that LED enhanced yellow traps could exhibit a greater effective radius and act over longer distances (Hartstack et al., 1971).

In the last experiment with all traps together in the open 35 m² crop stand, the high overall efficiency of the LED enhanced traps was shown most prominent. Two traps of each type were respectively placed on the northern and southern side of the area and significant influences of the site and interactions with the trap type were observed. Especially in the last two weeks, the LED enhanced traps on the southern side with the trapping surface turned away from the sun direction trapped much more whiteflies than LED traps on the northern side facing the sun. In contrast, yellow card traps trapped more whiteflies on the northern side facing the sun than yellow traps on the southern side, especially in the first weeks. This observation can be explained with different relative reflection intensity of the yellow traps which fluctuates with the sun illumination intensity, while the constant LED emission contributed relatively different to the overall intensity which is the force of attraction to yellow cards and green LEDs (Stukenberg et al., 2015; Stukenberg and Poehling, 2018). When traps faced the sun, the reflection from yellow card traps was relatively higher and LED enhanced and yellow card traps appeared more equal attractive than on the other side turned away from the sun. It is reported that whiteflies conducted more flights in the morning and evening hours in tomato greenhouses (Bonsignore, 2015), which is also the time when traps were exposed to a flat sun illumination angle since our experiment was conducted in autumn.

Moreover different flight and movement patterns of the Whiteflies might be involved in the different trapping patterns. In the first weeks only the released whiteflies were present in the system and quiet low numbers were overall recorded. LED trap catches on both sides of the

area were relatively equal while more whiteflies were trapped on the northern side on yellow traps facing the sun. This could be an indication that settled whiteflies did not move much and that trapping resulted mainly from nearby plants by short flights (< 40 cm) or long flights (< 2 m), as classified by Bonsignore (2015). In the last weeks with the emergence of new generations the trapping rate increased and much more individuals were trapped in particular on LED enhanced traps on the southern side. This is an indication for an increase of dispersal flights and a southward movement of the population which might be driven by UV radiation from that direction, which is known to be involved in migratory behaviour (Coombe, 1982). The advantage of LED enhanced yellow traps in darker or not directly illuminated areas could be shown in this experiment which could facilitate a more consistent whitefly monitoring compared to yellow traps. Moreover the increased trap efficiency might lead to a more precise detection of low population densities or a reduction of traps needed for an efficient monitoring.

With regard to the trapping of randomly occurring fungus gnats it can be stated that the LED enhanced traps showed an even higher efficiency as compared to whiteflies, corroborating other studies with LED equipped yellow traps in greenhouses (Chen et al., 2004). Fungus gnat numbers were not controlled which led to high variation of trapping events in the first cage experiment with traps presented in a no-choice situation. This most likely prevented the determination of significant results although on average twice as many individuals were trapped on LED enhanced traps. In experiment 2 traps were presented in a choice situation and LED traps were highly preferred. Similarly, in the last experiment in the larger crop stand yellow traps captured only low numbers whereas LED were extremely efficient. These observations support the superiority of LEDs and the extremely sensitive phototactic response of fungus gnats, although the mechanism and ecological reason of this behaviour is not understood (Cloyd et al., 2007; Stukenberg et al., 2018).

The implemented automatic image acquisition method during the night proved to be reliable at moderate numbers and allowed in particular the visual segregation of relatively small whiteflies from other insects on the trap, since the whitish colour is a unique feature among greenhouse pests. In combination with occasional visual observation and maintenance of traps, the developed method can reduce workload for monitoring because images are rapidly evaluated at a central location. Moreover daily assessment especially at critical times can improve timely accurate release of natural enemies (Böckmann et al., 2015). Finally, the on-site images could be a basis for the future development of image analysis methods for automatic identification and counting of whiteflies and other pests (Xia et al., 2012).

In future studies the traps should be tested in commercial greenhouses with different crops to evaluate if the increased efficiency really leads to an improved monitoring. A focus should be trap spacing and the number of traps needed per area for an effective sampling. Finally, trap

catches should be linked with economic thresholds and a decision support system in order to perform biological or chemical plant protection measures.

5 General Discussion and Outlook

With regard to the visual behaviour and colour processing of *T. vaporariorum*, the first chapter reveals a photoreceptor sensitive for blue light and an inhibitory blue-green chromatic mechanism which is used for host plant detection. After a long period with no basic research on the visual ecology of the greenhouse whitefly (Coombe, 1981; 1982), this study represents the first detailed characterisation and comprehensively links existing studies and fills a knowledge gap, although some indications for this mechanism were already present in the literature (Stukenberg et al., 2015). Because blue-green opponency is already described for other herbivorous insects (Döring et al., 2009), it can be suggested that it represents a relatively universal mechanism, especially for phloem-sucking insects. With this study, *T. vaporariorum* finally joins the list of herbivorous insects using this mechanism.

The presented colour choice models are empirical ad hoc models which rely on variable photoreceptor configurations based on template formulas (Govardovskii et al., 2000), rather than on fixed receptor sensitivities which were determined by physiological measurements. Their aim is to characterise the mechanism and to give an approximate estimation of the spectral photoreceptor sensitivities. Under different conditions their outcome might be slightly different and real receptor sensitivities might also slightly differ. Only one physiological electroretinogram study exists for *T. vaporariorum* which turned out to be not completely reliable because it lacks partial receptor adaptation and the blue receptor was not determined there (Mellor et al., 1997). A new and more precise physiological study which shows the real receptor sensitivities would be helpful for future investigations and modelling of the visual processing. More precise and established modelling approaches which are already used for well-studied insects like honeybees and butterflies could be applied then (Chittka, 1996; Kelber, 2001).

The initial main objective of this thesis in order to improve visual traps for the greenhouse whitefly was to screen for attractive LEDs regarding colour (wavelengths) and intensity. For the greenhouse whitefly *T. vaporariorum*, the first chapter gives answers to this fundamental question by the presented choice experiments and the determined LED based action spectrum of the ‘settling response’. A chartreuse green LED with a centroid wavelength around 550 nm proved to be most attractive while green LEDs in the range 520-530 nm did not exploit maximum response if compared at equal intensities. The determined intensity dependencies show that the lower attractiveness of green LED wavelengths which do not optimally fit to the maximum sensitivity can be relatively increased by increasing its intensity. The chartreuse green LED (550 nm) used in the experiments was a special multi-chip emitter which is not commercially available at reasonable prices and needed additional cooling. LED light is generated by electroluminescence based on different semiconductor materials and mixtures

which do not allow to adjust any desired wavelength at sufficient efficiencies. Especially in the green-yellow range (550-560 nm), the so called “green gap”, no efficient LEDs are available up to date (Laubsch et al., 2010), and sufficient intensities can only be obtained by special multi-chip arrangements such as used in chapter 1. Therefore we can state that perfectly fitting LEDs that could be used in reasonable traps for *T. vaporariorum* are currently not available on the market. This is in contrast to LEDs from the blue range which can be used to trap the western flower thrips (*F. occidentalis*). A recent study shows that the attractiveness of blue sticky traps could be increased by the amendment with only one single blue high-power LED which fits well to the maximum sensitivity (Otieno et al., 2018). Nevertheless, due to the rapid technical development, more efficient green-yellow LEDs might be available in the near future (Yeh and Chung, 2009).

For the later constructed LED enhanced yellow traps (chapter 3) commercial green LEDs of 530 nm peak emission were used. This commercial and cheap LED was among the two second most attractive LEDs in chapter 1, together with the yellow LEDs of 574 nm, which was also a special multi-chip emitter. Only one LED of this green LED type was sufficient to attract whiteflies in the small scale cage setup under greenhouse conditions in chapter 1. The obtained results in chapter 3 clearly show that these LEDs considerably increased the attractiveness of the LED enhanced traps, which corroborates other whitefly LED trap studies with similar wavelengths (Stukenberg et al., 2015; Chen et al., 2004). According to the illustrated mechanism of attraction, the attractiveness of the LED traps is determined by intensity and could theoretically be further increased by additional LEDs. Nevertheless, because of photoreceptor adaptation mechanisms (Laughlin and Hardie, 1978), there should be a limit where further increased intensities might not result in a further increase of the trap efficiency. Moreover, the third chapter shows that flight activity and flight distance is most likely the limiting factor for the trap efficiency and these factors cannot be influenced by the attractiveness of the trap. The constructed traps already emitted a high intensity by the six (trap version 1) and eight (trap version 2) high-power LEDs and it remains unclear if higher or lower intensities would have affected the results to a considerable degree. Future studies should evaluate the factor intensity from an economic point of view with regard to trap efficiency and trap reliability in commercial greenhouses. For commercial purposes, cost-benefit analysis must be performed, which should also include optimal trap spacing and numbers of traps for different greenhouse crops.

Another aspect which arises from the intensity dependent attraction is the possibility to enhance the reflection from the yellow trap itself by modifying the colour of the material. In a study on the colour choice behaviour of the pollen beetle (*Meligethes aeneus*), which is also based on blue-green opponency, a fluorescent yellow colour proved to be most attractive (Döring et al., 2012). Due to the fluorescent properties the relative reflection intensity in the

green range reached more than 120 %, while the commercial yellow trap showed only 80 %. The yellow trap by company Neudorff used for the LED enhanced yellow trap shows only 60 % relative reflection intensity. Therefore it can be assumed that a fluorescent yellow colour could further increase the trap efficiency due to higher sunlight reflection independent from LEDs. The LED emission from the trap would also improve slightly, since the construction with LED acrylic glass edge lighting also partially reflects the LED light from the yellow trap behind it.

Apart from trapping with appropriate wavelengths and intensities, the blue-green opponent mechanism shown for *T. vaporariorum* in chapter 1 gives rise to further considerations how blue light could be used to control whiteflies in greenhouses. In line with another study (Stukenberg et al., 2015), the presented LED mixing experiments clearly show the repellent effect of relatively small intensities of blue light. Therefore, one can think of using upwards shining blue LEDs arranged around or in between plants to deter whiteflies. *T. vaporariorum* has a divided compound eye with only 30 and 55 ommatidia in the ventral and dorsal region respectively, indicating a poor spatial resolution (Mellor et al., 1997). Due to this limited spatial separation, high intensity blue LED light might overlay the host plant stimulus, especially for young and small plants. According to the reported mechanism, the stimulus for the settling response to host plants might be disrupted, which could make plants more or less invisible. First small scale tests of such a light barrier not presented in this thesis are very promising and the principle was claimed by the Leibniz Universität Hannover and a patent application has been filed. Such a control measure could theoretically be combined with highly attractive LED enhanced traps as some kind of push and pull strategy for invading or dispersing whiteflies. Nevertheless, this approach needs detailed investigations under realistic and commercial greenhouse conditions in the future. Another plant protection measure which could be related to this mechanism is the use of reflective mulch films which are known to reduce whitefly infestation (Csizinszky et al., 1999). This effect is explained to be an effect of UV reflection in applied studies, but on the background of this study it is likely that the reflection of blue light might be the real cause. This is supported by the contradicting fact that the combined use of green and UV LEDs even increased the attractiveness of LED traps (Stukenberg et al., 2015).

The second chapter presents the first detailed LED based study about the visual behaviour in fungus gnats and in particular for the black fungus gnat (*Bradysia Difformis*). It has relevance for the basic understanding of the visual behaviour of *B. difformis*, which was rarely regarded up to now. Furthermore, the study provides implications for the use of LEDs in visual traps for monitoring and control of fungus gnats in greenhouses and in edible mushroom production. In summary *B. difformis* shows two different, probably wavelength specific behaviours to UV radiation and green-yellow light, with the former being the most attractive stimulus. These behaviours might be directly related to underlying photoreceptors, suggesting dichromatic vision in *B. difformis*.

The results clearly show the superior attractiveness of LEDs compared to conventional yellow card traps. A UV LED with a peak at 382 nm was clearly more attractive than any LED from the visible range, which is in line with the fact that fungus gnats are commonly trapped with UV fluorescent tube based traps in mushroom cultivation (Ishitani et al., 1997). It is in contrast to the results obtained for whiteflies and suggests that UV LEDs should be used in visual traps for fungus gnats instead of green or yellow LEDs. On the other hand, the LED enhanced yellow traps in chapter 3, were highly efficient to trap fungus gnats, even more efficient than for whiteflies. This corroborates other studies with LED equipped yellow traps (Chen et al., 2004), and the fact that fungus gnats are commonly trapped on yellow traps in greenhouses (Cloyd et al., 2007). Regarding the wavelength preference for LEDs in the visible range, an unspecific broad sensitivity for green and yellow LEDs (525 - 600 nm) was observed, while blue around 450 nm was most unattractive. This suggest that almost any green or yellow LED could be used in visual traps, which is in contrast to the results obtained for whiteflies. But because of the higher electrical efficiency of green LEDs (Laubsch et al., 2010), they appear most suitable to be used for LED enhanced traps. UV LEDs are still relatively expansive as compared to green LEDs and are therefore the better choice for LED enhanced yellow traps, since their efficiency appeared absolutely sufficient to trap fungus gnats. Nevertheless, for special cases such as mass trapping in high-value ornamental crops or edible mushroom production, UV LEDs alone or in combination with yellow or green LEDs should be considered.

Regarding the factor intensity no clear results could be obtained as compared to whiteflies, and fungus gnats were equally trapped with different intensities. Their response to LED light was very sensitive and already small intensities appeared sufficient which is also supported by the high efficiency of the LED enhanced yellow traps. This suggests an extremely sensitive phototactic response of fungus gnats which could be an indication that spatial vision is only weakly developed and no real object detection is present (Kelber and Osorio, 2010). Another interesting aspect was that a black surface was preferred when compared to the yellow LED trap screen. A similar contrast may be found in nature between colour-reflecting plants and the dark substrate. As adults of *B. difformis* are mainly searching for convenient substrates for oviposition, it is reasonable that rather dark areas next to plants are preferred targets and that our black surface was associated with a substrate for egg laying. Nevertheless, the mechanism and ecological reason of this behaviour remains unclear, but the use black contrasts may be a good possibility to further increase the attractiveness of visual traps. Maybe the black margin around the trapping surface of the LED enhanced yellow traps already contributed positively to their overall high efficiency.

As extensively discussed in chapter 3, the developed LED enhanced yellow traps show a high efficiency to trap whiteflies and fungus gnats as compared to conventional yellow sticky card

traps. The responsible behavioural mechanisms were extensively investigated and discussed in chapter 1 and 2, and were comprehensively discussed here.

The developed image analysis method allowed reliable counting of both pests which was comparable with manual counting on traps. This forms a basis for the development of automatic pest identification and has to be further developed regarding image analysis, software, user interface and applicability in the future. Furthermore the system has to be adapted and calibrated to certain crops and conditions. Moreover, correlations of whitefly trap catches and whitefly densities in the crop need to be drawn to provide decision support for biological or chemical control measures (Böckmann et al., 2015).

For a complete trapping device, the system should be further extended to other pest species. When knowledge on the visual behaviour and attractive wavelengths of certain species is not known, it can be evaluated in LED based colour choice experiments, as described in this thesis for whiteflies and fungus gnats. As an example, the LED wavelength preference of the western flower thrips was evaluated in a similar setup and subsequently blue sticky traps were equipped with one single high power LED (445 nm), resulting in an increased performance (Otieno et al., 2018). One could think of an LED enhanced trap that can be flexibly switched to certain species, depending on the pest pressure. Furthermore, the image analysis for automatic identification and counting should be extended to other pest insects than whiteflies and fungus gnats. In combination with such an automatic pest identification one could also think of a “smart trap” that adapts automatically to certain species. Because flight activity is limiting the efficiency of the constructed trap for whiteflies, the potential of optical or physical manipulation in order to initiate flight behaviour should be evaluated under laboratory as well as under greenhouse conditions. This could be for example UV or blue light above the crop plants (push and pull strategy) or mechanical rousing of pest (e.g. air pressure jets). Also the possibility of constructing the trap as a mobile system that moves automatically through the greenhouse should be taken into account. An experimental approach with a mobile greenhouse robot equipped with air nozzles, a trapping device based on yellow colour and an image analyses system has already been attempted (Xia et al., 2012). In its entirety, such approaches may lead to control strategies which could mainly be based on optical and mechanical manipulation and trapping. In a last step, such a monitoring or control system should be tested for its compatibility with biological control organisms (selectivity). Finally, the technology has to be implemented in comprehensive strategies for biological control and IPM.

In conclusion, this thesis connects unique basic experimental research with applicable aspects for biological plant protection. It has relevance for the basic understanding of the visual ecology of *T. vaporariorum* and *B. difformis* and provides implications for the use of LEDs in visual traps for monitoring and control in greenhouses. Since this thesis represents a connecting link

between visual ecology of pest insects and monitoring technology, it forms a profound basis for future investigations in both directions.

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Publications

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